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Abstracts

Selected short talks

P.59 **Breaking the Glycan Code: How Cell Cycle Timing Reveals Novel Lectin Checkpoint Regulation of T-cell-mediated killing**

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Background: T-cell function is regulated by immune checkpoints that ensure balanced immune responses and tolerance. Tumours exploit these checkpoints to supress tumour-infiltrating lymphocytes, limiting the efficacy of PD-1 and CTLA-4 therapies (20% efficacy). Siglec-15, a newly identified immune checkpoint, offers a promising therapeutic target, particularly in cancers resistant to PD-1/PD-L1 blockade. Upon binding its T-cell ligands, Siglec-15 suppresses antigen-specific T-cell responses, and monoclonal antibodies targeting Siglec-15 have demonstrated enhanced antitumor activity and growth suppression in murine models. However, much remains to be explored, including the mechanisms through which the Siglec-15 immunoinhibitory signal is delivered via T-cell ligand interaction. The aim of the work was to characterise cell cycle-dependent expression of Siglec-15 Ligands on CD8 T-cells, determine phase-specific CD8+ T-cell killing efficiency during cell cycle progression and evaluate the functional consequences of Siglec-15 checkpoint engagement on cytotoxic activity.

Methods: We employ a novel method combining EdU incorporation, DNA content analysis, and CTFR proliferation staining to accurately analyse glycan-ligand expression across cell cycle phases of CD8+ T-cells using flow cytometry, as well as granzyme B production with or without Siglec-15Fc treatment. Phase-specific CD8+ T-cell cytotoxic capacity was assessed using cell cycle inhibitors, aphidicolin and RO-3306, in conjunction with redirected lysis assays.

Results: Through this innovative approach, we provide the first evidence of cell cycle-dependent oscillations of Siglec-15 ligands on T-cells. Employing the same methodology, we revealed that granzyme B expression oscillates throughout the cell cycle in a pattern closely mirroring Siglec-15 ligand dynamics. CD8+ T-cell killing efficiency exhibits cell cycle-dependent variation, with peak killing activity observed during G1 phase.

Conclusion: Our study advances the understanding of T-cell glycosylation and its implications for immune checkpoint regulation. Overall, this opens the way for novel strategies in cancer immunotherapy, which include modulation of cycling glyco-immune checkpoints in addition to traditional protein-based therapies.

Chronic alterations in splenic B cells persisting after stroke are associated with the stromal niche and microenvironmental changes

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Stroke remains a leading cause of death worldwide, yet improved survival results in a growing population of stroke survivors with long-term complications. Stroke-associated infections (SAI) occur in 15-30% of patients during acute recovery, and contribute substantially to morbidity, mortality and prolonged disability. Despite this, it remains understudied beyond acute infection (2-5 days post-stroke). Clinical studies demonstrate broad loss of immune populations accompanied by marked splenic reorganisation. We have previously demonstrated depletion of marginal zone B-cells and impaired antibody production associated with spontaneous bacterial lung infections. Here we investigated splenic immune and stromal cell alterations at 3 months post-stroke.

Splenic immune populations were assessed in mice following transient MCAO (30 min). 5 days post-stroke: macrophages, dendritic cells, NK cells, T and B lymphocytes were significantly reduced, alongside decreased splenic mass. By 2 weeks, reductions persisted in T and B-cells. At 3 months, only B-cell populations remained significantly reduced and CD19+ cell numbers were negatively correlated with 2-day neurological Clark scores ($R2 = 0.45$), indicating an association between stroke severity and impaired B-cell recovery.

Bulk RNA-sequencing of spleens 3 months after tMCAO identified 209 significantly altered genes. Increased expression of Begain (synapse associated), mir142HG (inhibitor of Tnfrsf13cI) and Decorin (fibroblast marker). F10 was negatively correlated with acute Clark scores ($R2 = 0.69$) while Enpp2 was positively correlated with subacute Clark scores ($R2 = 0.85$).

To examine stromal alterations, transcriptomic data was integrated with a single-cell RNA dataset of stromal splenic cells (GSE156162). This revealed increased representation of white-pulp stromal cells, particularly follicular dendritic cells, critical for B-cell activation and differentiation, suggesting stromal remodelling as a potential driver of chronic B-cell loss post-stroke.

In conclusion, we identify persistent immune and stromal alterations in the spleen at chronic stages, implicating white-pulp fibroblasts in long term immune dysfunction and highlighting promising future clinical investigations.

The role and regulation of cholesterol metabolism in B cell survival and proliferation

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Aim: High cholesterol levels are a risk factor for developing cardiovascular disease (CVD); which causes approximately 17.9 million deaths per year. Fortunately, the progression of CVD can be prevented by drugs called statins which inhibit HMG-CoA reductase (HMGCR), a protein in the

cholesterol biosynthesis pathway. Studies suggest that elderly individuals taking statins have lower antibody titers and a higher chance of developing severe respiratory disease following influenza vaccination. This suggests a defect in the ability of these B cells to undergo antibody class-switch recombination (CSR). We hypothesised that inhibiting cholesterol metabolism would reduce proliferation, which is required for B cells to undergo CSR.

Methods: Research from our lab has shown that short-term lipopolysaccharide (LPS) and IL-4 stimulation upregulates proteins involved in cholesterol metabolism, including HMGCR. B cells were isolated from wild-type mice and plated in normal or cholesterol-free media to block cholesterol uptake through the low-density lipoprotein receptor (LDLR). The cells were treated with inhibitors to block rate-limiting enzymes in the cholesterol biosynthesis pathway, squalene monooxygenase (SQLE) or HMGCR, then stimulated using LPS and IL-4. Flow cytometry was used to quantify cell numbers, size, proliferation, and cholesterol content.

Results: Inhibition of HMGCR and SQLE reduced cholesterol content, survival, cell size and proliferation. Supplementing the growth medium with mevalonate (intermediate produced downstream of HMGCR) partially rescued the effects of blocking HMGCR in normal media. Blocking protein prenylation (another process downstream of HMGCR) only suppressed proliferation. The addition of geranylgeranyl pyrophosphate also rescued the effects of blocking HMGCR in normal media, potentially by increasing cholesterol uptake through the LDLR.

Conclusion: Our findings suggest that the activation of a feedback loop involving SREBP2 may be important for regulating changes in cholesterol levels. Both cholesterol metabolism and protein prenylation play important roles in B cell growth, survival and proliferation.

P.60 **The role of persistent antigen in sustaining lung CD8 memory T cells**

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Antigen can persist in the lung, even after the clearance of infection. However, what role this plays in shaping ongoing immunity is unclear. Indeed, there is conflicting evidence for the importance of antigen presentation in maintaining tissue-resident memory T-cells. As such, we determined to understand the contribution of persistent antigen in maintaining T cell responses.

By infecting mice with ZsGreen and OVA expressing Influenza A virus (IAV), we found influenza-derived antigen in the lung one-month post-infection. Using IAV-Cre infection of ZsGreen reporter mice with nucleoprotein staining, we observed this antigen across a range of cell types, though this was enriched in cells that had survived direct infection. Localisation of persistent antigen within surviving cells – which have enhanced antiviral protection – may provide a mechanism for its persistence upon ongoing immune challenge.

Demonstrating the ability for this persistent antigen to be presented, we observed that OTI T cells were primed when transferred to mice 1-month post-infection with IAV-OVA. Surprisingly, this presentation could be augmented upon a secondary Influenza B virus infection (IBV), leading to increased priming of OT1s. However, these newly divided T cells do not differentiate into memory populations and appear to be gradually deleted, suggesting that these signals are insufficient to lead to priming of new naïve responses.

In conclusion, antigen persists in the lung long after clearance, enabling ongoing T cell priming. While new responses are deleted, persistent antigen may support maintenance of existing memory, which require less stringent activation. To directly test this, we are developing a dual-TCR OT1/P14 system with tamoxifen-inducible Cre. Following IAV-Gp33 infection and memory establishment, tamoxifen will delete the P14 TCR, allowing us to test the requirement for persistent antigen in maintaining lung CD8 memory. We hope that understanding how antigen persistence shapes memory may help inform more robust vaccination strategies.

Diverse lung challenges elicit a conserved monocyte-to-macrophage differentiation blueprint

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Alveolar macrophages (AMs) form one of the first lines of defence against respiratory pathogens. Yet, a common phenomenon following infection is the loss of these cells and their replacement by monocyte-derived cells. Monocyte-derived AMs (mono-AMs) initially favour inflammatory responses over homeostatic functions but, over time, progressively adapt and integrate, taking on properties of their foetally-derived predecessors (fAMs). This involves changes in identity, metabolism, responsiveness, and antigen presentation. However, the transcriptional regulation of Mono-AM integration, and whether the unique features of mono-AMs are conserved or insult-specific, is not well understood.

Here, we used a combination of flow cytometric analysis, genetic lineage tracing and transcriptional profiling and showed that respiratory syncytial virus (RSV) infection in mice led to marked and long-term remodelling of the AM compartment. Mono-AMs progressively out-competed fAMs over the months following viral clearance and we demonstrated that fAM and mono-AMs followed distinct transcriptional trajectories in the post-infectious lung. Our transcriptional profiling revealed an integration checkpoint within mono-AMs involving EGR2-mediated transcriptional rewiring and acquisition of a transient, suprahomeostatic proliferative state. We demonstrated that remodelling of AM composition altered functionality at population level, and this could be entirely attributable to the presence of mono-AMs. To assess whether monocyte origin is sufficient to explain such altered functionality irrespective of infectious insult, we characterised fAMs and mono-AMs after RSV infection compared with influenza infection and clodronate liposomes. Comparative transcriptional, functional and metabolic signature profiling revealed conserved 'hard-wired' differentiation and integration of mono-AMs across contexts. Finally, we demonstrated that clodronate-elicited mono-AMs were sufficient for protection upon subsequent challenge with S. pneumoniae, highlighting that monocyte origin confers prolonged altered functionality, even in the absence of prior lung infection.

Our findings highlight that long-term changes in the AM compartment show origin-dependent phenotypic divergence and that mono-AM integration follows a hard-wired trajectory fine-tuned by environmental factors.

Decoding Sunshine: Multi-omic Dissection of UV-Associated Immune Reprogramming in Humansine

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Background:
Environmental exposures shape immune function, yet mechanistic links between population-level associations and immune cell states in humans remain poorly defined. Ultraviolet (UV) exposure represents a uniquely quantifiable and temporally structured environmental perturbation. To date, research on UV has largely focused on local cutaneous effects or UV-driven skin cancers such as melanoma, squamous cell carcinoma, and basal cell carcinoma, while the systemic immunological consequences of UV exposure in humans remain poorly characterised.

Methods:
(1) We performed an unbiased population-scale analysis by integrating satellite-derived ambient UV exposure estimates with plasma proteomics data from approximately 50,000 UK Biobank participants. Individual-level recent UV exposure was quantified using residential address and satellite UV measurements aggregated over the two weeks preceding blood sampling.

(2) Alongside the population-scale analysis, we conducted a longitudinal human pilot study involving 19 healthy volunteers. Paired blood samples were collected before and after a two-

week course of controlled artificial UV exposure conducted during the Scottish winter, when background ambient UV levels are minimal. Plasma proteomics was used to assess within-individual UV-associated molecular changes, and analyses were extended to bulk and single-cell RNA sequencing of peripheral blood immune cells to characterise UV-responsive immune states.

Results:
(1) In the UK Biobank analysis, recent UV exposure was associated with widespread but highly structured changes in the circulating plasma proteome, dominated by coordinated downregulation of immune and inflammatory pathways, including TNF–NF-κB signalling, interferon responses, innate immune sensing, and lymphocyte activation, defining a systemic UV–inflammation axis.

(2) These pathways were reproducibly suppressed in the longitudinal pilot study at the proteomic level, defining a robust UV-associated immune signature. Single-cell transcriptomic analyses revealed no changes in immune cell composition following UV exposure. Instead, UV induced pronounced transcriptional reprogramming within T cells. In $CD4^+$ T cells, UV exposure suppressed activation- and differentiation-associated programmes linked to helper function and cytokine regulation, consistent with reduced TNF–NF-κB and interferon signalling observed in plasma. In $CD8^+$ T cells, effector and inflammatory programmes were attenuated, paralleling the downregulation of immune effector pathways detected at the proteomic level.

Conclusions:
Together, these findings demonstrate that UV exposure acts as a systemic immune modulator in humans, retuning T-cell functional states without altering immune cell abundance and revealing a stable, low-inflammatory immune programme.

Poster presentations

P.01 **Pannexin 1–released apoptotic metabolites regulate immune cell recruitment and tissue repair following injury**

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Apoptosis is a mode of cell death, and it is known that apoptotic metabolites signal to phagocytes to encourage their migration to the dying cell and corpse clearance. A subset of apoptotic metabolites are released via Pannexin 1 (Panx1) channels and influence gene expression in nearby host tissues and immune cells to promote wound healing following tissue injury. We aimed to further characterise host utilisation of Panx1 released apoptotic metabolites. We initially investigated how the number of immune cells were affected by Panx1 metabolite release, and how this related to tissue repair.

Tailfin transections were performed on transgenic zebrafish featuring fluorescently labelled neutrophils and macrophages. Zebrafish were immersed in vehicle or 33uM spironolactone, or microinjected with 0.5nL Panx1a morpholino or dH20. A macrophage-deficient IRF8 minus zebrafish line was treated with vehicle or 33.3uM spironolactone. In all experiments fish were imaged at set intervals for 48 hours post wounding (hpw).

We show that Panx1 inhibitor spironolactone inhibits tissue regrowth following injury in the zebrafish tailfin vs control, and significantly reduces macrophages at the wound site over 48 hpw. Furthermore, in macrophage deficient zebrafish, there is no significant difference between spironolactone and vehicle treated fish in terms of tissue regrowth following injury. We show a significant decrease in neutrophils at the tailfin 5 hpw in Panx1a morpholino vs control injected zebrafish, despite no difference in tailfin regrowth.

We present an initial investigation into the relationship between apoptosis and host tissue repair. Currently we report that macrophages, and possibly neutrophils, may play a Panx1 related role in tissue repair following injury. We continue to complement these results in a mouse injury model using mice lacking vs with functional Panx1. We will use FACS to determine how Panx1 affects the proportions, and additionally the polarisation, of immune cells post wound in this model.

P.02 **RNA-protein Interaction and Translation Regulation in Ageing Immune Functions**

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Maintenance of a healthy immune system is key to healthy ageing. A decline in immune functions with age, termed immunosenescence, underlies the increased morbidity and disease susceptibility in the elderly. It has also been proposed as an explanation for reduced response to immune checkpoint cell-based therapy in cancer patients. It is therefore crucial to understand the basic biological events associated with the declined immunity, and the effects

of treatments which may reverse these processes, in order to improve the healthspan of the ageing population and patients.

Immunosenescence is at least in part driven by factors controlling mRNA translation. For example, translation factor eIF5a is reduced in lymphocytes from humans aged 65 or above. Environmental or dietary supplementation with spermidine, a natural polyamine required for eIF5a maturation, restored old T cell function and has rejuvenating and life-extending effects in multiple cells and organisms. Little is known about age-related changes in the production or RNA-binding targets for most translation regulators, since systematic, molecular comparisons between young/healthy and old/disease cells are lacking. T cells provide an ideal system for such analyses, since a population shift to senescent cells with ageing is well documented with known biomarkers, and recovery of T cell functions in the body has the potential to restore key immunity against diseases, especially cancer.

I have preliminary data suggesting a "first wave" of ageing already occurs in pre-middle aged T cells (1-year-old mice, eq. 40yo human), characterised by altered RNA-interactome, while the total proteome stayed unchanged compared to 4-month-old mice (eq. 20yo human). More understanding of this early defect, as well as a more comprehensive knowledge of ageing RNA-interactome in longitudinal mouse and human samples, will provide better definitions of various stages of cellular ageing, surpassing the current transcriptomic and proteomic approaches.

P.03 **Divergent Dendritic Cell Maturation and Cytokine Programming across Group A Streptococcus Clinical Variants**

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I would like to present my poster on my first recent independent funding from the Wellcome Trust Institutional Funding for Research Culture (IFRC) for the project entitled "Dendritic Cell Behaviour in a Microbial Environment". Group A Streptococcus (GAS), also known as Streptococcus pyogenes, is a human-exclusive Gram-positive bacterium that colonizes the skin and throat. GAS can cause infections ranging from mild to severe and, although rare, may trigger inflammatory diseases affecting the heart, joints, and skin, leading to rheumatic fever. While B- and T-cell-mediated responses to GAS are well studied, the role of professional antigen-presenting cells such as dendritic cells (DCs) remains poorly understood. Our project aims to explore whether clinical strain variation influences DC maturation in a GAS bacterial environment. Currently prevalent strains were used to identify increases in co-stimulatory molecules, analysed by flow cytometry. While M protein, a key virulence factor of GAS, is well studied for its role in inhibiting phagocytosis and neutrophil recruitment, its role in affecting DC biology is less understood. Here, we aim to address DC behaviour in an M protein environment. Strain-dependent modulation of DC function and cytokine programming is observed in the p40 subunit protein shared by Th1-releasing IL-12 and Th17-releasing IL-23. Overall, this project aims to identify whether GAS virulence factors contribute to defective adaptive immune responses in the host and to identify the cytokine markers associated with inflammation.

P.04 **Inflammation leads to disrupted development in the mammary gland**

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Tissue remodelling is a highly regulated process that is crucial for development, homeostasis and repair following injury or insult. Here we reveal that inflammation in the mammary gland during puberty disrupts the remodelling process, leading to developmental pathology. Inflammation blunts the formation of terminal end buds (TEBs), key developmental structures, within the mammary gland. Transcriptional profiling of the pubertal mammary gland during inflammation was performed using single cell RNA sequencing (scRNA-seq). Macrophages play a central role in development by contributing to tissue remodelling. During inflammation, reduced transcription of pubertal hormone genes by resident LYVE-1+ macrophages was observed. Reduced expression of key hormone related genes, known to regulate mammary development, may result in diminished TEB growth. Our previous work highlighted the importance of chemokine receptors on macrophage dynamics during mammary gland development, shedding new light on the molecular regulation of this process. scRNA-seq revealed that chemokine receptor expression by monocytes and macrophages was dysregulated during inflammation, suggesting they are involved in positioning cells during inflammation. Together, we aim to understand how inflammation alters tissue remodelling in the mammary gland, in particular during puberty, and if this results in an environment that is more receptive to tumour development. Given 1 in 7 women will be diagnosed with breast cancer in their lifetime and these rates are rising, dissecting the cellular and molecular basis of successful mammary gland remodelling and how this is corrupted by inflammation may reveal novel therapeutic targets.

P.05 **Single cell proteomic analysis defines discrete neutrophil functional states in human glioblastoma**

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Introduction:
Neutrophils are vital innate immune cells shown to infiltrate glioblastomas, however we currently lack the molecular understanding of their functional states within the tumour niche. Given that neutrophils are known to display a prominent discordance between mRNA and protein abundance, we developed ultra-sensitive mass spectrometry-based mini-bulk and single cell proteomic (SCP) workflows to study the heterogeneity of peripheral blood and tumour associated neutrophils (TAN) from patients with glioblastoma.

Methods:
Single neutrophils and mini-bulk samples (500 cells) were sorted into fresh 384 well plates each well containing 1 µL of master mix. The master mix contained 0.2% DDM, 100mM TEAB, and 3 ng/µL trypsin in ultra-pure water. Directly after sorting, samples were stored at -80°C. The samples were processed on the cellenOne X1 and were analyzed using a Vanquish Neo UHPLC coupled to an Orbitrap Astral mass spectrometer with a FAIMS Pro Duo interface using a 50 sample per day workflow. The data was searched with Spectronaut 19 and analysed in R using the Seurat package.

Conclusions:
The Mini-bulk analysis enabled a deeper protein coverage of circulating immature, mature and TAN populations. It determined the main component of proteomic variation was maturity and defining signatures of neutrophil maturity. It also demonstrated that TANs resemble mature circulating neutrophils. Analysis of the SCP data resulted in the detection of >1,100 proteins from a single TAN providing a detailed characterization of neutrophil subsets in glioblastoma. Our approach shows evidence of pathogenic and anti-tumorigenic clusters and discovers cell states invisible to scRNAseq, opening new opportunities to selectively target pro-tumoural neutrophil states.

P.06 **Characterising the impact of novel microbial-specific light mediated approaches for treating microbial keratitis on the cornea**

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Background: Microbial keratitis (MK) is an infection of the cornea, and can be sight-threatening. It is caused by many pathogens; however, bacteria and fungi are most common. During infection, microbes lead to a strong inflammatory immune response, including rapid neutrophil recruitment and activation. The combined action of pathogen and neutrophils can put patients at high risk of scarring, perforation or eye loss.

Photodynamic therapy (PDT) is a light mediated treatment, in which a photosensitive drug is activated by a specific wavelength to produce singlet oxygen species, effectively killing microbes. Rose Bengal (RB) is a photosensitiser under investigating for treating MK clinically, however, it is non-specific and has a degree of toxicity.

Hypothesis: Modification of RB to become microbe specific (Mi-RB) will improve efficacy and reduce off target effects in models of MK.

Methods: Antimicrobial activity of multiple formulations of Mi-RB were assessed against a panel of bacteria: Staphylococcus aureus (S. aureus); Pseudomonas aeruginosa (P. aeruginosa), and fungi (ongoing): Aspergillus flavus; Fusarium keratoplasticum. Colony forming units (CFU) were counted to quantify reductions in viability, with further confirmation conducted using qPCR.

Results: Mi-RB is more efficient at killing P. aeruginosa than RB in equimolar amounts. Mi-RB caused complete killing (5-log10 reduction) of P. aeruginosa at 5μM compared to 20μM RB alone. Improved efficiency may be attributed to the synergistic effect of the Mi-RB binding domain. Preliminary results evidence that S. aureus was highly sensitive to both compounds at the lowest concentrations to date.

Conclusion: Preliminary results indicate that bacteria may be more sensitive to Mi-RB compared to RB alone, in equimolar concentrations. Therefore, the use of Mi-RB could reduce the dosage of RB required for treating MK, which may lead to fewer sight-threatening side effects. Further evaluation is warranted to assess performance in more complex model systems, and characterise off-target cytotoxicity.

P.07 **Comparative transcriptomic analysis reveals shared senescence-associated inflammatory signatures in renal and cardiac cells**

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Chronic kidney disease (CKD) is a senescence-driven disease, yet over 50% of patients with advanced CKD die from cardiovascular complications rather than progression of renal failure itself. This disparity suggests the existence of shared, systemic mechanisms, particularly

inflammatory and immune pathways, that link renal and cardiac dysfunction through the cardiorenal axis. Cellular senescence, via the senescence-associated secretory phenotype (SASP), represents a strong candidate driver of this cross-organ pathology.

To define conserved senescence-associated inflammatory programmes, we performed a comparative transcriptomic analysis of irradiated human proximal tubular epithelial cells (PTECs) and human cardiomyocytes. Bulk RNA-sequencing was used to identify genes significantly modulated by senescence induction ($p < 0.05$), followed by overlap and concordance analyses to determine shared and directionally consistent gene expression changes across both cell types.

Senescence induction resulted in robust upregulation of canonical cell-cycle inhibitors and inflammatory mediators, including CDKN1A, CDKN2A, CXCL8 and GDF15, alongside repression of cell-cycle regulators and nuclear lamina components such as CDK1 and LMNB1. A comparative analysis identified 139 genes significantly altered in both renal and cardiac cells, of which 123 showed concordant directionalities. Pathway enrichment revealed dominant signatures related to inflammatory signalling, immune activation, and stress-response pathways characteristic of the SASP.

These data demonstrate that renal and cardiac cells share highly conserved senescence-associated inflammatory transcriptomic programmes. Such shared immune-driven pathways may drive the disproportionate cardiovascular mortality observed in CKD and represent promising targets for dual-organ therapeutic strategies aimed at treating senescence-driven inflammation.

P.08 **The impact of the cytokine environment and direct viral infection in shaping basal cell-driven repair of the respiratory epithelium**

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Respiratory viral infections such as influenza cause acute damage to the lung epithelium. Rapid repair is essential to restore breathing, but the quality of the repair can have long-term consequences for lung function and susceptibility to future disease. Our aim is to understand the regulatory pressures that affect epithelial repair in the lung during and after an acute infection with Influenza A Virus. We are focusing on basal cell progenitors of the upper airway epithelium, which have been shown to migrate into the lower airways in situations of extreme damage, like influenza. Whether this dysplastic basal cell response is a maladaptive or protective form of repair remains uncertain. In this project, we address how basal cells are shaped by the cytokine environment, direct viral infection, and immune-epithelial interactions. We are using a hybrid approach of air liquid interface models and in vivo infection models. Our initial bulk RNA-sequencing analysis suggested that both cytokine signalling and direct viral manipulation alters the transcriptional profile of basal cell progenitors. We are now testing

whether cells exposed to cytokines or directly infected by virus have altered differentiation trajectories, potentially leading to a functionally different lung epithelium. Together our data provide new insight into the process by which a mature epithelium regenerates from infected or cytokine conditioned parent progenitors, and how this repaired lung differs from uninfected lung functionally, structurally, and in susceptibility to reinfection and chronic lung disease.

P.09 **Long-range consequences of intestinal helminth infection**

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Infections rarely strike alone, and immune responses shaped by one pathogen can drastically alter the host's defence against another. Helminth infections induce strong type 2 and regulatory immune responses and can modulate host immunity even at distal sites. Intestinal helminths have previously been reported to alter immune responses in the lung; however, the focus is usually on immune cells, and the impact of intestinal helminth infection on the lung epithelial landscape remains poorly understood. The lung epithelium plays an essential role in barrier integrity, immune signalling and antiviral defence; therefore, changes in epithelial composition or maturation may have significant consequences for respiratory infection. This study aims to determine whether a gut-restricted helminth can alter the lung epithelial composition and maturation, and whether this impacts secondary influenza infection. Using a murine model of chronic helminth infection, Heligmosomoides polygyrus, changes in lung epithelial cell composition, maturation and gene expression will be characterised using spectral flow cytometry, transcriptional profiling, and immunofluorescence imaging. Air-liquid interface cultures and genetically modified mouse strains will be used to further investigate the pathways driving helminth-associated epithelial remodelling. In vivo coinfection models with influenza will be established to assess the functional consequences of helminth-driven epithelial changes to secondary viral infection. Preliminary data show that infection with H.polygyrus causes changes in the proportion of maturing epithelial cells in the lung. Ongoing and future experiments will further characterise these changes and assess their impact on secondary viral infection. Together, this work will provide insight into the long-range consequences of intestinal helminth infection on lung epithelial immunity. This will contribute to understanding changes in susceptibility and severity of respiratory infections in helminth-endemic populations.

P.10 **Exploring TMEM154 Function in Maedi Visna Virus Resistance for the Establishment of a Breeding Selection Programme**

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Maedi Visna (MV) is a chronic lentiviral disease of sheep that causes severe debilitation and production losses worldwide. Clinical disease is commonly fatal, after a long asymptomatic but infectious period. There are no vaccines for MV and control options are restricted to husbandry practice changes and test and cull programmes, which may have limited effectiveness.

Genetic selection for MV resistance has emerged as an attractive strategy for reducing losses to MV. A change of amino acid 35 from glutamic acid (E) to lysine (K) in the TMEM154 protein has been associated with significant reduction in MV virus (MVV) infection and clinical disease in homozygous sheep. However, the cellular function of TMEM154 and its role, if any, in MV pathogenesis are unknown. This knowledge is required to underpin a breeding programme to select for resistant sheep.

Our results show that TMEM154 is expressed on the plasma membrane of sheep cells, with an extracellular N-terminus containing amino acid 35E/K. Virus entry assays using MVV reporter viruses have identified enhanced infection in cells overexpressing either form of TMEM154, suggesting a role for TMEM154 in an early step of the MVV infection cycle. Work is ongoing to further characterise the role of TMEM154 in MVV entry. We are also seeking to identify TMEM154 binding partners, to understand the normal cellular function of this protein. Our findings begin to elucidate the structure, localization, and function of TMEM154 and will be combined with further work to determine its impact on both health and disease.

P.11 **Activin signalling by intestinal monocytes is indispensable for epithelial barrier repair**

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Background
Activin belongs to the TGF-β superfamily of growth and differentiation factors, encoded by INHBA. It has important roles in differentiation and cytokine production, however its role in the GI tract in during inflammatory bowel disease (IBD) is unclear.

We sought to characterise the localisation of activin-expressing inflammation-associated monocytes and their interaction with intestinal epithelial cells (IECs) during IBD.

Methods
We performed single-cell RNA sequencing scRNA-seq on FACS purified CD45+Lin-HLA-DRint cells from treatment naïve CD at index colonoscopy, health controls and CD patients undergoing ileal resection with complementary analysis in DSS treated C57BL/6J (WT) mice.

We then generated Cd64iCre.Inhbafl/fl(Cd64Δinhba) and VillinCreER.Acvr1bfl/fl(VillinΔAcvr1b) mice, treated with DSS in a colitis recovery model performing scRNA-seq, flow cytometry and confocal microscopy with validation in human colonic epithelial cells in vitro and public scRNA-seq IBD datasets.

Results
INHBA/Inhba was exclusively expressed by inflammation-associated monocytes with a conserved transcriptional repertoire across species. INHBA+ cells accumulated at sites of epithelial damage in IBD, with ligand-receptor analysis confirming activin-receptor (ACVR1B/2A) expression on IECs, validated by confocal microscopy, flow cytometry and scRNA-seq. In addition, scRNA-seq analyses of intestinal macrophages in independent cohorts, revealed expansion of INHBA+ monocytes correlated with histological severity (GHAS) in CD patients undergoing ileal resection.

Both macrophage activin-deficient (Cd64Δinhba) and IEC receptor-deficient (VillinΔAcvr1b) mice demonstrated marked susceptibility to 2% DSS colitis in a recovery model with >50% mortality versus controls. Analysis of FACS purified IECs from colitic Cd64Δinhba or WT mice revealed an Scd1+ population that was dependent on monocyte-derived activin. This IEC subset was absent in health, represented an early differentiation fate from stem cell progenitors, with an analogous SCD+ IEC population found in active IBD. Pathway analysis of Scd1+ cells showed enrichment for TGFβ and cytokine signalling, validated by recombinant-activin stimulation of healthy human IECs in vitro.

Conclusion
Intestinal monocytes recruited during inflammation are the exclusive source of activin, signalling through cognate receptors on IECs to induce an Scd1/SCD+ subset. These IECs are activin dependent and are required to enable recovery after barrier damage, however persistent activin-signalling may contribute to disease progression in CD.

P.12 **The impact of prenatal stress on fetal lung mast cells**

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It is well established that exposure to heightened maternal stress during fetal development can have far-reaching and potentially life-long consequences on the health of the child. Looking specifically at respiratory health, numerous epidemiological studies have demonstrated links between exposure to prenatal stress and increased risk of childhood respiratory disease. However, the precise mechanisms underlying these predispositions remain unknown. Mast cells are long-lived tissue-resident granulocytes whose aberrant activation is widely implicated

in asthma, allergy and atopic disease. They have a complex ontogeny, arising in distinct progenitor waves during fetal development, eventually colonising barrier-site tissues at precise timepoints. Evidence from a recent publication suggests that exposure to prenatal stress at these crucial developmental stages can reprogram fetal skin mast cells, causing an increased risk of eczema. We hypothesise that a similar mechanism may occur in the lung whereby fetal lung mast cells also become reprogrammed by exposure to prenatal stress, potentially altering their normal function and increasing susceptibility to inflammatory diseases such as asthma. To investigate this, we have performed a regimen of repeated restraint and bright light exposure to induce stress in pregnant mice, timed to coincide with colonisation of the developing fetal lungs by mast cell progenitors. By analysing offspring lung mast cells with flow cytometry, we have found that such exposure to prenatal stress does induce an altered mast cell phenotype in newborn mice, suggesting a shift towards hyperactivation. Our ongoing and future work will aim to determine if this altered mast cell phenotype persists into adulthood, and whether it would predispose individuals to a heightened response in an experimental model of asthma.

P.13 **Fibroblast subtypes, macrophage infiltration and matrix alterations in allergic airway pathology**

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Asthma, a chronic respiratory disease affecting 1 in 12 UK adults, is associated with sustained inflammation and bronchial hyperresponsiveness. Asthma results in structural remodelling changes, including irreversible reorganisation of the extracellular matrix (ECM), that impacts lung function. A heterogenous population of fibroblasts are primarily responsible for ECM deposition/turnover. Furthermore, macrophages can regulate ECM remodelling directly, or indirectly by influencing fibroblast activation. However, which specific macrophage and fibroblast populations influence remodelling in asthma is unknown. We therefore investigated spatial alterations in fibroblast subsets, alveolar macrophages (AMs) and interstitial macrophages (IMs) within ECM remodelling regions using a mouse model of allergic airway pathology that shares features of severe asthma in people.

Using immunofluorescence staining, pulmonary fibroblast localisation was assessed in mice repeatedly administered an allergen cocktail intranasally. Macrophage infiltration versus ECM changes were investigated using spatial imaging of 3D-lung slices.

Allergen administration resulted in AM and IM expansion in lung regions exhibiting increased collagen-1 and hyaluronan. After 2-weeks of allergen dosing, macrophages infiltrated the adventitial cuff, the airway-adjacent vascular region, a response that was maintained following 4-weeks of dosing. AMs within alveoli were greater at the initiation of inflammation 1-day post allergen administration versus 5-days. However, IM localisation around airways increased following the longer refractory period. At chronic time points (8-weeks) a shift from high IM density within the cuff to large multinucleated AMs within the alveoli was observed. Expanded

AMs and IMs correlated with increased Pi16^{+} Pdgfrα^{+} fibroblasts within the adventitial cuff whilst Npnt^{+} Pdgfrα^{+} fibroblast numbers around alveoli remained unchanged.

Together our spatial imaging data suggests that initially, macrophages infiltrate and accumulate around the vasculature before migrating to ECM-remodelled airway sites. Therefore, macrophages may contribute to enhanced fibroblast activation, further promoting fibroblast proliferation and ECM remodelling. Future work will explore whether macrophage-fibroblast interactions in regions of pathology drives airway remodelling.

P.14 **It takes two: exploring the functions of CD163+ and CD11c+ macrophage subsets in the homeostatic and post-irradiation salivary gland**

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Tissue-resident macrophages are increasingly being revealed as essential for organ homeostasis alongside their well-characterised function in repair. We have previously revealed a novel, reparative function for macrophages in the submandibular gland (SMG) following radiotherapy injury; however their homeostatic functions, and whether these differ across sub-populations, remain elusive. Using scRNAseq, we have identified distinct CD163+CD11c- and CD11c+CD163- sub-populations of macrophages in the SMG. Some macrophages appear anatomically close to nerves and blood vessels, an association which has been shown to instruct function in other organs, such as the intestine. Importantly, the CD163+CD11c- sub-population highly expresses genes such as Adrb2 and Mrc1, common in nerve-associated and blood vessel-associated populations in other organs. In order to assess whether this sub-population are reliant on nerves or neuronal signals, we ablated sympathetic nerves by administering 6-hydroxydopamine (6-OHDA). While effective at denervating the SMG, 6-OHDA administration did not affect the survival, replenishment or function of CD163+ macrophages, implying a lack of acute reliance on sympathetic nerves. We then explored whether these cells play a role in blood vessel surveillance. We found that intravenous injection of fluorescent-conjugated tracer molecules demonstrated highly increased phagocytic uptake from the blood by CD163+ macrophages when compared with their CD11c+ counterparts, suggestive of a novel barrier function for this subpopulation in the SMG. Ongoing work exploring whether radiotherapy injury affects blood vessel integrity and compromises the phagocytic function of CD163+CD11c- macrophages will ascertain whether this putative barrier function goes awry in the SMG following radiotherapy injury, and how this impacts SMG function and repair. Given that salivary gland dysfunction and resulting xerostomia (chronic dry mouth) is a common and debilitating side-effect of life-saving radiotherapy for head and neck cancer, this finding will contribute to improved outcomes for those living with the side-effects of their treatment.

P.15 **Epithelial Memory After Respiratory Viral Infection in Mice Results in Prolonged Enhancement of Antigen Presentation**

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Background
Several cohort studies have linked severe respiratory syncytial virus (RSV) bronchiolitis in infancy with a higher risk of developing recurrent wheeze and childhood asthma, yet the pathomechanisms behind this phenomenon remain undetermined. Here, we hypothesise that RSV infection can imprint lung epithelial cells (LECs), thus changing their responses to subsequent challenges.

Methods
We used an established murine BLAB/c model of RSV infection to investigate changes in LECs 28 days after infection (dpi). We employed our cold dispase digestion protocol and sorted large numbers of highly pure and viable CD45-CD31-EpCAM+ cells from murine lungs. Cleavage Under Targets and Release Using Nuclease (CUT&RUN) was used to determine epigenetic changes, while NanoString and qPCR were used to determine transcriptional changes. Finally, we used a combination of flow cytometry and confocal immunofluorescence microscopy to confirm observed changes at protein level.

Results
Assessing H3K4me3 and H3K27ac histone modifications by CUT&RUN, we identified epigenetic changes at 28dpi in LEC genes associated with major histocompatibility molecules (MHC) class I and II. We further confirmed transcriptional changes at 28dpi, including in B2m and H2-DMb genes, directly related to MHC-I and MHC-II respectively. We confirmed a substantial increase in the levels of MHC-I and MHC-II molecules at 28dpi and found that these increases differ between airway and alveolar spaces. Importantly, MHC upregulation was associated with increased antigen uptake and processing, as well as increased antigen presentation to T-cells.

Conclusions
In conclusion, we observed that RSV infection leads to longer-lasting epigenetic and transcriptional changes in LECs that result in prolonged increases in the levels of both MHC-I and MHC-II. We speculate that on one hand, these changes contribute to enhanced antiviral

immunity following RSV infection and that on the other hand, they could support T-cell activation to aeroallergens, thus promoting allergic airway inflammation and asthma development.

P.16 **Vascular remodelling in allergic airway inflammation and pathology**

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Asthma is a global health burden, affecting around 300 million people worldwide. Asthma is characterised by airway obstruction, hypersensitivity, and remodelling as well as alterations to the pulmonary vasculature. Vascular changes broadly include vessel wall thickening, angiogenesis, and extracellular matrix (ECM) remodelling. Vascular alterations have the potential to disrupt immune cell trafficking, compromise vascular integrity, and hence contribute to disease progression. However, dynamic changes to the ECM and vascular cells remain poorly understood. Using a mouse model of allergic airway pathology that shares features of severe asthma in people, we aimed to characterise vascular cell changes correlating with timing of immune infiltration.

Mice were intranasally administered an allergen cocktail (house Dust mite, Ragweed, and Aspergillus extracts (DRA)) twice weekly. Vascular cells were characterised by flow cytometry, with spatial changes assessed by histology and immunostaining.

Vessel wall thickening was evident morphologically after 2 weeks, coinciding with the presence of interstitial macrophages around the vessel. Immunofluorescence staining showed vessel wall thickening was specifically driven by vascular smooth muscle expansion, rather than thickening of the endothelium. Nuclear density within the smooth muscle compartment was reduced and expression of proliferation marker Ki67 unchanged after DRA-treatment, suggesting muscle expansion occurs predominantly through hypertrophy. Following DRA challenge, pericytes, a mural cell which envelop blood vessels and play a role in vessel development and stability, underwent phenotypic alterations. Between 2- and 4-weeks of treatment the proportion of pericytes expressing podoplanin, and smooth muscle actin increased, consistent with a migratory phenotype and a myofibroblast-like state.

Findings indicate that vascular remodelling occurs in parallel to or shortly following inflammation. Furthermore, vessel thickening is driven mainly by smooth muscle cell hypertrophy while altered pericyte phenotype may potentially contribute to vascular fibrosis and exacerbate airway remodelling. Investigating pulmonary vascular remodelling will aid our understanding of immune infiltration and ongoing lung pathology.

P.17 **5-Azacytidine enhances resolution of inflammation and modulates Ly6Chigh monocytes in a TET2-mutant model of pneumonia**

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Background
By 2050, over 25% of people in Europe and North America will be aged ≥65 years old. This will create a huge demand on health care services with increase in chronic conditions, such as cardiovascular disease and cancers partly driven by age-associated inflammation.

Clonal Haematopoiesis of Indeterminate Potential (CHIP) is a non-malignant condition, increasingly recognised as a precursor for many age-related diseases. CHIP arises from HSPC with somatic mutations conferring survival advantage. One third of CHIP related mutations involve loss of function mutations in Ten-Eleven translocation 2 (TET2), a cytosine demethylase. Therefore, therapeutics reversing LoF mutations might limit clonal expansion and CHIP-associated disease.

Methods
Young (5-6 months old) and old (18-23 months old) C57Bl/6J mice were treated 0.5 mg/kg/day of 5-azacytidine or vehicle for 5 consecutive days before being intranasally inoculated with 104 CFU Streptococcus pneumoniae on day 6. Blood was collected pre- and post-treatment, 14 days post-infection, and at sacrifice and analysed by flow cytometry, immunophenotyping and whole blood stimulation. Tissues were collected at sacrifice and fixed by H&E staining.

Results
Immunophenotyping demonstrated AZA reduces circulating numbers of Ly6Chigh monocytes and inflammation. Furthermore, AZA+ mice had an increased resolution of inflammation and Ly6C+ monocytes had a high expression of TNF receptor. ELISA showed elevated pro-inflammatory cytokines in old vs. young and reduced with AZA.

Histology analysis of colon from mice further identified structural changes in the TET2 mice, and metabolic analysis identified a more sedentary behaviour from the TET2 mice.

Conclusion
CHIP is currently managed with a "watch and wait" approach risking adverse outcomes. AZA may offer a therapeutic avenue, enhancing resolution of inflammation post-infection. Structural changes in the colon, and the possible resulting sedentary behaviour observed in TET2KO mice highlight the systemic issues related to CHIP. AZA may alleviate characteristics by reducing inflammation and restoring immune balance.

P.18 **Increasing Dendritic Cell Infiltration in Colorectal Carcinoma: Unravelling the Signals for Required for Enhanced Immunotherapy Responses**

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Colorectal carcinoma (CRC) ranks as the fourth most prevalent cancer in the UK, and mortality rates remain high, with just 53% of patients surviving a decade post-diagnosis. CRC patients, especially those with microsatellite instability (MSI-H) and defective mismatch repair(defective mismatch repair), have limited response to immune checkpoint inhibitors (ICI); thus, the potential to enhance the efficacy of immunotherapy exists. The abundance of type one conventional dendritic cell (cDC1) within tumours corresponds to improved responses to ICI. Such findings drive the pursuit of potential strategies to selectively recruit cDC1 to the tumour microenvironment (TME) as a way of increasing the range of patients that can respond to ICI. Our research thus encompasses three key objectives:

1. Deciphering Infiltration Signals: Identifying these signals is pivotal to unravel their recruitment mechanisms, paving the way for tailored strategies.

2. Enhancing Chemokine Signals: By altering the chemokine milieu in the TME, we aim to increase the recruitment of cDC1. This approach could counteract the exclusion of anti-tumour immune cells often imposed by local factors.

3. Synergy with Immunotherapy: Investigating the potential of increased cDC1 infiltration to improve ICI could render CRC tumours more responsive to treatment.

We will use a novel screening method involving chimeric mice lacking specific chemokine receptors that will help assess the significance of these receptors in cellular migration and seeding of tissues. Moreover, we have optimised a CRISPR-based system to knockout relevant genes in bone marrow-derived hematopoietic stem cells, offering a platform for investigating gene modifications of the hematopoietic compartment in the context of CRC. By elucidating the signals driving cDC1 infiltration, we aim to enhance and augment the immunotherapeutic landscape of CRC treatment.

P.19 **Tryptophan metabolism shapes intestinal barrier inflammation and integrity during Schistosoma mansoni infection**

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The intestinal immune system balances immunity against pathogens with tolerating harmless challenges such as food and commensals. The breakdown of this balance is a hallmark of intestinal diseases, including inflammatory bowel disease, Coeliac disease or tumours.

However, we do not fully understand the cells and signalling pathways that maintain this intestinal immune balance.

Here, we study the maintenance of immune balance using murine infection with the parasitic helminth Schistosoma mansoni (Sm). During infection, adult schistosomes reside in the mesenteric vasculature, where they release eggs. These eggs transit via the bloodstream into nearby organs such as liver and the intestines. Strikingly, even though Sm eggs rupture through intestinal tissues into the lumen, this breach does not result in sepsis. Thus, Sm infection generates a regulated barrier breach that can be studied to discover core mechanisms that initiate intestinal inflammation and regulate its resolution, together maintaining intestinal immune balance.

For the first time, we have used flow cytometry to characterise dynamics of Sm infection in intestinal tissues. Our work illustrates dynamic development of mixed Th1/2/17 and T regulatory CD4+ T cell responses during Sm infection that together correlated with the regulated barrier breach. Furthermore, metabolic analysis showed a dramatically altered tryptophan metabolism association with increased infection intensity and barrier disruption. Finally, by supplementation of dietary tryptophan, we were able to manipulate barrier integrity, myeloid cell profile and Th1/2 balance during Sm infection.

Together, our work has identified key metabolic pathways that regulate the immune response during Sm infection and contribute to the maintenance of intestinal balance and integrity. Furthermore, we have identified a dietary intervention approach as a strategy for modulating intestinal immune balance. Together, our work reveals potential for novel immunotherapies for intestinal helminth infection, as well as serving as a basis for immune balance modulation in other intestinal inflammatory diseases.

P.20 **Bone marrow neutrophil reprogramming contributes to NET-associated blood–brain barrier injury in pediatric cerebral malaria**

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Cerebral malaria (CM) is a leading cause of childhood mortality, driven by blood–brain barrier (BBB) breakdown and microvascular injury, yet the mechanisms underlying neutrophil-mediated pathology remain unclear. Although neutrophil extracellular traps (NETs) can cause endothelial damage, their cellular origin and regulation in CM are poorly defined.

Here, we show that pediatric-CM is associated with a marked expansion of circulating low-density neutrophils (LDNs), comprising a heterogeneous population of immature and activated subsets with enhanced NETotic capacity. Plasma levels of NETs were significantly increased in CM compared with non-CM coma and correlated with brain swelling. Elevated LDNs abundance correlated with delayed neurological recovery, linking this compartment to ongoing cerebral pathology.

Phenotypic, transcriptional, and functional analyses revealed that LDNs display features of immaturity, including altered nuclear morphology and increased expression of cell-cycle and chromatin-associated genes, consistent with dysregulated granulopoiesis. Compared with normal-density neutrophils, LDNs exhibited increased spontaneous and calcium-dependent NET formation ex vivo, accompanied by increased mitochondrial content. Immunofluorescence and correlative electron microscopy demonstrated NET deposition within cerebral microvessels in fatal CM, spatially associated with endothelial disruption and BBB breakdown.

A murine model of severe malaria recapitulated the expansion and functional properties of the LDNs compartment observed in children with CM. Single-cell transcriptomic analysis across bone marrow and peripheral compartments indicated that LDNs differentiation occurs early during neutrophil development, driven by disease-associated factors arising during infection. Among these, signatures linked to red blood cell breakdown products, including heme-responsive pathways such as HMOX2, were evident, although multiple inflammatory and metabolic signals likely contribute. Consistently, exposure of human $CD34^+$ hematopoietic stem cells to free heme reproduced a NET-prone neutrophil phenotype.

Together, these findings identify expansion of an immature, pro-NET LDNs compartment as a key feature of pediatric CM and suggest that dysregulated granulopoiesis, with contributions from haemolysis-associated signals, promotes neurovascular injury and BBB disruption.

P.21 **Heterogeneity within memory CD4 T-cell populations in the influenza virus infected lung**

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Influenza is a rapidly mutating virus, with infections currently manged with seasonal vaccination regimes that aim to predict forthcoming dominating viral strains. However, B cell responses primed by vaccination have a narrow protective range, resulting in vaccines with low levels of efficacy against alternative influenza strains. In comparison to vaccine antigen targets, T-cells can recognise conserved viral sequences present across virus strains. This make CD4 T-cells an attractive vaccine target to generate broader protection.

Our scRNA-seq data have defined a heterogenous memory T-cell pool from the lungs of mice infected with influenza A virus (IAV). Using TRACE reporter mice, we can identify IAV specific CD4 T-cells and subdivide them into different memory cell populations, including based on the cell's potential to produce cytokines. CD4 T-cells that can produce multiple different cytokines expressed the highest levels of pro-survival genes, Myc and Foxo1, suggesting these cells are both long-lived and functional and thus effective targets for vaccination.

We now aim to test the role of Myc and Foxo1 at different stages of the immune response using IAV infected conditional knockout mice. We also aim to establish when memory CD4 T-cell heterogeneity is established, and whether T-cells maintain this heterogeneity following secondary exposure to the virus or display functional plasticity.

This project will reveal novel insights into the processes that underpin the generation and maintenance of memory CD4+ T-cells, and how these cells can rapidly shift from rest as memory cells to activation during re-infection. A better understanding of the memory T-cell subtypes which provide protection in influenza will help to inform improved vaccine targets that can establish long-lived protection with broader efficacy.

P.22 **Macrophage Heterogeneity and Epithelial Regeneration in Lung Repair and Fibrosis**

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Purpose: Lungs are constantly exposed to environmental insults, necessitating robust repair. Dysregulated repair can lead to fibrosis, respiratory failure and death. One example – occupational silicosis – is experiencing a global outbreak, creating urgent need for anti-fibrotic

treatments. Interstitial macrophage (IM) expansion has been reported in clinical as well as experimental injury and fibrosis. We aimed to decipher the actions of IM and identify molecular targets contributing to repair and fibrosis.

Methods: IM phenotypes were studied by single cell RNA sequencing (scRNASeq) of macrophages (MΦ) from mice acute lung injury and fibrosis models, as well as human pulmonary fibrosis. To study MΦ-epithelial crosstalk, we generated CD64iCre-DTR mice, enabling diphtheria toxin-mediated lung MΦ ablation. Epithelial cells were interrogated by flow cytometry and scRNASeq from MΦ-sufficient and MΦ-deficient injured lungs. To unravel IM role in silicosis pathogenesis, a mice model was established exploiting intratracheal silica administration.

Results: Three IM subpopulations, conserved across species, were identified based on a set of representative genes including MΦ activation state markers and fibrosis-associated markers. Notably, Ccr2+ and Trem2+ IM, but not Folr2+ IM, expanded significantly in fibrotic lungs, suggesting distinct functions of the IM subpopulations. MΦ ablation attenuated epithelial progenitor cell expansion and their upregulation of oxidative phosphorylation (OXPHOS) genes after injury, highlighting the role of MΦ in supporting epithelial regeneration. Utilising our silicosis model, granuloma formation was observed with significant IM localisation, reflecting their involvement in the fibrotic process.

Conclusion: We identified three IM phenotypes with distinct profiles in repair and fibrosis. We found that MΦ are necessary for epithelial progenitor cell expansion after injury, potentially via altering their energy metabolism. The importance of IM in fibrotic silicosis was also confirmed using a mice silicosis model. Functional validation of molecular targets and pathways, and whether they are dysregulated in fibrosis, are the focus of ongoing work.

P.23 **The role of TGMs in macrophages**

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TGMs are known as TGFb-mimetic molecules secreted by the intestinal helminth parasite Heligmosomoides polygyrus. This helminth drives a type 2 immune response in the host, at the same time that it tries to avoid the immune system to prolong its survival and evade its expulsion. Several mechanisms are known to contribute, being the TFMs one of the main involved proteins secreted.

Here I will try to clarify which is its role in macrophages, characterising them in a more specific way than the canonical "M1" and "M2" subdivisions. My aims are based on identifying in vitro the effect of TGM1 in bone marrow-derived macrophages (BMDMs), determining if this effect changes in presence of a previous type 1 or type 2 immune response induced, and checking if different TGMs have a different impact. My future steps include verifying if these consequences observed in vitro are also happening in vivo.

P.24 **Investigating the cellular interactions of macrophages and T cells in alopecia areata**

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Alopecia areata (AA) is an autoimmune condition that affects hair follicle structures in the skin, resulting in hair loss. Pathogenically, AA involves an abnormal infiltration of immune cells around hair follicles and cytotoxic attack against these structures. This central role of immune cells has been reflected in the clinic, with JAK inhibitors now an approved treatment option for AA. Current research focuses on the activity of T cells in this hair loss. However, understanding the contribution of other immune cells may support new therapeutic approaches, and help understanding of disease heterogeneity. Therefore, we are characterising cellular interactions between macrophages and T cells in AA skin, and investigating systemic changes associated with these interactions.

We have used immunofluorescent staining of AA and healthy control skin biopsies to initially characterise the abundance and location of macrophages in AA-affected tissue. These data have been complemented by single-cell and spatial transcriptomic analyses, which have allowed gene-level analysis of macrophage populations in AA skin, and their interactions with T cell populations. Finally, analysis of serum from donors with AA was performed to identify systemic changes potentially associated with macrophage activity in disease.

This work has provided insights into the potential activity of macrophages in AA. We are now analysing our transcriptomic data to identify molecules involved with macrophage – T cell interactions. These will be investigated locally in skin by immunofluorescence, or systemically via blood-based assays.

P.25 **A mouse pathobiont protects against DSS colitis via CD4+ T cells**

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Pathobionts, such as Helicobacter hepaticus (Hh), are members of the microbiota that may drive intestinal inflammation in mice with immune dysregulation but are well tolerated in healthy individuals. However, we recently found that in wild-type mice subjected to dextran sodium sulfate (DSS) colitis, Hh-colonisation instead ameliorated disease.

Hh persistence was required for amelioration of DSS colitis, because clearance of Hh by antibiotic treatment returned DSS disease severity to that observed in naïve mice. We found that Hh-specific CD4+ T cells in the colon expanded upon DSS treatment, and this was dependent on persistent Hh-colonization and MHC-II presentation. In DSS-treated Hh-colonized mice, Th17 and regulatory T cells were enriched locally in the colon and depletion of CD4+ T cells in Hh-colonised mice ablated the protection from DSS colitis. Furthermore, at single-cell resolution, both regulatory and effector CD4+ T cells in Hh-colonised mice

upregulated an anti-inflammatory gene module characterized by Il10, Tgfb, Foxp3, and Izumo1r (Fr4) as well as multiple co-inhibitory receptors including Lag3, Havcr2 (Tim3) and Tigit.

Therefore, Hh-colonisation may ameliorate colitis by driving upregulation of a shared anti-inflammatory gene module across regulatory and effector CD4+ T cells in the colon. As DSS colitis is driven by innate inflammatory cells, these T cells likely modulate local myeloid cells. This model may reveal novel mechanisms by which a "pathobiont" protects against aberrant inflammation in the colon, rather than drive it.

P.26 **Allergic airway inflammation alters myeloid cell phenotypes within the murine lung**

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Background: Asthma is estimated to affect over 250 million people worldwide with 455000 deaths reported in 2019 due to asthma related complications. Aberrant immune responses to environmental stimuli are critical in the development and exacerbation of asthma, leading to the use of novel anti-cytokine biological drugs targeting core immune components. However, significant cohorts with severe allergic asthma do not respond to such immune-regulatory interventions. Using a mouse model of allergic airway inflammation (AAI) our research group has replicated this effect. Lung cell networks suggests that tissue resident (TR) macrophages may be critical for establishing and maintaining airway remodelling in treatment-resistant asthma.

Methods: Mice were intranasally instilled twice weekly for 1-4 weeks with house Dust mite, Ragweed, Aspergillus extracts (DRA) to induce AAI and remodelling. Longitudinal blood samples were collected. Following a 1-/5-day refractory period, lungs were collected for histological and flow cytometry analysis.

Results: During early DRA inflammation (1-2 weeks) circulatory monocytes, particularly Ly6c-lo non-classical, increased in proportion. This correlated with increased lung macrophage counts and significant phenotypic shift. Alveolar macrophages (AMs) gained CD11b expression and interstitial macrophages (IMs) reduced CD88 and increased CD16.2, suggestive of recent monocytic origin. Proliferation of IMs but not AMs was also observed 24-hours after DRA instillation. These changes only partially subsided after 4 to 8 weeks of DRA challenge, with a persistent shift to CD11b+ AMs and CD11c+ IMs. In formalin-fixed paraffin embedded lung sections large, multinucleated macrophages can be observed throughout the alveolar space at 4 weeks DRA.

Discussion: Our work suggests significant changes in lung macrophages during severe AAI, including partial monocytic replacement of resident macrophages. Phenotypic differences across these populations may drive the establishment of fibrotic macrophage-fibroblast-matrix networks that establish and maintain airway remodelling. Research is ongoing to dissect the contributions of resident and recruited cells to these long-term changes.

P.27 **Lung Natural Killer cells make a muted type I Interferon response following a second influenza A virus infection**

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Natural Killer (NK) cells rapidly respond to influenza A virus (IAV) infection by migrating from the blood into the lung. There they kill infected cells and participate in co-ordinating anti-viral immunity. What role, if any, NK cells play in re-infection is not clear.

To examine this, we have performed single cell RNAseq on NK cells isolated from lungs from naïve and influenza A virus (IAV) infected mice at primary, memory, and re-infection timepoints. Additionally, anti-CD45 was administered intravenously prior to sacrifice which allowed for differentiation between circulating and tissue NK cells in flow cytometric analysis. Lung NK cell localisation was determined by confocal imaging.

A comparison of NK cells from naïve and primary infected mice demonstrated a substantial response with 1069 differentially expressed genes (DEGs). In contrast, there were 726 DEGs between NK cells from the memory and re-infection timepoints. Gene ontology analysis revealed that the primary response was characterised by increased type I Interferon gene expression which was still present, although muted, following re-infection.

UMAP analysis pinpointed type I Interferon responding cells to a single cluster characterised by Ly6a and Cxcl10 that lacks Klrg1 expression. Flow analysis indicated that KLRG1 was most prominently expressed by circulating cells, suggesting that type I Interferon responsive cells were localised within the lung. Confocal imaging indicates that many NK cells are localised together during primary and re-infection timepoints. Increased expression of CCR2 and CXCR3 chemokine receptors suggest NK localisation may be driven by inflammatory chemokine gradients.

Together these data demonstrate that NK cells respond similarly during primary and re-infection timepoints. However, reduced pro-inflammatory cytokine production upon re-infection could indicate that other memory immune cell populations are imparing NK cell activity in the re-infected lung. Alternatively, NK cells may adapt their response and reduce inflammatory responses to future infections to prevent excessive inflammatory damage.

P.28 **ACKR3 regulates CXCL12-dependent migration of peritoneal macrophages**

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ACKR3 is an atypical chemokine receptor (ACKR) that primarily binds CXCL12, a chemokine crucial for the retention of hematopoietic stem cells in the bone marrow. ACKR3 regulates cell migration by scavenging and internalizing CXCL12, thereby shaping local chemokine concentrations and gradients. Complete ACKR3 knockout (KO) mice are embryonically lethal

due to severe defects in heart and brain development, and the developmental roles of ACKR3 have been extensively studied. However, the function of ACKR3 in macrophages remains largely unexplored.

Using an ACKR3-reporter mouse model, we identified ACKR3 expression in F4/80high macrophages within the peritoneal and pleural cavities. Bulk RNA sequencing and flow cytometry analyses revealed that ACKR3-expressing macrophages exhibit higher expression of Tim4 and CD102, markers associated with yolk sac-derived macrophages, compared to ACKR3-negative macrophages. In contrast, ACKR3-negative macrophages displayed higher expression of MHCII, CCR2, and IRF4, which are characteristic of monocyte-derived macrophages. To directly assess whether ACKR3 expression reflects macrophage developmental origin, we analyzed CCR2KO ACKR3-reporter mice, in which monocyte-derived macrophages are severely reduced. This analysis demonstrated that ACKR3 expression does not strictly distinguish macrophage origin, whether embryonic or monocyte-derived.

We therefore investigated the functional significance of ACKR3 expression in F4/80high peritoneal macrophages. Notably, ACKR3-expressing macrophages also express CXCR4, the canonical receptor for CXCL12. To assess their migratory response to CXCL12, we performed migration assays. ACKR3-positive macrophages exhibited more rapid and directed migration toward CXCL12 compared to ACKR3-negative macrophages. These findings suggest a previously unrecognized role of ACKR3 in regulating macrophage chemotaxis toward CXCL12.

P.29 **Elucidating the Immunomodulatory Mechanism of Dithranol in Alopecia Areata via Blood-Based In Vitro Assays**

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Alopecia areata (AA) is a chronic autoimmune condition characterised by immune-mediated hair follicle destruction, resulting in non-scarring hair loss. While a novel controlled-release nanoparticle formulation of topical STS01 (dithranol/ProSilic cream) has shown promising clinical efficacy in a recent phase 2 trial, the underlying immunological mechanisms remain poorly defined.

This study investigates dithranol's immunomodulatory effects on peripheral immune cells using in vitro assays with peripheral blood mononuclear cells (PBMCs). PBMCs from biobank-sourced AA patients and healthy donors were exposed to graded concentrations of dithranol to establish a non-cytotoxic working dose, which was optimally determined as 0.02 µg/ml.

Plasma cytokine profiling of trial participants revealed increased levels of IFN-γ, IL-6, IL-17, and TNF-α in AA samples relative to healthy controls. Functional assays demonstrated that LPS-stimulated monocytes treated with dithranol showed significantly reduced secretion of TNF-α and IL-6, as confirmed by ELISA and flow cytometry. In parallel, PMA/ionomycin-stimulated T cells exhibited decreased IFN-γ and TNF-α production when treated with dithranol. To delineate lineage-specific effects, naïve CD4+ T cells were differentiated under Th1-polarising conditions

with dithranol exposure, resulting in reduced TNF-α expression and diminished proliferation, indicating suppression of pro-inflammatory effector T cell responses.

Collectively, these findings suggest that dithranol modulates key innate and adaptive immune pathways by dampening monocyte and T cell-derived cytokine activity. Ongoing studies aim to corroborate these observations in ex vivo skin explant models using patient-derived biopsies to further elucidate dithranol's localised immunomodulatory effects in AA.

P.30 **Prevalence and predictors of hepatitis B virus infection among sellers and workers at West Africa's largest market, Kejetia, Ghana: a cross-sectional study**

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Hepatitis B virus (HBV) infection remains a major public health challenge in sub-Saharan Africa, including Ghana, despite the infant vaccination programs. Limited data exist on HBV prevalence and predictors in informal sector populations, who may face unique occupational and behavioural exposures. This study assessed the prevalence and predictors of HBV infection among sellers and workers at Kejetia Market, Ghana's largest commercial hub.

Methods: A cross-sectional study was conducted among 489 adult market workers from 4th December, 2024 to 25th January, 2025. Participants were selected through stratified random sampling across occupational groups. Data on sociodemographic, occupational, and behavioural factors were collected using structured questionnaires. On-site testing for hepatitis B surface antigen (HBsAg) was performed using the Hightop One Step Rapid Test kit. Bivariate and multivariate logistic regression analyses were used to identify independent predictors of HBV infection. $P < 0.05$ was considered statistically significant.

The overall prevalence of HBV infection was 7.36% (36/489), consistent with intermediate-to-high endemicity. Multivariate analysis identified three independent predictors of HBV infection. Female gender (aOR = 0.455, 95% CI: 0.221–0.937; p = 0.033) and absence of tattoos (aOR = 0.283, 95% CI: 0.110–0.730; p = 0.009) were associated with lower risk of HBV infection, while

unvaccinated individuals had 3.37-fold increased odds of getting the infection (95% CI: 1.395–8.142; p = 0.007). HBV prevalence declined progressively with increasing vaccine doses, from 9.2% in unvaccinated individuals to 2.3% among those who had completed three or more doses.

HBV infection is common among Kejetia market workers, with prevalence exceeding both continental and global estimates. Gender, tattooing, and vaccination status were significant predictors of infection. Strengthening adult vaccination programs, promoting safe tattooing practices, and implementing male-focused screening and prevention interventions are critical to reducing HBV burden and achieving Ghana's contribution to the WHO goal of eliminating HBV by 2030.

P.31 **Schistosomiasis drives regional changes in intestinal myeloid populations**

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During Schistosoma mansoni infection, parasite eggs transit from the mesenteric blood vessels, rupturing through the intestinal wall and into the gut lumen. Despite the ongoing tissue damage caused by this process, immunocompetent hosts rarely progress to sepsis. The host must carefully balance the immune response against the parasite with tissue repair. However, the mechanisms that regulate this balance, particularly those governing intestinal tissue immune responses during schistosome infection, remain poorly understood.

Here we investigated the nature and regional variation of schistosome-induced intestinal immune responses by comparing low and high intensity infections in mice, and over time. At 4-15 weeks post-infection, following the onset of egg laying, RNA was isolated from intestinal tissues (ileum and colon) for bulk sequencing to identify key cellular and molecular networks involved in inflammation, barrier repair and defence during infection.

Our results provide the first comprehensive transcriptional profiles of the ileal and colonic environments during experimental schistosomiasis, demonstrating how infection and its intensity or chronicity alter the intestinal immune and transcriptional landscape. We identify region-specific signatures that reveal key differences between the colon and ileum. Notably, infection induced significant changes in macrophage function across all tissues, with consistent upregulation of genes linked to type 2 activation of macrophages in both high- and low-dose infected mice.

Collectively, these findings demonstrate that S. mansoni induces distinct tissue-specific gene expression changes, likely driven by differences in infection chronicity and intensity as well as the character of the immune response, microbial load, and local tissue damage. Our data serve

as a valuable resource for understanding intestinal immunity during schistosome-induced tissue injury and beyond.

P.32 **IL-25 Suppression by Secretins of a Parasitic Nematode**

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Helminth infections pose a significant health and economic burden. Host responses to helminths rely on a type 2 immune response that is initiated by alarmin cytokines such as IL-25, IL-33, and TSLP. These cytokines activate innate and adaptive immune cells, including ILC2s and Th2 cells, resulting in the production of IL-4, IL-5, and IL-13 that drive the characteristic 'weep and sweep' response. However, helminths can actively suppress this response to evade immune clearance. Heligmosomoides polygyrus bakeri secretes immunomodulatory proteins, such as the HpARI and HpBARI families that inhibit IL-33 signalling, yet no immunomodulators targeting the IL-25 pathway have been identified. Given IL-25's importance in type 2 immunity, we hypothesised that the parasite may also suppress this pathway. We investigated the effect of H. polygyrus excretory-secretory products (HES) on IL-25 signalling. IL-25 responses were measured by treating an IL-25-responsive reporter cell line or bone marrow-derived (BMD) ILC2s with IL-25 in the presence or absence of HES. Downstream reporter expression or cytokine (IL-5 and IL-13) production was measured as an indicator of IL-25 signalling. Flow cytometry was also utilised to examine changes in IL-25 receptor expression. HES suppresses IL-25 signalling in a dose-dependent manner. Reporter expression decreased with increasing concentrations of HES, and IL-5 and IL-13 production by BMD ILC2s was similarly reduced. IL-25 stimulation decreases IL-25 receptor expression on BMD ILC2s, which is reversed by addition of HES. This suggests HES may be interfering with IL-25 or downstream signalling, rather than the IL-25 receptor. Overall, these findings indicate that HES can suppress IL-25 signalling and reveal a potential new mechanism of helminth-mediated immunomodulation. Ongoing and future work can focus on identifying the protein(s) within HES that are responsible for this and determining the mechanism of suppression of the IL-25 signalling pathway.

P.33 **Anti-Inflammatory Neutrophil Functions in Rheumatoid Arthritis**

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Neutrophils are the most prevalent circulating leucocyte, and in rheumatoid arthritis (RA) they are responsible for driving inflammation and tissue damage within synovial joints. Neutrophils are short-lived and typically die via apoptosis, an anti-inflammatory form of cell death that preserves membrane integrity to prevent release of cytotoxic cell contents. Apoptotic neutrophils are cleared via efferocytosis by macrophages, which drives pro-resolution macrophage polarisation. RA patients typically have high production of autoantibodies, which bind to antigens to form immune complexes (ICs). ICs are powerful stimuli for neutrophils,

promoting various pro-inflammatory effector functions. However, previous work from our group recently showed that insoluble ICs (iICs) also promote anti-inflammatory neutrophil functions; neutrophil apoptosis and iIC clearance via macropinocytosis. We hypothesized that constitutive and iIC-induced neutrophil apoptosis is impaired in RA, resulting in reduced neutrophil death, reduced efferocytosis, and impaired macrophage polarisation towards a pro-resolution phenotype, ultimately promoting inflammation. However, performing co-cultures of healthy donor (HD) or early RA patient-derived neutrophils with monocyte-derived macrophages (MDMs), our work identified that neutrophils from RA patient blood maintained their ability to undergo constitutive and iIC-induced apoptosis, regardless of patient treatment status. Moreover, both HD and RA patient iIC-apoptotic neutrophils were cleared more efficiently than constitutively apoptotic counterparts by MDMs. iIC-apoptotic HD and RA neutrophils maintained their ability to repolarise inflammatory MDMs towards a pro-resolution phenotype. The increased efferocytosis of iIC-apoptotic neutrophils was not driven by phagocytosis, increased cell death, nor increased production of extracellular vesicles (EVs), suggesting a possible improvement to eat-me signals by a yet-to-be-determined mechanism. These unexpected results suggest that iIC-driven anti-inflammatory neutrophil functions are preserved in RA patients, regardless of treatment status. We propose these anti-inflammatory pathways downstream of iIC stimulation may be involved in keeping tissue damage to a minimum within the synovial joint in RA, through enhancing neutrophil apoptosis and driving macrophage repolarisation.

P.34 **TFH cells in a Th2 context have high lipid metabolism which regulates CXCR5 expression**

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T follicular helper (Tfh) cells play a central role in antibody-mediated immune responses. Tfh activation involves extensive metabolic reprogramming to support their growth, proliferation, and function. Tfh cells are reported to be highly glycolytic, but emerging evidence suggests additional reliance on lipid and mitochondrial metabolism. This study aimed to identify the metabolic pathways supporting Tfh cell function in Th1 and Th2 cytokine environments, and to evaluate their potential as therapeutic targets.

Mice were infected with Heligmosomoides polygyrus or Influenza A Virus (IAV) to induce Th2 and Th1 immune responses. Tfh cells from H. polygyrus-infected mice showed significant upregulation of genes associated with glycolysis and lipid metabolism compared to naïve T cells. Tfh and Th2 cells were metabolically similar, although Tfh cells displayed higher expression of glycolytic genes and reduced sterol metabolism gene expression. Tfh cells during IAV infection similarly demonstrated upregulation of genes associated with glycolysis and lipid metabolism, but the dominance of lipid-dependent metabolism in Tfh cells was weaker than in

the Th2 context. To assess the functional relevance of lipid metabolism, we targeted stearoyl-CoA desaturase (SCD). Acute inhibition of SCD significantly reduced expression of the chemokine receptor CXCR5 in human Tfh cells in vitro.

In conclusion, Tfh cells exhibit active glycolytic and lipid metabolic profiles. Lipid metabolism is partially regulated by SCD, and its inhibition disrupts CXCR5 expression. Lipid metabolism is enhanced in Tfh cells in a type 2 immune response, and is less prominent in Tfh cells in a Th1 response. We hypothesise that the regulation of chemokine receptor expression by lipid metabolism may control Tfh positioning in and around the germinal centre, thereby influencing antibody output. Together, our findings highlight lipid metabolic pathways as potential targets for therapeutic modulation of aberrant Tfh cell responses in immunopathology.

P.35 **Metabolic Reprogramming of Tumour-Associated Macrophages by TripleNegative Breast Cancer Cells and Its Modulation by PTP1B Inhibition**

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Background: TAMs are key tumour-promoting cells in breast cancer, comprising up to 50% of the tumour mass. A high infiltration of TAMs in tumours is associated with enhanced growth, poor prognosis and therapy resistance, making them therapeutic targets. Targeting TAM metabolism is considered one potential therapeutic strategy. PTP1B inhibitor, MSI-1436 lactate, has progressed to clinical trials for metastatic breast cancer. However, the effect of PTP1B inhibition on TAMs specifically remains unclear. This study investigated the effects of co-culture of a triple-negative breast cancer cell line (MDA-MB-231) on the metabolism of TAMs and PTP1B-inhibited TAMs.

Methods: Human blood derived monocytes were differentiated to macrophages in the presence of MDA-MB-231 (triple-negative) breast cancer cell-conditioned media to drive a TAM phenotype. MSI-1436 Lactate was used to inhibit PTP1B within MDA-MB-231 conditioned TAMs. Metabolism of TAMs and PTP1B-inhibited TAMs were assessed using the Seahorse XF MitoStress test andglucose and lactate assays; viability was determined by a live/dead staining kit.

Results: Macrophages conditioned with MDA-MB-231 conditioned media did not alter their basal mitochondrial respiration but significantly increased maximal respiration, spare respiratory capacity and extracellular acidification rate. Glucose consumption was increased in MDA-MB231 conditioned TAMs as indicated by decreased glucose and increased lactate levels in culture media, indicating an overall more metabolically active TAM. TAM viability was maintained post conditioning. PTP1B inhibition in MDA-MB-231 conditioned macrophages decreased maximal respiration, spare respiratory capacity and glucose consumption with no change in overall viability, suggesting a less functional TAM.

Conclusions: The predominance of TAMs observed in triple negative breast cancer may relate to their more robust metabolic characteristics and viability. PTP1B inhibitors decrease breast

cancer cell proliferation but also decrease the metabolic integrity of macrophages, hence could be an adjuvant therapy for this cancer cell type.

P.36 **Intestinal virome transcytosis drives innate immune activation in Metabolic Dysfunction-Associated Steatotic Liver Disease**

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Background: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is the most common chronic liver disease, affecting one in four individuals. Patients with MASLD exhibit distinct gut virome signatures, including reduced diversity and enrichment of Crassvirales, though the impact on disease progression and pathogenesis remains unknown. This study aimed to characterise the gut virome in MASLD and determine their ability to cross the gut barrier and trigger inflammatory responses.

Methods: Faecal samples were collected from 32 MASLD patients and 16 healthy individuals at Aberdeen Royal Infirmary. Samples were enriched for virus-like particles (VLPs), quantified using fluorescence microscopy and morphotypes were identified using transmission electron microscopy. Gas chromatography was used to measure short-chain fatty acids (SCFAs). VLP enrichments were screened for the presence of crassphage (ϕcrAss001) and exposed to polarised CaCo-2/HT29-MTX-E12 intestinal epithelial cocultures. Barrier integrity was assessed using transepithelial electrical resistance and dextran diffusion, while viral transcytosis was measured by fluorescence microscopy. Inflammatory responses were evaluated by exposing THP-1 macrophages to VLPs, followed by qPCR analysis of cytokine and innate immune gene expression, alongside phagocytosis assays.

Results: TEM revealed a reduction of Myoviruses in MASLD samples. SCFA analysis showed significantly increased propionate ($p=0.032$) in patients with moderate to severe fibrosis (F2-F4). The presence of ϕcrAss001 was confirmed by PCR. VLP enrichments demonstrated active transcytosis across epithelial layers without compromising gut barrier integrity. Exposure of epithelial-macrophage cocultures to MASLD-derived VLPs resulted in significantly increased TLR-9 expression, alongside a trend towards elevated TNF-α, IFN-β, and IFN-γ, consistent with antiviral innate immune activation.

Conclusions: MASLD is associated with compositional changes in the gut virome and increased propionate in advanced cases. Crucially, disease-associated gut viruses can traverse an intact intestinal barrier and engage innate immune sensing pathways, which, together with accompanying metabolic shifts, may amplify macrophage activation and inflammation in MASLD

P.37 **Characterising the binding and opsonophagocytic activity of monoclonal antibodies as a novel therapeutic for the treatment of invasive candidiasis**

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Invasive fungal infections are responsible for over 2.5 million global deaths annually. Currently there are several major challenges complicating the treatment of fungal infections. These include a concerning rise in antifungal drug resistance, the emergence of multidrug-resistant fungal species, drug toxicities and drug-drug interactions. In parallel there is an ever-increasing number of immunocompromised patients and those at high risk of invasive infections. As a result, there is an unacceptably high mortality rate associated with these infections, reaching over 40% even with treatment. There is an urgent clinical need for alternative antifungal treatment strategies such as immunotherapies.

Here we investigate monoclonal antibodies (mAbs) specifically targeting the surface exposed regions of two key cell wall proteins (CWPs), Pga31 and Utr2 of the major human fungal pathogen Candida albicans. These mAbs were isolated from a human antibody library using phage display technology. These CWPs play a key role in cell wall remodelling and the maintenance of wall integrity when cells are exposed to antifungal agents. The mAbs have demonstrated protection in a mouse model of systemic candidiasis, with our lead mAb achieving 83% survival. Importantly, our mAbs have demonstrated recognition of several clinically significant Candida species, including drug-resistant and drug-susceptible isolates of C. albicans, C. auris, C. parapsilosis and C. tropicalis. Furthermore, antibody binding assessed through Candida whole-cell ELISA and fluorescence microscopy has demonstrated preferential binding to the C. albicans hyphal morphology when compared to yeast cells, suggesting these mAbs could be effective in the treatment of disseminated infection. To further interrogate the mechanisms of protection offered by our mAbs, immune cell interaction assays have been performed, showing enhanced phagocytosis by macrophages following opsonisation of fungal cells with mAbs. Taken together, this demonstrates the potential of our mAbs as a novel first-in-class therapy for invasive and drug-resistant fungal infections.

P.38 **Immunogenicity of Helminth Proteins Associated with Heparin-Sulfate Binding**

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The inflammation associated with obesity is primarily a type 1 immune response. As such, the induction of type 2 immunity has been suggested as an option to counter obesity-associated inflammation. An important initiator of the type 2 immune response is the alarmin cytokine IL

33. IL 33 has been shown to reduce weight gain in mice on a high fat diet (HFD). The murine intestinal nematode Heligmosomoides polygyrus bakeri (Hpb) has been characterised secrete various proteins, including HpARI2 which suppresses IL 33 activity, and HpARI3, which stabilises and enhances IL 33 activity. As such, it was hypothesised that the administration of HpARI2 to mice on HFD would worsen weight gain and obesity-induced inflammation, while HpARI3 would protect from it. While neither protein resulted in changes in weight gain, HpARI2 led to eosinophilia in adipose tissue in mice given HFD. This induction of eosinophils was found to be independent of ST2 and MyD88, but dependent on the heparan sulfate (HS)-binding domain of HpARI2. Splicing the HS-binding domain of HpARI2 onto HpARI3 led to the fusion protein similarly inducing eosinophilia, while neutralising the positive charge of the HS-binding domain of HpARI2 (and thus it's HS-binding ability) abolished HpARI2's ability to induce eosinophilia. In addition, mice treated with Hpb proteins that bound HS showed an antibody response, producing antibodies against the respective Hpb proteins. This suggests that HS-binding increases the immunogenicity of a protein, and may be useful for the further design and development of vaccine targets or drugs.

P.39 **The contribution of Kupffer cells and gut macrophages to the progression of acute liver injury**

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Liver disease is a major cause of global mortality, causing two million annual deaths globally. Acute liver injury (ALI) involves inflammatory responses mediated by both resident and recruited macrophage populations resulting in cycles of tissue damage and repair. In addition to liver inflammation, increased intestinal permeability has been described in ALI patients with an increased risk of developing lethal systemic infections. However, the role of both liver and gut macrophages in this process has not been fully described.

Kupffer cells (KCs) are liver resident macrophages are crucial for initiating immune responses by efficiently phagocytosing pathogens that enter via the portal vein (gut-derived pathogens) or hepatic artery (systemic infections). However, during ALI, the KC population decreases, leaving patients at risk of infection. Gut macrophages significantly contribute to gut barrier integrity yet the effect of ALI on gut macrophages and their contributions to ALI-associated intestinal permeability are poorly understood. The interactions between gut and liver macrophages and their contributions to ALI are poorly understood.

The first year of this project will be focused on achieving two aims:

1. To characterise the composition and phenotype of gut macrophages during the progression of ALI.

2. To delineate the contribution of long-lived gut and liver resident macrophages to ALI-associated intestinal permeability.

A Timd4CreiDTRfl/fl mouse model will be used to specifically deplete Tim-4+ macrophages in both the liver and the gut following diphtheria toxin (DTx) administration post ALI induction via paracetamol overdose. Tim-4 is expressed on both KCs and long-lived gut macrophages allowing for specific depletion of these cells in to investigate their roles in the gut-liver axis. We aim to titrate the dose of DTx to selectively deplete KCs and compare the outcome of ALI in in mice depleted of long-lived macrophages in the gut and liver and those with only KC depletion.

P.40 **Mitochondrial quality control meets antiviral defence: PINK1–MAVS crosstalk links innate immunity and mitophagy in macrophages**

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Mitochondrial antiviral signalling and mitochondrial quality control are traditionally viewed as distinct processes, despite both being coordinated at the mitochondrial outer membrane. MAVS, a central adaptor of antiviral innate immune signalling, mediates RNA-sensing dependent responses, while PTEN-induced kinase 1 (PINK1), a Parkinson's disease–linked mitochondrial kinase, regulates mitophagy. How these pathways communicate to coordinate immune signalling with mitochondrial quality control remains unknown.

An unbiased in-vitro proximity labelling study using TurboID identified MAVS as a candidate proximal interactor of PINK1, which was validated biochemically. Bone marrow derived macrophages were used as an immune-relevant model, where both PINK1 and MAVS are functionally active. Mitochondrial stress alone failed to induce interferon stimulated genes (ISGs), indicating that activated PINK1 is not sufficient to directly activate MAVS. In contrast, immune stimulation elicited a robust antiviral response that was attenuated in PINK1-deficient cells.

Unbiased transcriptomic profiling revealed that while the core ISGs were largely preserved in PINK1-deficient macrophages following stimulation, these cells preferentially induced negative regulators, including repressors of RIG-I and JAK–STAT signalling. At the basal level, wild-type macrophages showed enrichment of chromatin modulators from the PAD family absent in PINK1-deficient cells, suggesting additional epigenetic regulation. These results indicate that PINK1 deficiency does not abolish MAVS signalling directly but enhances negative feedback that may dampen pathway output.

Reciprocal regulation was examined using mitophagy reporter mice. Activation of antiviral signalling suppressed mitophagy, implicating a role of innate immune pathways in mitochondrial turnover. Experiments are ongoing to determine whether this effect requires PINK1.

Collectively, these findings identify bidirectional PINK1–MAVS crosstalk as a mechanism linking mitochondrial quality control to innate immune signalling in macrophages.

P.41 **Investigating the effect of WDFY4 on endosomal trafficking of antigen in cDC1s**

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Background: Cancer is one of the leading causes of death worldwide. Although immunotherapy has led to better outcomes for a subset of patients, more work remains to be done to ensure that a greater percentage of patients benefit from such treatment. In both melanoma and head and neck squamous cell carcinoma, the abundance of type 1 conventional dendritic cells (cDC1) has been shown to correlate with response to immune checkpoint blockade. These cDC1 are essential for initiation and maintenance of antitumour CD8+ T cell immunity through cross-presentation of tumour-derived antigens, yet the molecular mechanisms governing this remain unclear. Understanding cross-presentation could, therefore, lead to improved treatments driving more effective antitumour CD8+ T cell-mediated immunity. One molecule shown to be necessary for cross-presentation is WD Repeat and FYVE Domain-Containing Protein 4 (WDFY4), but the mechanisms by which WDFY4 carries out this function are not yet understood.

Methods: To investigate how WDFY4 affects cross-presentation, we modelled its structure using Alphafold. GST fusions of several WDFY4 domains were synthesised, purified, and assessed for phosphatidyl inositol phosphates (PIP) binding. To investigate the possibility that WDFY4 exerts its control on cross-presentation via endosomal trafficking of antigen, the endosomal compartments of wild-type and WDFY4-knockout cDC1 were analysed by flow cytometry.

Results: Alphafold modelling revealed that the PH domain of WDFY4 may have the capacity to bind to PIPs. The synthesised GST-fused WDFY4 PH domain displayed a high affinity for phosphatidyl-inositol-3-phosphate (PI3P), a PIP enriched on early and recycling endosomes. With flow cytometry, it was found that MHC-I accumulated in recycling endosomes in the absence of WDFY4.

Conclusion: Our preliminary data suggest that WDFY4 associates with early and recycling endosomes and may control the recycling of MHC class I to facilitate cross-presentation. We are now generating cDC1 with modified WDFY4 to identify localisation and binding partners.

P.42 **Deciphering the role of lymph node immune memory in cancer metastasis**

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Background- Tumour-draining lymph nodes (tdLN) facilitate interactions between antigen-presenting cells and specific lymphocytes, driving the activation of anti-tumour adaptive immune responses. However, tdLNs can also act as an early site for metastasis, with tumour colonisation promoting an immunosuppressive environment, or leading to metastasis. Clinical removal of regional lymph nodes along with the tumour reduces the risk of relapse, but worsens the risk of distal metastasis, with loss of immune memory being implicated as a potential factor.

While T cell memory populations could play an important role in controlling metastasis, it is unclear what the impact of LN treatment has on the maintenance of these populations.

Methods-TdLN samples were obtained from a murine subcutaneous B16F10 ZsGreenminOVA melanoma model with a control, endpoint, and tumour-resected experimental group. Drainage of antigen into the tdLN was visualised using immunofluorescence staining and microscopy, while the immunophenotyping of T and B cell subsets in the tdLN was achieved using flow cytometry. Anti-tumour serum IgG titres were quantified from murine blood through ELISA.

Results-We observed the presence of tumour antigen in the tdLN which colocalised with the follicular dendritic cellular (FDC) network, decorating its membrane surface. Immunophenotyping revealed that tdLN retain more tumour-specific CD8 effector memory populations, which reduce upon tumour resection. However, its CXCR6+ subset maintains stable numbers even after tumour resection. A strong germinal centre (GC) B cell response is also observed in the tdLN, with high cell numbers and tumour specific IgG serum titres, both maintained high even after tumour resection.

Conclusion- The retention of antigen on the FDC network, and sustained presence of tumor-specific T and B cell populations following tumor resection presents a compelling opportunity to investigate their antigen dependency for continued maintenance, and how lymph node (LN) resection/irradiation influences these subsets—and the possibility of inducing these subsets in non-draining nodes.

P.43 **From cases to adventures: using gamified case-based learning for enhancing Immunology teaching**

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Immunology is central to medical sciences, but it is a challenging discipline to teach and learn due to its complexity. For many students, it can feel abstract and disconnected from its real-world impact. Making this subject more accessible and engaging can be a challenge for immunology educators.

The use of case-studies helps bridge the gap between theory and real-world application. To further enhance engagement and motivation, I transformed a case-study into an interactive, mystery-style game. In “The case of the mischievous B cells” students become immune detectives to solve an immunopathology case based on real clinical data. Clues and hints help students reach the final diagnosis while exploring concepts such as B cell activation, antibody production, and immune dysfunction. This game immersed third-year undergraduate medical sciences students in problem-solving tasks, fostering teamwork and active participation.

The game was developed on the Genially platform. Anonymous post-session feedback was collected in the form of a survey (Microsoft forms) that contained both Likert scales (5 points) and free text questions. All respondents (n=11) reported that the sessions helped them consolidate immunology concepts and gained a better understanding of the topics, and 10 respondents enjoyed the game approach as revision of key concepts. Moreover, most respondents (9) felt integrated in the session.

This gamification approach integrates real-world scenarios with immersive storytelling to enhance immunology teaching. By transforming complex immunology concepts into accessible and meaningful learning experiences, it supports both student participation and knowledge consolidation.

P.44 **A Mechanistic Understanding of Fuelling Plasma Cells**

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The activation of naïve follicular B cells, through a T-cell dependent manner, results in the selection of B cells that differentiate into plasma cells (PCs) that produce high affinity antibodies. Each plasma cell can secrete up to 10,000 antibodies per second, and thus their morphology, transcriptional and metabolic profiles are developed to allow them to support this production. There is some evidence that defines the nutrient and metabolic requirements of PCs, however, critical gaps exist including the repertoire of nutrient transporters required to fuel the production of antibodies.

The 40LB feeder cell system, which express B-cell activating factor and CD40L, allows the differentiation of IgM+ B cells, plasmablasts and PCs from naïve B cells in vitro. Using cell sorting and quantitative mass spectrometry I have mapped the proteome of differentiating cells and provided insights into the metabolic profile of these cells, from naïve B cells to PCs. Importantly, B cells expand their protein synthesis machinery, including ribosomes, endoplasmic reticulum and Golgi apparatus through the differentiation process. The proteomic data has given some insight into the nutrient transporters expressed by these cells across the stages of differentiation.

To address the gap in understanding of plasma cell biology, I am investigating how nutrient deprivation affects the PC proteome and their capacity to secrete antibodies. Proteomic analysis suggests that the deprivation of glutamine or methionine shifts the cells' machinery towards supporting lipid metabolism in comparison to control cells. This is notable because plasma cells depend on lipid synthesis to expand the endoplasmic reticulum, which is essential for antibody production. The next phase of this project will examine how tissue-resident PCs and PCs in disease context fuel their activity. Together my data will provide new insights into the metabolic demands of plasma cells and the mechanistic basis of plasma cell fuelling.

P.45 **How TLR Signals Influence Antigen Trafficking and Cross-Presentation in cDCs**

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To drive T cell activation, conventional dendritic cells (cDCs) present antigen in the context of MHC with associated costimulatory signals. There is selectivity about which antigens are presented on MHCII, with TLR ligand presence driving presentation of endosome contents. However, it is unclear whether a similar mechanism dictates whether antigens are cross-presented by type1 cDCs (cDC1s). While cDC1s may cross-present through the cytosolic or vacuolar pathway it is unclear whether these pathways are regulated by PAMPs within phagosomes. This has consequences for cancer immunotherapy where different types of cell death may produce different qualities of TLR ligands, altering the CD8+ T cell response.

Firstly, I will investigate the cytosolic pathway. I have designed a split-GFP approach whereby the 11th helix of GFP (GFP11) is expressed in tumour cells and the GFP1-10 in cDCs. Upon phagosome rupture and release of GFP1, cDCs should become fluorescent, revealing whether addition of TLR ligands leads to increased cytosolic escape. I also developed a fluorescent antigen which was transduced into B16 melanoma cells which can be killed and fed to BMDCs, allowing quantification of antigen uptake and MHCI/MHCII presentation. These tumour cells will be killed by different mechanisms and with/without loading cells with TLR ligands. As TLR ligand presence can alter MHCII presentation which relies on endosomes fusing with the MHCII loading compartment, I will also determine how antigen is trafficked differentially depending on phagosome contents. This will be carried out in cDC1s and cDC2s to understand MHCI/MHCII presentation in both cells. I have optimised a method of flow cytometry to analyse isolated endosomes, allowing determination of the different compartments antigen is trafficked to depending on the associated TLR ligands.

Ultimately, this will allow me to understand the signals driving MHCI presentation and the impact this may have on anti-tumour immunity.

P.46 **CFTR modulators alter macrophage function and Aspergillus fumigatus growth**

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Background: Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CFTR gene, resulting in thick, sticky mucus on mucosal surfaces, including the lungs. This creates an ideal environment for opportunistic pathogens, including Aspergillus fumigatus. CFTR modulators improve lung function and lifespan by correcting epithelial defects. This study examined the direct effects of CFTR modulators on human macrophage function and on Aspergillus fumigatus viability, swelling and phagocytosis by macrophages.

Methods: Clinical Aspergillus fumigatus isolates from CF patients (11361, 15115; Radboud University Medical Centre, Nijmegen, NL) were cultured with or without CFTR modulators (ivacaftor, tezacaftor, elexacaftor, or their triple combination). Fungal viability and killing was measured using XTT assays and hyphal growth was evaluated through microscopy. Fungal swelling was analysed with ImageStream cytometry and TEM. Monocyte-derived macrophages from healthy donors or CF iPSC-derived macrophages (CF Canada-SickKids Program) were pre-treated with modulators and then exposed to Aspergillus fumigatus conidia. Phagocytosis was quantified via microscopy.

Results: Pre-treatment of CF macrophages with CFTR modulators increased their phagocytic index (isolate 11361), indicating an enhancement in CF innate immune responses in individual macrophages. Conversely, pre-treating healthy macrophages with CFTR modulators decreased the overall percentage of macrophages phagocytosing conidia (isolate 11361), though it did not impact their killing capacity. CFTR modulators did not significantly affect the viability of Aspergillus fumigatus. However, isolate 11361 showed delayed conidial swelling and hyphal formation when exposed to modulators.

Conclusion: CFTR modulators increase the phagocytic ability (index) of CF macrophages but reduce the percentage of phagocytosing macrophages in both healthy and CF macrophages. Although CFTR modulators do not directly impair Aspergillus fumigatus viability, they delay early fungal morphogenesis, suggesting that CFTR modulators could shape host-pathogen interactions through influences on both. Further mechanistic studies are underway to determine whether and how effects extend beyond their corrective role in CFTR function.

P.47 **Immune control of the provisional matrix: macrophage regulation of fibroblast dynamics and hyaluronan in asthma**

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Extracellular matrix (ECM) is a complex, dynamic structure that plays a pivotal role in maintaining microphysiological conditions in any tissue. During early stages of regeneration, a primary matrix is formed that differs from the mature, self-sustaining ECM. Cell-matrix crosstalk is essential for tissue function, but during pathology chronic inflammation can alter these interactions and the biochemical and biomechanical properties of the matrix, thereby disrupting tissue function.

As macrophages are known to regulate the ECM, we aimed to investigate the relationship between macrophages and early pathogenic ECM formation in the lungs, using a mouse model of allergic airway pathology that shares features of severe asthma in people. Because type 2 cytokines such as IL-13 can induce an ECM-regulatory profile in macrophages, we hypothesised that macrophage inflammatory programmes set the trajectory of primary matrix deposition.

We performed time-resolved analyses of lung inflammation together with ECM composition, organisation and biomechanics. We quantified collagens 1, 3 and 4 and hyaluronan (HA),

alongside ECM mechanical properties, and found that HA accumulates in the lung prior to extensive ECM remodelling. HA is synthesised by HAS enzymes. Analysis of Has mRNA expression, together with visualisation of the cells expressing Has, revealed stage-dependent patterns consistent with cell-type-specific control. Specifically, macrophages appear to be key regulators of HA accumulation during allergic airway pathology and may therefore influence the immunomodulatory properties of HA across disease development. In parallel, macrophage-associated inflammation coincided with ECM change, including shifts in the collagen/HA balance and matrix reorganisation, accompanied by altered mechanics. Future work will help us determine whether key type 2 immune mediators affect HA production, with help of 3D cultures of primary murine fibroblasts stimulated with Il-13, Ym1/Chil3 and Tgf-β.

Our data suggest that macrophages during allergic airway inflammation may reprogramme early ECM assembly through regulation of HA. Defining this immune-to-matrix axis may identify time-sensitive intervention points to restore regenerative remodelling and prevent persistent, mechanically abnormal ECM states.

P.48 **Pharmacological modification of muscarinic pathways via pilocarpine disarms neutrophils during fungal challenge**

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The most common fungal pathogen, Candida albicans, is associated with a broad spectrum of infections. Among the first immune cells to respond are neutrophil granulocytes, which migrate to the site of infection to prevent the pathogen from spreading. They phagocytose small yeast, but larger hyphae are targeted by extracellular defence mechanisms, such as neutrophil extracellular traps (NETs), released granule components, and reactive oxygen species (ROS). While the immune response aims to eliminate invading fungi, it simultaneously results in tissue destruction.

Current treatment for fungal infections does not focus on modulating the host immune response. However, a therapeutic agent that promotes the resolution of inflammation while exerting antifungal activity could accelerate healing and mitigate further tissue damage. One drug candidate is pilocarpine hydrochloride, which has been shown to inhibit hyphae formation of Candida albicans. Its effect on the immune response however remains unclear.

In this study, we investigate the effect of pilocarpine on neutrophil responses during C. albicans infection. Pilocarpine induces apoptosis in neutrophils in a concentration-dependent manner. It further downregulates neutrophil activation as measured by CD11b and CD66b receptor expression, but in a calcium-independent way. Chemotaxis towards both chemoattractant, and fungal hyphae was reduced by pilocarpine treatment. The drug further influences neutrophil-mediated antimicrobial activities against C. albicans, including killing, ROS production, degranulation and phagocytosis. While the drug itself does not induce NETs, it is able to

downregulate ROS-dependent NETosis. We further investigated NET-release in response to heat-inactivated yeast, dead hyphae, and C. albicans during its transition from yeast to hyphae under pilocarpine treatment.

In summary, pilocarpine inhibits fungal metabolism while downregulating neutrophil activities and inducing apoptosis. Hence, pilocarpine could serve as a therapeutic option for local fungal infections, as it provides a dual benefit by targeting fungi and immune regulation. We suggest muscarinic receptor M3 antagonism as potential pathway.

P.49 **Directed modulation of TGFb signalling using helminth derived TGFb mimic-ST2 fusion proteins**

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Heligmosomoides polygyrus is a helminth parasite with extensive immunomodulatory capabilities allowing suppression and evasion of host type 2 immune responses. One family of proteins important for this immunomodulation is the TGF-β mimic (TGM) family, with 10 currently known members found to have diverse effects on TGF-β signalling including both agonistic and antagonistic interactions with the TGF-β receptor, TGFBR. TGF-β shows a high degree of pleiotropy, in part due to cell type specific co-receptors, which has made understanding of its roles complex. Similarly, TGMs have their own co-receptor interacting domains which allow them distinct functions.

TGF-β pleiotropy can be seen in the differing responses of fibroblasts and immune cells. Fibroblasts are stimulated to proliferate, and fibrosis can result from this proliferation. However, in the context of immune cells, TGF-β is generally immunosuppressive. Expression of ST2, the ligand binding component of the IL-33 receptor and an important activating receptor, has also been found to increase on group 2 innate lymphoid cells (ILC2s) in response to TGF-β signalling.

By fusing an ST2 specific ScFv to TGMs, TGFBR agonism or antagonism can be specifically directed to ST2 expressing type 2 immune cells, such as ILC2s. This will allow control of pathological cytokine responsivity and release, relevant to allergy and asthma, while avoiding unwanted activation of other cell types such as fibroblasts. This work aims to further understand the role of TGFBR signalling in ST2 expressing cells and investigate the potential of these fusion proteins as a therapeutic strategy.

P.50 **Translational High Throughput Adjuvant Screening Pipeline**

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Adjuvants are essential components of many vaccines, as they enhance the antigen-specific immune response, contributing to overall vaccine effectiveness. Although adjuvant

mechanisms of action are complex, ultimately they support enhanced Dendritic cell -T cell (DC-T) interactions to increase T cell responses leading to adaptive immune memory. Consequently, we have developed high-throughput imaging to screen compound libraries for agents that enhance DC-T interactions, and therefore may be vaccine adjuvants. Using mouse DC and T cells, we identified a series of candidate adjuvants, and confirmed their effective adjuvant activity in vivo, in mice.

The aims of this study are to develop a humanised version of this assay to facilitate the translation of existing adjuvants and screen for novel human adjuvants. Here we will show the results of this project towards creating a new model to examine human T-DC interactions.

Fresh blood was collected from healthy donors. Monocytes were isolated by CD14+ selection, and the remaining cells were stored at -80˚C for later T cell isolation. Monocytes were differentiated into moDCs. The response of moDC to PRR agonists as model adjuvants were analysed with Flow cytometry. Subsequently, we examined the immune synapses formed by moDCs with autologous T cells using a Thermo CX5 high-content screening (HCS) platform. Finally, we investigated the effects of PRR agonists on T-cell proliferation using flow cytometry.

Our data demonstrate that the use of Thermo CX5 HCS together with flow cytometry can successfully identify adjuvant candidates. MPLA and Poly I:C cause strong moDC activation, but ODN-1018 does not cause strong moDC activation. Although they differ in their ability to stimulate moDCs, they all significantly enhance moDC-T cell interactions, which in turn increases T cell proliferation. Through the application of these models to our adjuvant screening pipeline, we aim to combine mouse and human models to discover novel, translatable vaccine adjuvants.

P.51 **Loss of TGFbR signalling leads to alveolar macrophage 'de-differentiation' and inflammatory reprogramming**

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Alveolar macrophages (AMs) are traditionally viewed as "terminally differentiated", homeostatic tissue-resident cells. However, upon bacterial, viral, fungal or even sterile challenge, AMs rapidly adopt a proinflammatory state and, in many cases and somewhat counterintuitively, diminish in number at a time you expect highly phagocytic and microbicidal cells to be needed. The molecular pathways regulating these responses remain poorly understood.

Using a variety of single-cell RNA-sequencing (scRNA-seq) datasets, we show that within AMs, one of the most consistently downregulated gene modules across disease states includes TGFβ receptor (TGFβR)-associated genes, suggesting that abrogated TGFβR signalling during challenge leads to altered AM identity, behaviour and survival.

To test this, we generated Csf1rMer-Cre-Mer.Tgfbr2fl/fl to allow inducible deletion of Tgfbr2 in macrophages. Consistent with our hypothesis, loss of TGFbR led to a hyperinflammatory state in AM characterised by high IL-6, IL-1B and CXCL10, and increased ability of control

pneumococcal infection. However, strikingly, TGFbR-deficient AM appear to lose their characteristic SiglecF-hi CD11b-lo identity, instead adopting a SiglecFloCD11b+ profile. Mapping of transcriptional signatures from TGFbR-deficient AM to monocyte-to-AM developmental trajectories in the context of influenza shows that SiglecFloCD11b+ adopt a transcriptional signature of transitional monocyte-derived macrophages. However, using complementary fate-mapping approaches, we show that SiglecFloCD11b+ AM are tissue resident cells that have 'de-differentiated'. Moreover, loss of TGFbR led to reduced proliferation and increased levels of apoptosis, showing TGFbR is a direct pro-survival signal for AMs. Assessment of our 'TGFbR deficiency gene module' demonstrates that TGFBR signalling may be abrogated across disease states.

Together, our findings demonstrate that TGFβR signalling is not only essential for AM development but remains dynamically regulated in adult tissue, controlling identity, function, and fate. Ongoing work seeks to identify the upstream cues that regulate TGFβR signalling during infection and inflammation, with implications for therapeutic modulation of AM responses in lung disease.

P.52 **Understanding mechanisms of prostate mediated immune evasion and suppression**

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Background- Prostate cancer (PC) is the most common form of male cancer in the West. In the UK 1/8 men will be affected across their lifetimes. Despite having high success rates with localised treatment, many men experience recurrent cancer that is unresponsive to all clinical options. Immunotherapy in PC has minimal success and the understanding of PC as an 'immune cold' tumour remain to be fully understood, to identify actionable strategies to improve anti-tumoural phenotypes.

Methods- Utilising an inhouse murine PC line (CP2) we orthotopically transplant cells into the anterior lobes of the prostate, to form tumours measurable by ultrasound after one week. CP2 cells expressing ZsGreen with the model antigen ovalbumin (ZsGminOVA) are implanted as subcutaneous and intraprostatic tumours. OTI cells are injected after tumour establishment, or 3 days before cull. Endogenous and OTI specific populations from tumours and tumour draining lymph nodes are immunophenotyped using flow cytometry and immunofluorescence.

Results- OTI cells were enriched in the lumbar and renal nodes, identifying these as the tdLN of intraprostatic tumours. The prostate tumour immune infiltrate harboured significantly fewer CD8+ T cells, with fewer effector CD8+ seen in the tdLN compared to subcutaneous tumours. Intraprostatic tumour growth was reduced after administration of ex-vivo activated and expanded OTI cells. Decreased tumour volume as measured by ultrasound resulted in a significantly improved survival for OTI receiving mice.

Conclusions- Comparing the CP2 ZsGminOVA line in subcutaneous and intraprostatic tumours has allowed us to elucidate prostate specific immune composition and phenotypes, aiding understanding of how to sensitize the immune cold prostate tumour to have a greater anti-

tumoral phenotype. Ongoing work will determine if endogenous T cells can be sensitised to induce a cytotoxic anti-tumoral response, and to characterise new murine cell lines with patient relevant oncogenic and tumour suppressor alterations in the orthotopic prostate model.

P.53 **Identifying germline encoded antigens in colorectal organoids to develop cancer vaccination approaches**

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Cancer vaccines provide an exciting frontier in treatment, with both therapeutic and prophylactic uses as showcased by the HPV vaccine and current clinical trials with 'neoantigen' vaccines – which are created from mutated sections of the genome meaning they are individual and personalised to a patient's specific mutational burden. Recent studies show that many immunogenic peptides expressed on MHC-I arise from unmutated and non-coding sections of the genome, and there is an interest in discovering further antigen targets shared between tumour types and patients to provide cancer vaccine equity which may increase efficacy of current drugs on the market, thus lowering costs for the healthcare system in the long-term.

The field of immunopeptidomics utilises liquid chromatography-mass spectrometry (LC-MS) and data pipelines and can thus identify novel de novo peptides via cross-referencing with transcriptomic data originating from RNA- and Ribosome-sequencing to find likely targets. Peptides can then be synthesised and immunogenicity tested using ELISpot. Utilising murine colorectal cancer-derived organoid cell lines AKPT and VKPN arising from different mouse models, cells were grown as standard organoid cultures and as monolayer cells and treated with interferon-gamma to upregulate MHC-I expression; a comparison was drawn between them to investigate the differences in peptide expression with monolayer cells showing increased MHC-I expression compared to standard organoid. Murine organoids can be transferred back to the original mouse model and collected for subsequent analysis and comparison of peptides originating from the same tumour in 2D monolayer, 3D organoid, and in-vivo tissue to provide future immunopeptidomic studies with a strong background when choosing model systems.

P.54 **Induction of pulmonary type 2 responses by the helminth Schistosoma mansoni**

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Schistosoma mansoni (Sm) is responsible for the majority of cases of schistosomiasis, which affects over 150 million people worldwide. The immunopathogenesis of this parasitic infection is directly related to the progression of the helminth's life cycle and its location in the host's

organs. The initial Th1/Th2-balanced response to migrating schistosomula shifts drastically towards Th2-type inflammation post egg deposition by the worms. The lung environment is believed to be imperative in developing resistance to a schistosome infection after vaccination with radiation-attenuated larvae. However, translation of these findings into the human setting has not been successful. In endemic regions, majority of people become infected with schistosomes multiple times throughout their lives leading to suppressed adaptive responses to cercariae in the skin. T cell hyporesponsiveness in the lung has not been studied in this context. Moreover, immune responses responsible for pulmonary symptoms, as well as protection in the lung remain elusive. Here, we modelled schistosome-driven inflammation in the lung by intravenous injection of Sm eggs and investigated the subsequent immune response. We observed stark induction of eosinophil and conventional type-2 dendritic cell numbers, as well as ablation of alveolar macrophages. Using a lineage-tracing transgenic mouse line we concluded that post egg injection the majority of macrophages in the lung were interstitial and monocyte-derived. In the adaptive immune compartment, we showed egg-induced increase of CD4+ T cell frequencies and their enhanced expression of activation and T helper (Th)1 and Th2 markers – CD44, T-bet and GATA-3 respectively. Together, our data presents a strong case for Sm egg inducing a type 2 immune responses in the lung. In ongoing work we are interrogating the effect of multiple exposures to cercariae on the systemic and lung-localized immune response. We are also investigating features that are crucial for eliciting protection in the lungs following vaccination with irradiated cercariae.

P.55 **Profiling HMGB1 during chronic airway disease exacerbations using sputum proteomics: results from the EMBARC-BRIDGE study**

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Background
High-mobility group box 1 (HMGB1) acts intracellularly as a DNA chaperone and extracellularly as a danger associated molecular pattern (DAMP) released by activated or necrotic cells. HMGB1 can promote inflammation and airway hyperresponsiveness in people with asthma and COPD, however its role in bronchiectasis remains unclear.

We investigated the relationship between airway HMGB1 and inflammatory endotypes during bronchiectasis exacerbations using sputum proteomics.

Methods
People with CT-confirmed bronchiectasis at Ninewells Hospital (Dundee, UK) and Sant Pau Hospital (Barcelona, Spain) enrolled in the European multi-centre EMBARC registry were included. Spontaneous sputum samples collected during exacerbation were analysed using label-free liquid chromatography-tandem mass spectrometry (LC-MS/MS) to profile sputum proteome, alongside 16S rRNA sequencing to characterise microbiota.

To investigate HMGB1 associations, proteins and bacterial abundance was dichotomised into 'high' and 'low' groups using median splits. Wilcoxon rank-sum tests and Spearman correlation with FDR adjustments were used to determine protein co-abundance. Exacerbation endotypes were categorised as neutrophilic or eosinophilic/type 2 based on established proteomic markers.

Results
854 proteins were identified from 52 participants (age 67(64-70) [mean,95%CI], 50% female) of which 396 were positively correlated with HMGB1. HMGB1 abundance was significantly higher in samples with elevated neutrophil elastase (ELANE; high=17.76(17.25-18.56) (median, IQR), low=17.23(16.18-17.75); ρ=0.008; FDR-corrected p=0.016), interleukin-8 (IL-8; 18.06(17.45-18.56), 17.12(16.21-17.51) respectively; ρ=0.004; p=0.0005), matrix metalloproteinase-9 (MMP9; 17.74(17.23-18.42), 17.17(16.35-17.75); ρ=0.009; p=0.043) and neprilysin (MME/CD10; 17.82(17.34-18.42), 17.15(16.26-17.70); ρ=0.004; p=0.035), linked to a neutrophilic endotype. However, no associations with neutrophil granule proteins azurocidin-1 (AZU1) or myeloperoxidase (MPO) were found. There were no significant associations between HMGB1 and markers of eosinophilic/type 2 inflammation (RNASE3, RNASE2, EPX), or with Pseudomonas aeruginosa or Haemophilus influenzae dominance.

Conclusions
Airway HMGB1 is selectively associated with neutrophilic inflammation during bronchiectasis exacerbations. These results highlight the complexity of inflammatory signalling in bronchiectasis and support further investigation of HMGB1 as a mechanistic contributor to disease activity.

P.56 **You can't choose your neighbours: Understanding salivary gland macrophage crosstalk to aid regeneration**

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Background
Therapeutic radiation used to treat head and neck cancer frequently results in salivary gland (SG) injury. This causes reduced saliva production leading to chronic dry-mouth, tooth-decay, and increased infections. There are currently no effective treatment options. Recent work from our lab using a mouse model of targeted SG irradiation (IR) found an initial acute injury which appears to fully regenerate prior to a delayed functional decline in the chronic phase. Macrophages, the most abundant immune cell in the SG are essential for the initial regeneration. However, why they eventually fail to maintain normal SG function is unclear. We hypothesised that in the chronic phase of IR-injury, disruption to normal crosstalk between macrophages and their neighbouring epithelial and stromal cells results in their altered potential for tissue repair.

Methods and Results
To establish the effect of the IR on the macrophages and their surrounding cell types we have used a combination of single cell/nucleus RNA sequencing, bone-marrow chimeras and multi-parameter imaging. Intriguingly we have found broad cellular changes beginning in the months preceding the SG functional decline, including downregulation of the macrophage mitogens Csf1 and Il34. Indeed, the resident macrophages themselves appear to be more proinflammatory and have altered communication with epithelial and stromal cell types.

Conclusions
Whether the disrupted crosstalk between macrophages and their neighbouring cell types following IR is sufficient to alter their function is unclear. Ongoing work aims to unpick the importance of this communication on macrophage behaviour and SG function, in part through deletion of CSF1 using cell specific Cre-drivers.

P.57 **Does critical illness-induced hypoxia drive long-lasting immune dysfunction and morbidity by shaping the mononuclear phagocyte landscape?**

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Critical illness survivors face long-lasting increased susceptibility to infections, suggestive of protracted immune dysfunction. Insufficient blood oxygenation is present in > 50% of critically ill patients, and its severity associates with increased risk of mortality. Previously, we showed

that hypoxia impairs inflammation resolution following acute lung injury (ALI; a preclinical model of lung-associated critical illness), by hindering emergency monopoiesis and monocyte recruitment to the lung. We hypothesise that resulting lung monocyte-derived cells (MdCs) are long-lived and phenotypically imprinted by the hypoxic insult, contributing to persistent changes in host immunity.

ALI monocytes were fate mapped by pulsing Ccr2CreERT2.Rosa26LSL-tdTomato mice with tamoxifen one day before induction of lung injury by nebulised lipopolysaccharide. Thereafter, mice were housed at 10% O2 (hypoxia; ALI-H) or room air (normoxia; ALI-N) for 5 days to model a period of critical illness, followed by recovery in room air.

ALI tdTomato+ MdCs adopted markers consistent with interstitial (SiglecF-CD64+) and alveolar (SiglecF+CD11c+CD64+) macrophage identity and persisted in the lung for up to three months post injury. Abundance of tdTomato+ macrophages was increased by ALI-N compared to naïve mice for up to 35 days in the interstitial compartment, and at 35 and 90 days in the alveolar compartment; however, this was heavily blunted in ALI-H mice. Additionally, tdT+ (recruited) alveolar macrophages had increased MHC-II expression compared to their tdT- (resident) counterparts in ALI-N, but not ALI-H lungs at 35 days post-injury.

Therefore, ALI monocyte-derived lung macrophages are long lived, with evidence of phenotypic imprinting based on bone marrow ontogeny. We are currently exploring the long-lasting impact of hypoxia on ALI macrophages using transcriptomics, microscopy and ex vivo functional assays. Future work will employ in vivo secondary challenge models and chimera/adoptive transfer approaches to ascertain the contribution of short-lived hypoxia to persisting post-critical illness immune dysfunction.

P.58 **The Cellular Analysis Facility at the University of Glasgow**

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