Oral presentations

Bi-directional signalling between the intestinal epithelium and type-3 innate lymphoid cells (ILC3) via the Notch pathway

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Background: Type-3 innate lymphoid cells (ILC3s) are tissue-resident cells enriched at mucosal surfaces where they act as central coordinators of communication in the intestine, responding to localised environmental cues to regulate homeostasis and orchestrate immune responses. There is a bidirectional interaction between the epithelium and ILC3s, but the pathways underpinning this interaction are not fully understood.

Methods: To elucidate this interaction, we employed a co-culture system of small intestinal epithelial organoids (SIOs) with ILC3s.

Results: We found significant global transcriptional changes to intestinal epithelial cells upon co-culture with ILC3, including the enrichment of secretory Paneth and goblet cell signatures. Given that conventional SIOs display reduced proportion of goblet and Paneth cells, we enriched SIOs for these secretory cells. In co-culture with goblet cell enriched SIO, ILC3 expression of NKp46 and IL-22 is upregulated in a contact dependent fashion. In turn, ILC3s induce expression of IL-22 target genes in epithelial cells, including upregulation of antimicrobial Reg3b. Through reanalysis of published datasets, we uncovered that goblet cells are an intestinal source of Delta-Like-Canonical-Notch-Ligands (Dll). Using our organoid co-culture models, we determined that Dll are important for the induction of IL-22 production by ILC3 specifically in T-bet+ ILC3. Finally, we found upregulation of Atoh1, a Notch regulated transcription factor that is crucial for secretory lineage determination, in SIO co-cultured with ILC3, which further highlights the importance of the Notch pathway in ILC3-intestinal epithelial interactions.

Conclusions: Collectively, our findings uncover complementary Notch mediated pathways between ILC3s and the epithelium, which are relevant in intestinal homeostasis and disease.

Segmented filamentous bacteria-induced epithelial MHCII regulates cognate CD4+ intraepithelial lymphocytes and epithelial turnover

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Intestinal epithelial cells have the capacity to upregulate MHCII molecules in response to certain epithelial-adhesive microbes, such as segmented filamentous bacteria (SFB). However, the mechanism regulating MHCII expression as well as the impact of epithelial MHCII-mediated antigen presentation on T-cell responses targeting those microbes remains elusive. Here we identify the cellular network that regulates MHCII expression on the intestinal epithelium in response to SFB. Since MHCII on the intestinal epithelium is dispensable for SFB-induced Th17 response, we explored other CD4+ T-cell-based responses induced by SFB. We have found that SFB drive the conversion of cognate CD4+ T-cells to Granzyme+ CD8α+ intraepithelial lymphocytes. These cells accumulate in small intestinal intraepithelial space in response to SFB. Yet, their accumulation is abrogated by the ablation of MHCII on the intestinal epithelium. Finally, we show that this mechanism is indispensable for the SFB-driven increase in the turnover of epithelial cells in the ileum. This study identifies a previously uncharacterized immune response to SFB, which is dependent on the epithelial antigen presentation.

Dysregulation of intestinal Vδ1 T cell compartmentalisation and functionality in inflammatory bowel disease

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Human gut barrier function is maintained by mucosal lymphocytes enriched in MHC-unrestricted Vδ1+ γδ T-cells, including Vγ4+ subsets that recognise BTNL3 displayed on the intestinal epithelium. However, our understanding of Vδ1+ T-cell regulation in intestinal homeostasis and inflammation remains poor. Here we probed Vδ1 immunobiology in healthy individuals and patients with inflammatory bowel disease (IBD), both within distinct gut mucosal compartments and at different regions of the intestine, combining flow cytometry, phenotype-linked index-sorting, single cell TCR analysis and functional assays. Despite substantial inter-individual heterogeneity in frequency and phenotype, mucosal Vδ1 T lymphocytes were universally Teffector cells and commonly utilised Vγ4, particularly in NKp46+ subsets. The Vδ1 TCR repertoire was private and typically highly clonotypically focussed, within both Vγ4+ and Vγ4- subsets. Clonotype mapping indicated Vδ1 T-cells traffic readily within sub-mucosal regions and between the caecum/ileum but in healthy individuals exhibit strong segregation from blood Vδ1 T-cells. In IBD, mucosal Vδ1 T-cell compartmentalisation was

compromised, accompanied by enhanced proliferation, increased TEMRA-to-TRM ratio, and functional exhaustion. These results suggest a paradigm involving local generation of migratory but mucosa-restricted Vδ1 T-cells clonally adapted to maintain gut barrier protection from specific microbial challenges, dysregulation of which has implications for IBD aetiology

Vitamin D bioavailability regulates microbiome-dependent cancer immunity

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Studies in mice and humans have shown gut commensals to influence anti-cancer immune responses and impact the efficacy of immune checkpoint blockade therapy. The host factors that allow gut-resident microbes to modulate systemic anti-cancer immune responses remain elusive. Here, I report the serendipitous finding that mice with enhanced vitamin D (vitD) bioavailability following genetic deletion of the vitD blood carrier “group-specific component" (Gc) protein or increased vitD dietary supplementation display increased resistance to challenge with transplantable tumours. This resistance is attributable to changes in the microbiome that regulate cancer immunity and can be transferred in dominant fashion to wild-type animals by fecal transplantation. In humans, we show that vitamin D-induced genes correlate with signatures of immunity to cancer, as well as with superior responses to checkpoint blockade inhibitor treatment across seven cancer types, with higher overall survival and lower tumour stage in some cancers. Further, analysis of health records of nearly 1.5 million Danish individuals confirms that a low vitamin D measurement is associated with increased risk of cancer development over the subsequent decade. These findings indicate a previously unappreciated connection between vitamin D bioavailability and microbial commensal communities that act as a potential determinant of cancer immunity and immunotherapy success.

Alterations in B and T cell profiles associate with long COVID

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Long COVID is a highly heterogeneous debilitating disease that develops in at least 10% of individuals following a SARS-CoV-2 infection, and currently affects over 65 million people worldwide. Despite improved understanding of disease pathogenesis, the immune mechanisms driving these persistent symptoms remain ill-defined.

Here, we analysed the phenotypic and functional characteristics of the lymphocyte compartment of hospitalised COVID-19 patients at 12 months of convalescence. We also performed comprehensive immune repertoire analyses using high-throughput sequencing of T and B cell receptors (TCRs, BCRs).

We report that most alterations in B and T cell subsets observed in acute disease are resolved by 12 months of convalescence. However, frequencies of plasmablasts, T follicular helper (Tfh) and T

regulatory cells (Tregs) are reduced in all convalescent individuals compared to controls. Importantly, the production of TNFα by B and T cells was significantly elevated in convalescence and associated with a good clinical outcome. Those with long COVID had lower TCR repertoire diversity, lower somatic hypermutation (SHM) across most IGHV genes, decreased IgG2/IgA2 BCRs, and increased gene usage of IGHV3-48, compared to recovered individuals.

Our data show long-term alterations in lymphocyte subtypes in individuals previously hospitalised with COVID-19. The association of increased TNFα with a good clinical outcome supports a protective role for TNFα in recovery from COVID-19 infection. The BCR/TCR repertoire analyses suggest an impaired SARS-CoV-2 specific humoral response in long COVID individuals that is unable to mediate disease resolution. We propose that these lymphocyte alterations could contribute to persistent symptoms associated with long COVID.

Pannexin 1 drives efficient epithelial repair after tissue injury

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Aims: Epithelial tissues such as lung and skin epithelium are environment-facing barrier cells and are therefore particularly vulnerable to injury or infection. How epithelial regeneration is coordinated is incompletely understood, with dysregulated regeneration and repair occurring in several human disease states. Pannexin-1 (Panx1) channels are activated during cell death to release soluble mediators (including ATP) into the extracellular space. We hypothesised that pannexin-1 channels may represent an evolutionarily conserved method of coupling cell death during tissue injury at barrier sites to pro-regenerative cell proliferation pathways.

Methods: Airway epithelial injury was modelled by naphthalene administration to globally deficient or lineage-restricted Panx1 deficient (Panx1-/-) and sufficient (Panx1+/+) mice. Injury-induced epithelial proliferation was assessed, and RNAseq identified candidate Panx1-regulated pro-regenerative proliferation pathways. In parallel, Panx1 and Panx1-regulated genes were antagonised pharmacologically by small molecule inhibitors or genetically by morpholino knock-down in the regenerating zebrafish tailfin.

Results: We found that Pannexin1 drives efficient epithelial regeneration after tissue injury, by regulating injury-induced epithelial proliferation in mouse lung and zebrafish tailfin. Mechanistically, epithelial injury promoted the Panx1-dependent release of factors, including ATP, from dying epithelial cells. In turn, this induced a reparative response in tissue macrophages that included the induction of the soluble mitogen amphiregulin. RNASeq of regenerating epithelium identified Panx1-dependent induction of Nras and Bcas2 which positively promoted epithelial proliferation and tissue regeneration.

Conclusions: Pannexin-1 has a conserved role in epithelial regeneration across tissue types and organisms. Pannexin-1 regulates expression of Nras and Bcas2, with these genes required for timely proliferation and regeneration of injured barrier epithelial tissue, and a reliance on tissue macrophages for maximal regeneration.

Dermal γδ17 T cells orchestrate innate and adaptive immunity in distal organs during nematode infection

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γδ T cells are preferentially located at barrier sites where they exhibit innate-like features and are thus among the first lines of defence of the mammalian immune system. However, their role in coordinating systemic immunity remains largely unexplored. Experimental nematode infections provide a great tool to investigate this process, as infective larvae migrate across different tissues. Moreover, many nematode parasites, such as Nippostrongylus brasiliensis, have the skin as their entry site – a tissue harbouring a major population of IL-17-producing γδ (γδ17) T cells. We have established a natural N. brasiliensis infection model that allows us to fully recapitulate all the steps of the infection and investigate the role of γδ17 T cells in inter-tissue communication. Bypass of the skin phase of infection, or conditional ablation of IL-17 on skin-resident lymphocytes resulted in marked transcriptional and functional changes in the innate immune response to migrating larvae in the lungs. Interestingly, employment of tissue-restricted gene reporters suggested that dermal γδ17 T cells migrate from the skin to the lungs to kick-start the early response against N. brasiliensis. Furthermore, ablation of skin-derived IL-17 at the skin migration phase resulted in a subsequent decrease in lung Th2 immunity and impaired T cell priming in lymph nodes. Together these data indicate that dermal γδ17 T cells not only affect the development of innate immune responses at distal sites but also adaptive immunity during N. brasiliensis infection. Thus, dermal γδ17 T cells may have previously undiscovered roles in mediating immune-communication between mucosal tissues upon pathogen invasion.

Integrated spatial and single cell transcriptomic analyses reveal a critical role for Vg6 gd T cells in the control of skin inflammation during infection

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African trypanosomes colonise the skin in a process critical for disease transmission, but skin responses to infection remain unexplored. Here, combining spatial and single cell transcriptomics, we investigated local immune responses of the skin in a murine model of infection. Our results reveal an unexpected crosstalk between subcutaneous adipocytes and gamma-delta (gd) T cells

during infection. During chronic infection, we detected an expansion of IL-17-producing Vg6 gd T cells in the infected murine skin compared with naïve controls. In silico cell-cell communication analyses suggest that adipocytes trigger Vg6 gd T cell activation via Cd40, Il6, Il10, and Tnfsf18 signalling, indicating a role for adipocytes in controlling T cell activation locally. In vivo, the infected skin of Vg4/6-/- mice displays more inflammation compared with infected wild type controls, correlating with an elevated capacity of dermal CD8+ T cells to produce IFNg, independently of dermal TH1 CD4+ T cells. Intriguingly, the Vg4/6-/- mice do not experience subcutaneous adipose tissue wasting to the same extent as the FVB/N controls, indicating that Vg4/6 gd T cells might also control this process. Based on these observations, we propose a model whereby adipocytes and Vg4/6 gd T cells act concertedly in the skin to limit CD8+ T cell-mediated inflammatory responses, imposing an immunological barrier for parasite transmission. These studies shed light onto the mechanisms of gd T cells-mediated immunity in the skin in the context of African trypanosome infection, as well as a potentially novel role of adipocytes as regulators of skin immunity during chronic infection.

A specialised keratinocyte niche permits differentiation of monocytes into Langerhans cells despite ongoing inflammation

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Langerhans cells (LC) are a unique population of macrophages that acquire characteristics more commonly associated with dendritic cells (DC) upon residency in the skin epidermis. Graft-versus-host disease (GVHD) after haematopoietic stem cell transplant results in destruction of the LC network in patients. The empty niche is replenished by recruited monocytes that differentiate into LC that are transcriptionally and functionally identical to the embryo-derived cells they replace. Strikingly, we have shown that restoration of the resident LC niche by monocyte-derived cells occurs despite ongoing inflammation. However, the molecular mechanisms by which the LC network is repaired during immune injury remain unclear.

Transcriptomic analysis of epidermal myeloid cells in a murine model of GVHD revealed unexpected heterogeneity; while some monocytes appear to undergo an intrinsic programme of differentiation to tissue repair phenotype macrophages, others upregulate the cell adhesion molecule EpCAM before differentiating into populations of resident and migrating LC. Consistent with the acquisition of a more DC-like function, commitment to a LC fate is linked to loss of the macrophage-defining factor Zeb2, while migrating LC downregulate LC-defining genes but upregulate a core migratory DC programme. Interrogation of the keratinocyte niche predicted that monocyte converting cells interact with specialised follicular keratinocytes via the Notch ligands Jagged-1 and -2; and we demonstrated that provision of Jagged-1 signalling was sufficient to drive differentiation of monocyte-derived LC in culture.

Lightning presentations

P.01 **Aberrant B cell responses in chronic obstructive pulmonary disease**

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Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease characterised by uncontrolled inflammatory responses that lead to emphysematous destruction of the lungs, which ultimately results in airflow limitation and decline in pulmonary function with limited reversibility. Despite the increased accumulation and persistence of B cells in the COPD lung, their roles in disease pathogenesis remain understudied. Here, we characterize B cell subsets and their spatial distribution in the lung of COPD patients and non-COPD controls. We report alterations in B cell profiles of COPD patients compared with controls. Histology staining confirmed previous studies showing an increase in lymphoid follicles in the lung tissue of COPD patients compared with non-COPD controls. Using Cytof mass cytometry, we found that lung samples from COPD patients displayed an expansion of plasmablasts, plasma cells, memory B cells and Tim1+ Bregs. Furthermore, Hyperion mass cytometry imaging analysis revealed an increase in airway-associated B cells in the COPD lung, found to be in close proximity to bronchioles and blood vessels, as well as other immune cells such as T cells and macrophages. The data presented here suggest an increased density of organised B cell follicles and an expansion of IL-10-producing regulatory B cells in the COPD lung. Whether B cells are directly or indirectly involved in disease pathogenesis remains unclear. However, excess B cells would likely affect gaseous exchange and lung elasticity. Therefore, future investigations will be required to determine the therapeutic potential of targeting B cell subsets in COPD.

P.02 **Skin Immuno-matrix: The role of the endothelial glycocalyx and heparan sulfate in psoriasis-like skin inflammation**

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Psoriasis is a chronic inflammatory skin disease, the pathogenesis of which relies upon leukocyte recruitment into skin. The endothelial glycocalyx is a layer of glycoproteins and glycolipids lining the lumen of blood vessels, which can act as a barrier against leukocyte migration. Under various inflammatory conditions the glycocalyx is shed, exposing underlying endothelial cell adhesion molecules, allowing them to bind to circulating leukocytes, facilitating leukocyte migration into the tissue. To investigate the role of the glycocalyx on immune cell migration in psoriasis-like skin inflammation, mice were treated with Aldara cream to stimulate psoriasis-like skin inflammation. Decreased expression of the glycocalyx components heparan sulfate and syndecan-1 were found on endothelial cells in Aldara treated mice, whilst increased concentrations of these components were present in the serum, suggesting the glycocalyx is degraded in response to inflammation and shed into the blood. To further elucidate the role of heparan sulfate in psoriasis-like skin inflammation, mice were treated with Aldara cream alongside an inhibitor of the heparanase enzyme, the enzyme responsible for cleavage of heparan sulfate from the glycocalyx. Heparanase inhibition caused significantly increased clinical signs of inflammation in Aldara cream treated mice, however despite this, flow cytometry revealed fewer immune cells present in the skin. This reduction was particularly prevalent in migratory populations such as neutrophils and regulatory T cells. These findings suggest

that inhibiting glycocalyx remodelling using heparanase inhibitors during skin inflammation affects the recruitment of immune cells, providing a potential therapeutic target for inflammatory skin conditions.

P.03 **A matrisome-enriched co-expression module associated with the perivascular niche is upregulated in treatment non-responsive inflammatory bowel disease**

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Inflammatory bowel disease is a prevalent, multifactorial disease which remains

Extensive tissue remodelling occurs in the pathogenesis of inflammatory bowel disease (IBD) pathogenesis. However, the genetic and molecular changes that occur in disease are not fully understood, nor are the effects of this changing microenvironment on cellular behaviour. Characterizing these processes holds potential to uncover novel mechanisms of ECM-mediated pathology in IBD.

Differential expression of matrix and matrix-related (matrisome) genes was analysed in a bulk RNA sequencing (RNAseq) dataset of IBD and non-IBD patients. Weighted gene correlation network analysis (WGCNA) was used to identify co-expression modules1. Module expression was compared in public single cell RNAseq datasets of IBD and selected gene expression was validated in murine dextran sodium sulphate (DSS) colitis by qPCR.

Bulk RNAseq indicates transcriptional regulation of 167 matrisome genes. A WGCNA module comprising 43.6% matrisome correlates with IBD occurrence, Nancy Index, macroscopic inflammation and predicts non-response to anti-TNF and corticosteroid therapies. In single cell RNAseq datasets, expression of this module is restricted to endothelial cells, pericytes, fibroblasts, activated/inflammatory fibroblasts and was upregulated in disease. Analysis of colon tissue from murine DSS colitis shows conservation of this module, with a number of genes upregulated before peak colitis.

These data suggest 1) tissue remodelling is orchestrated by a local cellular ecosystem of diverse stromal cells, 2) remodelling events precede, and may be required for, inflammation, and 3) dysregulation of this niche is associated with inflammation and treatment-nonresponsive IBD.

Poster presentations

P.04 **CXCL4 drives wide-spread non-receptor mediated immune cell recruitment via interactions with extracellular matrix proteoglycans**

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Leukocyte recruitment from circulation into tissues is a complex process that is central to a functioning healthy immune system and developing inflammatory diseases. Despite chemokines which are small, secreted proteins (8-10 kDa) being key to this process, they have yet to be therapeutically targeted during inflammation. Whilst there are many reasons for this, the overarching explanation for this shortfall is the lack of basic understanding for how the chemokine system functions. For example, our lab has recently demonstrated that CXCL4 (platelet factor 4, PF4), unlike other chemokines, does not drive leukocyte recruitment by binding to a G protein-coupled receptor (GPCR), but functions primarily through glycosaminoglycans (GAGs). This work highlights that inhibiting GAG binding represents a potential new way to therapeutically target CXCL4.

To build on this, the objective of this project is to define the main immune cell sources of CXCL4 in different tissues, to establish the role of CXCL4 in health and disease and ultimately identify how extracellular matrix components including GAGs influence CXCL4-mediated cellular recruitment. We hypothesize that macrophages are a significant source of CXCL4 and that GAGs contribute substantially to the critical functions of CXCL4 at steady state and during disease. We anticipate that such knowledge will inform the development of novel therapeutic targets.

P.05 **Investigating Host Susceptibility to Infection**

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Why do some people get severely ill when infected, whilst others do not? In order to attempt to address this question and mechanistically study host factors underlying disease severity we used Citrobacter rodentium (CR), a natural mouse-adapted pathogen that leads to different host-dependent infection outcomes. When infected with CR, C57Bl/6 mice develop mild, self-limiting colitis; conversely C3H/HeN mice succumb to infection and represent a severe model of colitis that resembles life-threatening human infection.

Given the early deterioration shown by C3H/HeN mice, we focussed in characterising innate immune responses in these hosts. Despite their inability to control the infection, we observed significantly higher neutrophil recruitment to the colon of C3H/HeN mice that coincided with tissue damage, heightened G-CSF levels and expansion of the neutrophil compartment in the bone marrow (BM). To investigate the intrinsic factors contributing to this response we performed proteomic profiling of BM and colonic neutrophils. While similar in homeostasis, BM neutrophils had distinct signatures in infected mice, where C3H/HeN neutrophils showed defects in terminal differentiation, including pathways such as chemotaxis. Colonic neutrophils from C3H/HeN mice also displayed differences, reflected in lower Ly6G expression, impaired migration to the infection site and increased pro-

inflammatory cell death away from this site, thus contributing to the increased mucosal pathology and failure to control infection.

By providing insights into the regulation of neutrophil function and increased susceptibility to infection, we hope to pave the way to more targeted therapies that can reduce neutrophil-related tissue damage without impacting on their antimicrobial capabilities.

P.06 **Alterations to circulating myeloid cells and bone marrow hematopoiesis following intestinal worm infection**

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Following infection, the bone marrow (BM) adapts to the increasing demand for functional immune cells, altering the steady-state hematopoiesis towards an “emergency” one. This process is accompanied by changes in the output of monocytes and other myeloid cells into the circulation which adopt different flavours and shape the immune response at the site of infection. Most understanding of infection-induced myeloid cell production has been following life-threatening Th1-driving infections, such as bacteria or intracellular parasites. How the BM is altered in response to Th2-driving chronic helminth infections remains unknown.

In this work, we use experimental infection with Heligosomoides polygyrus bakeri (Hpb), a chronic helminth parasite that infects the small intestine of mice. Hpb is entirely enteric and triggers a type 2 inflammatory response. We show that early after Hpb-infection, monocytes acquire a previously undescribed phenotypic signature - Ly6Clow/Sca-1hi - with a global interferon transcriptional profile. This response is distinct from that occurring following Th1-driving infections and is dependent on type I IFN signals conveyed by T cells, in a microbiome-dependent way. Moreover, these alterations occur with concomitant changes in the hematopoietic stem/progenitor cell (HSPC) compartment within the BM. Particularly, the number of MPP2 (multipotent myeloid cells) is significantly increased in the Hpb-infected animals when compared to the naive, and this is also dependent on type I interferon signalling.

Our findings demonstrate specific alterations to circulating myeloid cells and BM progenitors in response to a chronic intestinal infection. Better understanding of infection-induced hematopoiesis could aid in identifying pathways important to treatment of helminth infection but more broadly in chronic inflammatory states.

P.07 **Mucosal organoids capture tissue-specific innate lymphoid cell development**

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ILC-restricted precursor (ILCP) populations arise from common lymphoid precursors (CLP) in the bone marrow, however the final stages of adult ILC-subset maturation likely occur upon exiting this niche. Deciphering what local stimuli drive the differentiation and function of ILC in these tissues remains a pressing question, as ILC frequencies can become dysregulated in disease and promote inflammation.

Here, we introduce murine and human co-culture systems of ILCP with organoids that faithfully capture the maturation of ILC within mucosal tissues.

This approach promotes significant expansion and functional maturation of all ILC subsets in parallel. Notably, gut and lung organoids cells are sufficient to induce tissue-specific patterns of ILC subset frequencies and phenotypes, even in the absence of organ-specific microbial tropism or additional cells. For example, like their ex vivo counterparts, ILC2 are KLRG1high when developed on intestinal organoids and a ST2high when generated on lung organoids. This lung phenotype, is dependent on IL-33, demonstrating the importance of tissue derived factors on ILC modulation.

Importantly, using hIPCS-derived organoids we show that human gut epithelium, not stroma, supports proliferation and maturation of human ILCP.

Taken together, our work provides unprecedented insight into in situ ILC maturation and function. Moreover, our work introduces a modular organoid platform, which provides exquisite control over both environmental stimuli and host genetics, making it powerful tool for dissecting immune-epithelial interactions in health and disease. Finally, this system has the potential to be used for the generation of ILC for human treatment opening the door to autologous ILC therapies.

P.08 **Understanding the role of the endothelial glycocalyx in regulating immune cell recruitment during lung inflammation / infection**

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Lung viral infections are a leading cause of death worldwide with an estimated 3.5 million deaths annually. Leukocyte recruitment to the lung is critical for clearing infection and inflammation resolution. However, excessive leukocyte recruitment promotes lung injury.

One component of leukocyte recruitment which has been overlooked is the endothelial glycocalyx (eGC). The endothelial glycocalyx is a carbohydrate-rich structure lining the luminal surface of the vascular endothelium, that regulates the access of cells and molecules in the blood to the endothelium. However, there is a lack of understanding of the mechanisms that regulate the eGC-leukocyte recruitment axis in lung infection. We sought to characterise the composition and presence / absence of the eGC, in addition to clarifying its functional importance in influenza A infection.

To recapitulate IAV infection in humans, mice were inoculated with PR8 IAV. Lungs were harvested at acute and chronic timepoints post infection from C57BL/6 mice and flow cytometry and IHC performed.

We show that a dispase II tissue digest is superior to a collagenase digest for analysis of HS, HA, and syndecan-1 in the resting and infected lung. We also found that eGC components displayed unique expression profiles over the time course of IAV infection in both endothelial cells and a subset of EpCam+CD31+ cells.

We propose that there is a complex interplay occurring between the eGC and leukocyte recruitment during IAV. These studies provide a foundation for future work to understand how the eGC changes during lung infection and whether these changes remain post viral infection.

P.09 **A feedback loop between epithelium and Innate Lymphoid Cells controls intestinal TGF-β1 dynamics**

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Background: Innate Lymphoid Cells (ILCs) are involved in intestinal homeostasis and pathogenesis. Type-1, 2 and 3 ILCs, can transdifferentiate into one another leading to their altered distribution in Inflammatory Bowel Disease (IBD). We have shown that ILC express, Transforming Growth Factor – beta 1 (TGF-β1), a pleiotropic cytokine secreted in an inactive form, leading to modulation of intestinal and matrix remodelling and contributing to the development of fibrosis, a sequela of IBD. Here, we aimed to determine how ILC-produced TGF-β1 is activated and how its levels are regulated in the intestine.

Methods: Lamina propria ILCs and small intestinal organoids were cultured with Transformed Mink Lung Cells to detect bioactive TGF-β1. To identify TGF-β1 activators by ILCs and organoids, publicly available data sets were screened, and potential target genes validated by RT-qPCR. ILC-organoid co-cultures were used to study the immune-epithelial interactions.

Results: In addition to possessing the capacity for Tgfb1, expression, lamina propria ILCs respond to, produce and activate TGF-β1. When stimulated with recombinant TGF-β1, organoids also increase their production of this cytokine. The expression of TGF-β1 activators changed when ILCs and organoids were co-cultured or stimulated with recombinant TGF-β1.

Conclusions: Collectively, these data demonstrate the ability of ILCs and organoids to produce and activate TGF-β1, reveal possible activators and suggest that TGF-β1 levels in the intestine are controlled by an ILC-epithelial feedback loop. Dissection of the mechanics of these TGF-β1-dependent pathways may facilitate their targeting and therapeutic modulation to promote intestinal health.

P.10 **Maintenance and Metabolism of Intestinal ILC3**

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Group 3 innate lymphoid cells (ILC3) are potent innate effector cells with critical roles in enforcing immunity, barrier integrity and tissue homeostasis along the gastrointestinal tract. ILC3 are considered to be primarily tissue-resident cells, seeding the gastrointestinal tract during embryonic stages, however the mechanisms through which ILC3 are maintained within these tissues are poorly understood. Here, we report that ILC3 are minimally replenished from bone marrow precursors in healthy adult mice, persist in the tissue for extended periods of time in the gut, and display a quiescent phenotype. Strikingly, despite robustly producing cytokines, LTi-like ILC3 remain non-proliferative during enteric bacterial infection. Here we report survival of LTi-like ILC3 is dependent upon the balance of the metabolic activity required to drive effector function and anti-apoptotic programs. Notably, the pro-survival protein Bcl-2 was required for the survival of LTi-like ILC3 but was rendered partially dispensable if mitochondrial respiration was inhibited. Together we demonstrate LTi-like ILC3 are a quiescent-like population that persists independently of haematopoietic replenishment to survive within the tissue microenvironment.

P.11 **Investigating how Heligmosomoides polygyrus infection affects gut barrier integrity**

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Helminth infection is a major concern across the globe, with nearly a quarter of the world population infected. Research has improved our understanding of the immune response to infections with single species of helminths but, in intestinal infections, worms are accompanied by multiple bacterial colleagues, and we still understand very little about the interplay between the immune responses to each stimulus. Data has shown that helminth infection can attenuate a host's response to concurrent infections, but it is not yet clear whether bacteria or other pathogens can potentiate or alter immunity against the helminths.

Our research aimed to investigate whether helminth infection is associated with breaches in the gut barrier and whether these breaches allow bacteria to cross, increasing susceptibility to co-infection. Using a mouse model of Heligmosomoides polygyrus infection, we show that the early entry and exit of the helminth into the wall of the small intestine is accompanied by spikes in local inflammation, tissue remodelling, and expression of the pro-inflammatory cytokine, IFN-γ. Our data suggest that bacterial translocation may not occur, although further research is required. Using IFN-γ blockade in Heligmosomoides polygyrus infection, we are currently assessing whether the IFN-γ signature in the intestinal wall is initiated by bacterial or helminth antigens, and whether its presence regulates tissue repair, host immunity, parasite clearance and/or bacterial spread. Our most recent

experiment has shown that IFN-γ is responsible for increased tight junction protein expression and therefore plays a role in the repair process during helminth infection. Our continued research will allow further development in understanding the immune response to Heligmosomoides polygyrus, and the tissue-based interactions that affect how intestinal parasites can alter the host response to other infections.

P.12 **IL-13 as a modulator of the hyaluronan extracellular matrix in lung**

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Accumulation of the extracellular matrix (ECM) polysaccharide, hyaluronan (HA) in the lung is associated with pathologies such as influenza, SARS-CoV2 infection and asthma. We recently demonstrated that accumulation of HA in the lungs following SARS-CoV2 infection was reversed when infected mice were treated with IL-13 neutralising antibodies. Furthermore, intranasal delivery of IL-13 alone was sufficient to drive HA accumulation in the lung. Our aim is to investigate the mechanisms by which IL-13 contributes to the formation and regulation of the HA ECM and the consequences of this for pulmonary health in different biological contexts.

To study the role of IL-13 as a modulator of HA, IL-13Fc was delivered intranasally to naïve mice. Following treatment, HA was increased in lung tissue and bronchoalveolar lavage fluid. Differences in surface HA staining on lung macrophage populations were also observed. Alveolar macrophages were less positive for HA whilst the number of positive cells and intensity of HA staining on interstitial macrophages increased with IL-13 treatment. In lung tissue sections, IL-13Fc led to HA accumulation around the vasculature and airways. IL-13Fc treatment was associated with increased expression of Has2 (a HA synthase) but decreased expression of the HA degrading enzymes, Hyal1 and Hyal2. Overall, our results suggest IL-13 alters HA metabolism, which in some contexts could lead to HA accumulation. Modulation of HA by IL-13 represents a novel mechanism by which immune-mediators influence the ECM. Further characterisation will allow us to understand how IL-13 contributes to the composition of HA matrices during health and disease.

P.13 **Myeloid cell suppressive receptor, CD200R1, promotes IL-17 production by unconventional lymphocytes**

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Limiting immune responses is critical for preventing inappropriate inflammation at barrier sites where environmental stimuli can contact immune cells. Multiple mechanisms suppress inflammatory responses, one important example being CD200R1 and its ligand, CD200 which limits myeloid cell cytokine production. The CD200R1:CD200 pathway suppresses immune responses against self-antigens, allergens, infectious agents and cancer. However, the role of this pathway in inflammatory skin diseases is under explored. We previously observed reduced CD200 levels in the inflammatory

skin disease psoriasis, thus here we examined the effect of reduced CD200R1 on psoriasis-like skin inflammation.

We used CD200R1-deficient mice, flow cytometry, in vivo models of psoriasis-like skin inflammation and in vitro models of unconventional lymphocyte activation to examine the role of CD200R1 in regulating skin inflammation. Surprisingly, CD200R1 is required for optimal IL-17 production in skin inflammation models, where it promotes IL-17 production by type 3 innate lymphoid cells (ILC3) and gamma delta T cells. ILC3 subsets and cell surface phenotype are unaffected by CD200R1, but CD200R1-deficient ILC3 are transcriptomically distinct from WT, and are less able to activate STAT3 downstream of IL-23, accounting for their reduced IL-17 production.

CD200R1-deficient gamma delta T cells also have a reduced ability to produce IL-17, which is observed in skin and thymus. The IL-17-producing Vgamma4+ subset is also reduced in skin and lymphoid organs demonstrating a critical developmental role for CD200R1.

Therefore, CD200R1 is a critical factor promoting IL-17 production, and targeting this pathway may be a novel therapeutic strategy for inflammatory diseases.

P.14 **Gut lymphoid tissues and group 3 innate lymphoid cells are regulated by a conserved microRNA**

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Situated and primed for rapid responses at barrier tissue sites, Group 3 Innate Lymphoid Cells (ILC3s) mediate critical roles including maintenance of mucosal tissue homeostasis, modulation of adaptive immunity, tissue repair and lymphoid tissue organogenesis. ILC3s are dependent on the canonical transcription factor RORγt (encoded by Rorc), however mechanisms imprinting broader ILC3 functionality are poorly understood.

We have identified the evolutionarily conserved, immune system-associated, microRNA-142 (miR-142) as a critical regulator of intestinal ILC3s. Using germline knockout (Mir142-/-) and ILC3-conditional (RorcΔMir142) models of miR-142 deficiency, ILC3 cellularity was found to be reduced within the intestinal lamina propria and gut-associated lymphoid tissues (GALT) and associated with functional defects in ILC3 effector cytokine responses, including IL-22 production, at steady state and during αCD40-mediated innate cell-driven colitis. Analysis of ILC3 subsets revealed that post-natal CCR6+ lymphoid tissue inducer-like cells (LTi-like ILC3) were severely impacted in response to miR-142 deficiency, with the CD4+ LTi-like subset especially affected. Furthermore, Mir142-/- mice failed to develop critical structures associated with GALT including small intestinal Peyer's patches and caecal patches – although mesenteric lymph nodes (mLNs) were present. However, ILC3-conditional deletion of miR-142 was not sufficient to recapitulate absence of these structures in RorcΔMir142 mice, despite the described impact on adult LTi-like cells. This suggests broader, as-yet unappreciated roles for miR-142 in the regulation of GALT development.

Collectively, our findings further the understanding of the cell-intrinsic regulatory pathways governing ILC3s and their critical functions at the intestinal barrier.

P.15 **Imaging mass cytometry highlights a novel role for chitinase-like proteins in airway remodelling**

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During airway inflammation, the composition and organisation of the lung extracellular matrix (ECM) is altered contributing towards airway remodelling. The mechanisms driving remodelling remain ill-defined due to lack of understanding of the interactions between the immune system, stromal cells, and the ECM. Chitinase-like proteins (CLPs), including murine Ym1 and Ym2 and human YKL-40, are strongly associated with asthma development and are thought to play a role in regulating both airway inflammation and ECM remodelling. Using Imaging Mass Cytometry (IMC) we will interrogate how immune cells and ECM components are spatially organised during allergic pathology in the presence or absence of CLPs.

To study the role of CLPs in airway remodelling, FFPE lung tissue sections from WT and Ym2-knockdown mice following allergy challenge were stained with metal-labelled antibody panels against 44 immune, epithelial, stromal and ECM markers.

IMC staining panels successfully detected a range of ECM, stromal and immune components across alveoli, airway, and vasculature. An infiltration of B220+ cells and CD11b+ cells associated with areas of remodelling were observed during allergy. Furthermore, Ym2 knockdown resulted in changes to the spatial organisation of B220+ cells and collagen VI.

Our ongoing analysis of high dimensional IMC will allow the identification of lung cells that are associated with specific ECM components. IMC will aid our understanding of immune-matrix interactions in the lung and how cellular organisation changes from health to disease. Our work will also yield a novel insight into how CLPs may regulate the development of airway remodelling and local inflammation.

P.16 **CD200R1 is required for efficient wound healing in middle-aged mice**

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Wound healing requires a transition from acute inflammation to a proliferative phase where keratinocytes proliferate and migrate. However, in older individuals, wounds often fail to progress and arrest in chronic inflammation. This impairs wound closure resulting in chronic wounds. We propose that in chronic wounds, pathways which restrain immune responses are dysregulated. Over-activation of such pathways could suppress a productive inflammatory phase in healing, conversely under-activation could prevent inflammation resolution. To test this hypothesis, we investigated the immune cell-expressed, suppressive receptor CD200R1, and its ligand, CD200, which is expressed by epithelial stem cells involved in re-epithelialisation.

Excisional wounding was performed on young adult and middle-aged WT (C57/B6) and CD200R1KO mice. Wound closure was measured by planimetry and histology. Wound tissue was collected and isolated cells were analysed by flow cytometry.

Despite significant upregulation of CD200R1, particularly by ILC2 (type 2 Innate Lymphoid Cells) but also γδ and αβ T cells, modulating this pathway did not alter skin healing in young adults. However, middle-aged animals lacking CD200R1 displayed delayed healing with impaired wound contraction and increased fibrotic processes. Excessive type-2 immune responses were observed in wounds and skin of middle-aged CD200R1KO mice, but not in young counterparts. This could suggest that lifetime exposure to CD200R1 signalling protects against age-related healing decline or that CD200R1 compensates for age-related loss of other protective mechanisms. Therefore, promoting CD200R1 signalling in ageing may promote efficient healing, with potential as a novel therapeutic strategy for non-healing wounds in the elderly.

P.17 **The immunological links between inflammation in the skin and the respiratory system**

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Guttate psoriasis is an inflammatory skin disease, driven by T cells, IL-17 and IL-23. It is triggered by streptococcal respiratory tract infections. Plaque psoriasis patients are also more susceptible to these infections, suggesting an immunological association between respiratory and skin inflammation. Additionally, respiratory allergies frequently occur with atopic dermatitis (AD). It is apparent that inflammation at one tissue site can regulate the immune system in distal tissues, but it is unknown how they are linked and what local changes lead to inflammation susceptibility.

To determine the effects of S. pyogenes respiratory tract infections on psoriasis-like inflamed skin, pre-clinical mouse models were used. Skin inflammation was induced after infection using Aldara cream (containing 5% Imiquimod). Immune cells and their mediators were analysed within the skin and draining lymph nodes by flow cytometry to determine immunological changes and which infections exacerbated psoriasis. Uninfected lungs were also analysed following psoriasis-like or AD-like (using topical Calcipotriol) skin inflammation.

Intranasal administration of S. pyogenes caused an increase in steady-state ear skin thickness but did not affect subsequent Aldara-induced skin inflammation. However, topical Aldara application reduced CD4 T cell and T cell numbers in the uninfected lung and increased T cell IL-17 production. Calcipotriol-induced skin inflammation led to increased lung neutrophil frequency and CD4 T cell IL-5 production. Neither caused overt lung inflammation.

Our finding that skin inflammation induces subsequent changes in the lung could be targeted therapeutically to develop early interventions for guttate psoriasis patients or AD patients prone to respiratory allergies.

P.18 **Tissue damage and microbiota modifications provoke intestinal type 2 immunity during Schistosoma mansoni infection**

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During mammalian infections with Schistosoma mansoni, hundreds of parasite eggs rupture through the intestinal wall and into the lumen. Although this destructive process is central to schistosomiasis-associated pathology, there are limited studies addressing the impact of egg migration on the intestinal interface, including barrier integrity, the microbiota, and mucosal immunity. Herein, we provide a high-resolution image of the intestinal environment during murine schistosomiasis, using a combination of egg-producing vs non egg-producing, and high vs low dose infections. We demonstrate a significant reduction in intestinal barrier function during patent infections, with evidence for systemic responses to gut bacteria, and infection intensity altering the kinetics of intestinal 'leakiness'. Due to the exuberant anti-parasitic response elicited by schistosome infection, it has proven notoriously challenging to extract live immune cells from schistosome-infected murine intestine, with researchers often using the mesenteric lymph nodes (MLNs) as a proxy for intestinal responses. Here, we show patent infections to induce Type-2 dominated immune responses in the MLN and colonic lamina propria, as evidenced by enhanced Th2 cell cytokine production, transcription factor expression, and increased proportions of alternatively activated macrophages and DCs. We highlight discrepancies between colonic and MLN responses, and show the dramatic Type-2 shift to coincide with alterations in microbiota composition. Finally, through the use of germ free mice and faecal transplants, we provide evidence that the schistosome infection-associated microbiota may promote the emergence of a unique population of CD4+ T cells in recipients. In ongoing work we aim to further dissect the immune environment during schistosomiasis and determine which bacterial species (and/or their products) contribute to schistosome-mediated immunomodulation.

P.19 **Localised intestinal radiotherapy causes systemic immune changes in the lung and thymus**

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Radiotherapy remains the mainstay anti-cancer treatment, being part of curative therapy in 40% of patients. In addition to the well-documented direct cytotoxic effects, radiotherapy can have profound immunomodulatory effects on the tumour and surrounding normal tissues, both locally and systemically. Patients receiving pelvic radiotherapy often develop acute intestinal damage that can progress to long-term side-effects. Moreover, radiotherapy can induce immune-driven distal

effects that are proposed to be beneficial in controlling metastases. However, these local and distal immune responses to radiation, have not been fully characterised.

Using targeted CT-guided X-irradiation of the mouse abdomen and flow cytometry we investigated how localised radiation modifies immune cells within the irradiated intestine but also at distal non-irradiated sites, to understand how irradiation of non-cancerous tissue causes immunomodulation.

During the acute phase (2-7 days) the irradiated intestine showed marked transient increases in numbers of several innate cell subsets, including monocytes, macrophages, granulocytes, NK and dendritic cells. At the same time, in distal non-irradiated lung we observed a significant depletion of eosinophils and dendritic cells, as well as B and T lymphocytes. Additionally, the thymus showed a dramatic shrinkage 7 days after intestine-specific irradiation, and a reduction in CD4+CD8+ thymocytes.

Our findings suggest that radiation induces strong acute immune responses within the targeted normal tissues but also in distal non-irradiated organs. We are currently working to identify the key immune mechanisms by which radiotherapy leads to these systemic effects, including DAMP/microbiota-driven signalling, and what are the functional and long-term consequences of these distal changes.

P.20 **Targeting integrin αvβ8 to promote TGF-β activity in IBD**

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Background: Inflammatory bowel disease (IBD) represents a global health problem, with increasing incidence and significant morbidity. There is no cure, and current treatments aim to inhibit inflammatory pathways. Current treatments often show low efficacy, lose efficacy or are not tolerated. There is a clinical need for new therapeutic targets and treatments in IBD. One potential target is the cytokine TGF-β, a potent regulator of immunity, particularly in dampening T cell responses. TGF-β is produced by cells as an inactive complex, and we have previously shown that monocytes and macrophages activate TGF-β via expression of an integrin, αvβ8. We have shown this to be an important anti-inflammatory pathway, and that αvβ8 expression is downregulated in patients with active IBD. However, the mechanisms that promote downregulation of this pathway in IBD are unknown. Here, we aim to identify such mechanisms, with the aim of potentially preventing loss of expression as a target for IBD therapy.

Results: αvβ8 expression on human monocytes and macrophages is increased with LPS stimulation ($p<0.01$), decreased via treatment with IL-4 ($p<0.05$), and IFNγ ($p<0.05$). Single nucleotide polymorphisms in the region of integrin genes including ITGB8 have been identified as risk alleles for IBD. In human monocytes αvβ8 expression and TNF-α production in response to LPS stratify according to genotype for the SNP rs149169037 ($p<0.01$, $p<0.05$).

Conclusion: Cytokine signalling, microbial antigens, and genetic pre-disposition all contribute to human αvβ8 expression. Targeting mechanisms that regulate αvβ8 expression to promote anti-inflammatory TGF-β activity represents a novel approach in IBD.

P.21 **Is mitochondria striving to rescue the murine lupus lung?**

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Introduction: Altered pulmonary manifestations in lupus is reported to be associated with morbidity and mortality. Since mitochondrial dysfunction is known to be involved with lupus aetiology, its master regulator was analysed for its role in regulation of pulmonary manifestation in lupus. Present study is based on understanding association of AMP-activated protein kinase/Peroxisome proliferator-activated receptor-gamma co-activator/ Sirtuin1 (AMPK/PGC-1/SIRT-1) axis in lupus associated lung.

Methodology: Pristane induced Balb/c mice lupus model (LM) was evaluated for anti-oxidants (MnSOD and catalase) in plasma/tissue, mitochondrial complexes (MCs), pro-inflammatory cytokines (IL-1β, IFN-ϒ, IL-17A) levels in lung tissue lysates and plasma using standard procedures. Immunohistochemistry of tissue sections assisted in analyses of presence of autoantibodies. Respiratory capacities were measured by plethysmography. The AMPK/PGC-1/SIRT-1 signalling expression were analysed by quantitative PCR.

Results: Significant presence of immune complexes (ICs) in lung tissue sections with high antinuclear antibody (ANA) in serum and enhanced pro-inflammatory cytokine levels in plasma were observed in LM. Lung respiratory capacities were found to be aberrant in LM vs. control. Activity of MCs was also enhanced in LM, suggesting energy stress in lung cells. Mitochondrial anti-oxidant enzymes such as Mn superoxide dismutase (SOD) was elevated in lung mitochondrial and tissue lysates of LM vs. control, however significantly reduced level was observed in plasma of LM as compared to control. AMPK/PGC-1/SIRT-1 signalling axis was significantly high in lungs from LM.

Conclusion: Study points towards connection between elevated mitochondrial activity, AMPK/PGC-1/SIRT-1 expression & autoantibody associated stress in lungs of LM.

P.22 **Investigating the Effects of Ultraviolet Radiation in Manipulating Skin Microbiome-Host Interactions**

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Skin is a major interface with the external environment, acting as a first line of defence against environmental insults including ultraviolet radiation (UVR) as well as participating in a unique dialog with its microbiota. Some effects of UVR on skin are attributed to the induction of interleukin-10 (IL-10) mediated immunosuppression but understanding of how the skin microbiome may manipulate this response remains limited. Here, we investigate the potential skin commensal-dependent modulation of UVR induced immunosuppressive (IL-10) and pro-inflammatory (IL-6/IL-8) mediators. We hypothesise that any modulation may be due to UVR-induced adherence of bacteria to keratinocytes. NHEKs were inoculated with Micrococcus luteus, Staphylococcus hominis or Cutibacterium acnes for 1h to facilitate adherence (1 x 10^2 – 10^6CFU/ml), and consequently exposed to narrowband UVB (erythemally weighted, 20mJ/cm²). Microbial adherence and secretion of IL-10, IL-6 and IL-8 were assessed 24h post-irradiation. Exposure to UVB induced 2.1- ($p<0.01$) and 220.2- ($p<0.05$) fold-increases in M. luteus and S. hominis adherence to NHEKs respectively. M. luteus and C. acnes partially abrogated UVB induced IL-10 secretion, resulting in 37.4% ($p<0.05$) and

19.5% ($p<0.0001$) decreases in UVB induced IL-10 secretion respectively, relative to irradiated controls. This was accompanied by commensal dependent increases in pro-inflammatory mediators for C. acnes (IL-6, $p<0.05$) and M. luteus (IL-8, $p<0.0001$) treated NHEKs, relative to irradiated controls. In conclusion, specific skin commensal bacteria are capable of differentially regulating UVR-induced responses in keratinocytes; perhaps resulting from UVR induced changes in microbial adherence. These data provide novel insight into the contribution of skin commensals to UVR responses.

P.23 **The role of chemokines in driving normal tissue toxicity in radiotherapy-induced bowel inflammation**

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Radiotherapy (RT) is delivered to 60% of cancer patients and is associated with increased cancer survival rates. However, radiation injury to the intestines often leads to acute and chronic inflammation; limiting the dose that can be safely administered and affecting the patient's quality of life. We hypothesise that locally produced chemokines are key in facilitating leukocyte recruitment and that understanding how RT alters chemokine production and drives RT-induced intestinal inflammation will reveal novel therapeutic targets to reduce side effects. A radiation platform (SARRP) was used to deliver CT scan-guided X-rays to the intestine of C57BL/6 and specific receptor knock-out (iCCR and CCR2 KO) mice. Flow cytometry, Luminex and histological analysis were used to characterise and delineate the chemokine-driven mechanism of immune cell recruitment and the implications for radiation-induced toxicity. Local and systemic tissues were harvested at acute and chronic time points post-RT. Flow cytometric analysis of the small intestine revealed a pronounced increase in the innate immune cell compartment at day 7 post-RT with significant elevations in CCL2, CCL3 and CCL11 concentrations. Notably, we demonstrate that a lack of CCR2 increases susceptibility to RT-induced toxicities. We also highlight that local gut irradiation induces a systemic innate immune response in the lungs driven by a similar chemokine-dependent mechanism. We reveal the importance of chemokines in driving RT-induced inflammation and demonstrate that CCR2-mediated leukocyte recruitment is key for minimising RT-induced toxicity. These new insights provide an enhanced understanding of the mechanisms driving radiation-induced side effects and will benefit patients' recovery following radiotherapy.

P.24 **Ym1 as a Regulator of IL-17**

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Ym1 is a key component of the host response to helminth infection. In addition, it is substantially upregulated in many inflammatory settings, being implicated in IL-17 induction and tissue remodelling. Nevertheless, the exact functions and mechanisms of action of Ym1 remain to be defined. Therefore, we generated a Ym1 knockout (KO) mouse line to enable us to assess the different aspects of Ym1 biology. During infection with lung migrating nematode Nippostrongylus brasiliensis, Ym1 KO mice display a reduction in IL-17 producing γδ T cells in the lungs, when compared with their wild-type counterparts. This, alongside a trend towards fewer neutrophils in the BAL of infected Ym1 KO mice, supports a role of Ym1 in the induction of IL-17 and neutrophil recruitment. As most of our current understanding of Ym1 induction of IL-17 stems from type-2 immunity models, we employed exogenous LPS delivery as an additional model to understand the interactions of Ym1, γδ T cells and IL-17 production. Mice treated intraperitoneally with LPS together with Ym1 showed increased recruitment of neutrophils and monocytes to the peritoneal cavity, when compared with those that received LPS alone. Furthermore, Ym1 was found to enhance IL-17 production by γδ T cells when peritoneal exudate cells were stimulated in vitro. Importantly, Ym1 fails to enhance IL-17 production, in vitro, on purified γδ T cells, suggesting it acts through a yet unidentified player. Thus, our results suggest that Ym1 promotes myeloid cell migration into inflamed tissues via indirect enhancement of IL-17 production by γδ T cells.

P.25 **Lung basal cells stimulate the immune system to clear influenza virus then then calm the immune system to repair the damage**

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Despite anti-viral drugs and vaccines, influenza viruses are still poorly controlled and pose a threat to those who suffer chronic diseases such as COPD and asthma. The most dangerous influenza symptoms are caused by damage to the lung tissue, and repairing this damage is essential for survival. Lung repair is driven by activation of epithelial progenitor cells, but their exact role is not well understood. We infected mice with influenza virus and studied the behaviour of epithelial progenitors and consequent effects on the cellular composition of the recovering lung. We found that the epithelial composition of the lung is changed during the peak and recovery phases of influenza. There is a loss of ciliated and alveolar cells at day 6 post infection, but by day 10 these populations are being restored. This recovery correlates with increased activation and proliferation of basal cells. Using differential expression and pathway analysis, we found lung basal cells activation and proliferation is fuelled by a switch to high energy yield oxidative phosphorylation. We show that lung basal cells produce inflammatory cytokines such as TNF; but they also make anti-inflammatory factors such as IκBα. Our findings confirm that lung epithelial precursor activation occurs during recovery from influenza, and the reshaping of the lung after infection is fuelled by a change in

progenitor metabolism. We suggest a careful balance is struck by lung progenitors after infection: they assist in clearing the infection in the short term, while also preparing to recover the damaged epithelium in the long term.

P.26 **The Role of Lipid Metabolism in an Active T Follicular Helper Cell Response**

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

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

P.27 **Microplastics induce a pro-thrombotic phenotype and disrupt cellular integrity of respiratory epithelial cells in vitro**

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Inhaled airborne microplastics have potentially significant health effects, initiating inflammatory processes and inducing cellular dysfunction. Microplastics have been shown to induce morphological and proliferative changes on lung cell lines and are associated with respiratory disease morbidity in vivo. There is an association between disruption of the epithelial cell barrier and perturbation of the immune system. The purpose of this study was to investigate the effects of microplastics on respiratory epithelial cell phenotype and integrity.

A549 respiratory epithelial cells were incubated with TNFα or microplastics at ratios of 5:1, 10:1 or 20:1 microplastics:cells for 24hrs. Cells were harvested and phenotyped using flow cytometry. Further, A549 cells were grown on Transwell® membrane supports and treated as above, integrity was assessed by the movement of Lucifer Yellow into the basolateral chamber.

Results are expressed as Δchanges compared to incubation with media. Incubation with 20:1 microplastics significantly increased both the percentage of cells expressing Tissue Factor (TNFα - 2.86(±1.26)% vs 20:1 3.56(±0.55)%, $P<0.02$) and its expression (TNFα 2.88(±8.15)RFU vs 20:1

(75.15(±7.53)RFU, P<0.0008). Incubation of cells with 20:1 microplastics increased the percentage of cells expressing VE-cadherin (TNFα 2.88(±1.56)% vs 20:1 13.79(±1.33)%, P<0.002). Integrity as Lucifer Yellow OD530nm was significantly decreased upon incubation with all microplastics (TNFα 480.05(±4.85) vs 5:1 (-156.60(±33.16), 10:1 (95.32(±35.73), 20:1 (-441.80(±44.91), all P<0.003).

Microplastics significantly induced a pro-thrombotic respiratory epithelial phenotype, increased the cellular junction adhesion molecule VE-cadherin, perhaps by de novo compensatory synthesis, and decrease cellular integrity. These effects indicate effects on barrier disruption and perturbation of inflammatory responses requiring further investigation.

P.28 **Allergic Airway Matrix Remodelling Develops Independently of Type 2 Inflammation**

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Asthma is a global health concern which affects ~300 million people worldwide. Symptoms are associated with airway inflammation alongside remodelling of the local extracellular matrix (ECM). New therapeutics such as monoclonal antibodies to IL4, IL13, and IL17 have been trialled with promising results. However, studies have often ignored matrix remodeling, focussing instead on readouts of inflammatory resolution and amelioration of symptoms.

We have utilised a mouse model of chronic allergen exposure, involving the coadministration of three clinically relevant allergens over several weeks. This model recapitulates many characteristics of steroid-resistant Asthma in humans. including mixed Th2/Th17 inflammation and remodelling of the pulmonary ECM involving smooth muscle cell hyperplasia, and collagen and hyaluronan deposition around the large airways.

Utilising both Th2 and Th17 deficient mice we found that pulmonary neutrophil and eosinophil recruitment was deficient in the absence of both IL-17a and IL-13, analogous with the efficacy of targeting these pathways in humans. Surprisingly, we found no change in the airway ECM composition in either strain, despite clear differences in mucus production and smooth muscle hypertrophy.

Together our data suggest that in this model airway remodelling progresses independently of the Th2/Th17 immune response. These results highlight a potential separation between the inflammatory progression and alterations in the ECM leading to impaired lung function. Understanding how inflammation and remodelling coordinate in Asthma will be key to developing effective therapeutics and understanding the side effects of current treatments.