

Tissue Immunology 2023

Friday 7th July 2023
Holiday Inn Bloomsbury, London

08:15 Registration

09:00 **Welcome address & Polling for 2024 meeting**
George Finney, University College London, UK
Laura Pallett, University College London, UK

SESSION 1 – T cells in the tissue

Chairs: Stephanie Kucykowicz, University College London, UK, George Finney, University College London, UK
and James Harker, Imperial College London, UK

9:15 **Tissue T cells: Lessons from life in lockdown**
Mala Maini, University College London, UK – *invited speaker*

09:45 **The role of type I interferons in the generation of tissue-resident memory CD8+ T cell responses during RSV infection**
Joy Nakawesi, Imperial College London, UK – *selected short talk*

10:00 **Putative intestinal-derived resident memory T cells can be identified in human blood (ex-Trm) and are altered in Crohn's disease**
Beverley Rodger, Queen Mary University of London, UK – *selected short talk*

10:15 Flash Talks

Tumour-infiltrating double negative B cells give rise to regulatory plasma cells in response to Toll-like receptor 7 signals in renal cell carcinoma
Zara Baig, University College London, UK

Simultaneous CD38 and PD-1 blockade eliminates tissue-resident memory T cells in ART1+ murine non-small cell lung cancer
Ricardo Sainz, The Institute of Cancer Research

Exploring Novel Approaches for Developing Immunotherapeutic Interventions in a Mouse Model of Colorectal Cancer
Jake Scott, Cardiff University, UK

Efferocytes Release Extracellular Vesicles to Promote Inflammation Resolution via Prosaposin-GPR37 signalling
Manikandan Subramanian, Queen Mary University of London, UK

10:35 **Regulatory T cells in tissue**

Adrian Liston, Babraham Institute, Cambridge, UK – *invited speaker*

11:05 Refreshment break and meet the exhibitors

SESSION 2 – Innate and adaptive immunity in the tissue

Chairs: Fränze Prohatzky, The Francis Crick Institute, UK, Roel de Maeyer, University of Oxford, UK and Will Traves, Imperial College London, UK

11:30 **Pivotal roles for type 2 immunity in epithelial immune surveillance**
Jessica Strid, Imperial College London, UK - *invited speaker*

12:00 **Viral-associated COPD exacerbations drive ILC subset plasticity and an ILC1-favoured microenvironment**
Kyle Mincham, Imperial College London, UK – *selected short talk*

12:15 **Flash Talks**

Utilising Vitamin D3 to enhance antigen-specific immunity in the skin and lung of older adults
Emma S Chambers, Queen Mary University of London, UK

Overzealous degradation of bioactive collagen fragment Pro-Gly-Pro by leukotriene A4 hydrolase (LTA4H) perpetuates fibrosis in Idiopathic Pulmonary Fibrosis (IPF)
Kornelija Suveizdytė, Imperial College London, UK

The role of innate signaling pathways on initial responsiveness to house dust mite exposure and their link to development of allergic airway inflammation
Anne-Marie Teresa Cosima Levins, Imperial College London, UK

Decoding host-microbiome interactions that regulate cancer immunity. Killing cancer with a gut instinct
Evangelos Giampazolias, CRUK Manchester Institute, UK

12:35 **Utilising epigenetics to understand macrophage ontogeny**
Naomi McGovern, University of Cambridge, UK – *invited speaker*

13:05 Lunch, poster viewing and meet the exhibitors (poster viewing from 13:50–14:45)

SESSION 3 – Multi-tissue immunology

Chairs: Kyle Mincham, Imperial College London, UK, Louisa James, Queen Mary University of London, UK and Katie Flaherty, Kings College London, UK

14:45 **Teasing out the T cells in the inflamed joint**
Leonie Taams, Kings College London, UK – *invited speaker*

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- 15:15 **Macrophage-secreted DNase prevents neutrophil extracellular trap mediated impairment of efferocytosis in atherosclerosis**
Umesh Kumar Dhawan, Queen Mary University of London, UK - *selected short talk*
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- 15:30 **Adventitial mast cell activation causes pericyte loss and vascular remodelling during early life asthma**
Régis Joulia, Imperial College London UK - *selected short talk*
-
- 15:45 **CD14 marks tissue CD8+T-cells instructed by myeloid cells and modulated by LPS**
Laura Pallett, University College London, UK - *selected short talk*
-
- 16:00 **The changing landscape of tissue immunity over age**
Donna Farber, Columbia University, USA – Keynote speaker
-
- 16:30 **Polling for 2024 meeting** and Closing Remarks – Laura Pallett, University College London, UK
-
- 16:45 Networking reception
-
- 19:00 Close
-

Poster Presentations

P.01 Neonatal skin Tregs regulate melanocyte function via PPAR γ pathway

Inchul Cho, King's College London, UK

P.02 Too big to fail: How do lung progenitors repair the lung after influenza virus infection?

Patrick Shearer, University of Glasgow, UK,

P.03 Lung antigen presenting cell-CD 4^+ T cell interactions are required for the optimal generation of influenza virus specific memory CD 4^+ T cells

Megan KL MacLeod, University of Glasgow, UK,

P.04 Understanding Dynamic Immune Responses within 3^{D} in vitro Human Skin Models

Sarah Hindle, Centre for Cell Biology and Cutaneous Research, Blizard Institute, Queen Mary University of London, UK

P.05 Therapeutic STm Δ aroA preferentially invade proliferating cells in colorectal cancer

Gillian Mackie, University of Birmingham, UK

P.06 Type I interferons induced upon RSV infection change the lung microenvironment and impair seeding of lung metastatic breast cancer cells

Ana Farias, Respiratory Infections Section, National Heart and Lung Institute, Imperial College London, UK

P.07 The induction of IL- 10 producing B regulatory cells leads to immunotolerance recovery with grass pollen subcutaneous immunotherapy

E. Palmer, Imperial College London, United Kingdom and NIHR Imperial Biomedical Research Centre, United Kingdom

P.08 Senescent cytotoxic CD 4^+ T cells cause skin pathology in human cutaneous leishmaniasis

Senescent cytotoxic CD 4^+ T cells cause skin pathology in human cutaneous leishmaniasis

Luciana Polaco Covre, Núcleo de Doenças Infecciosas, Universidade Federal do Espírito Santo, Vitoria, Brazil and Division of Medicine, University College London, London, United Kingdom

P.09 A putative piglet model of environmental enteric dysfunction in infants: an exploration of immune development and gut barrier function

Zeynep Hayirli, Department of Food and Nutritional Sciences, University of Reading, UK

P.10 Long-term lung inflammation after SARS-CoV-2 infection of mice

Sophie Guan, Imperial College London, UK,

P.11 Investigating the developmental dynamics of TRM cells across organs using a novel Ki 67 fate reporter mouse strain

Jodie Chandler, University College London, UK

P.12 The Evolution of a Transplantable Tumour

Ahmed Rokan, Division of Infection & Immunity and Institute of Immunity and Transplantation, the Pears Building, University College London, UK

P.13 Tissue-specific dysregulation of the immune system in Crohn's disease

Lucia Ramirez-Navarro, Wellcome Sanger Institute, UK

P.14 Stable neonatal natural Bregs contribute to immune regulation in adulthood.

Anna Vallduriola Martin, Institute of Immunology and Transplantation, Royal Free Hospital, University College London, United Kingdom

P.15 Deciphering the tissue B cell landscape

Isabella Withnell, University College London, UK

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SONY



Selected talks abstracts

The role of type I interferons in the generation of tissue-resident memory CD8⁺ T cell responses during RSV infection

Joy Nakawesi¹, Augusto Varese², Freja C M Kirsebom¹, Michelle Paulsen¹, Ana Farias¹, Rinat Nuriev¹, and Cecilia Johansson¹.

¹ Imperial College London, UK, ² Instituto de Investigaciones Biome´dicas en Retrovirus y SIDA (INBIRS), Facultad de Medicina Universidad de Buenos Aires, Argentina.

Respiratory viruses, most commonly respiratory syncytial virus (RSV), constitute major burdens on healthcare systems. RSV causes severe lower respiratory tract infections, especially in children and the elderly. Tissue-resident memory T cells (TRMs) are key players in memory responses at mucosal surfaces and play a central role in protecting from reinfection by respiratory viruses in mice and humans. In human experimental RSV infection, the presence of lung CD8⁺ TRMs correlates with a better outcome, however, the requirements for adequate lung TRMs responses during RSV reinfection are not fully understood. Type I IFNs are one of the early drivers of inflammation during viral infections and are critical anti-viral cytokines with pleiotropic effects.

We use mouse models to assess the role of type I IFNs in the generation and subsequent expansion of the TRMs pool during RSV infection.

We show that lung resident CD8⁺ TRM cells expand independently from systemic CD8⁺ T cells after RSV reinfection. Reinfected MAVS, MyD88/TRIF, and IFNAR1 deficient mice lacking key components involved in innate immune recognition of RSV and induction of type I IFNs, display impaired expansion of CD8⁺ TRM cells. IFN- α treatment of MAVS deficient mice during primary RSV infection restores the TRM cell expansion. Furthermore, bone-marrow chimeric mice show that the effect of type I IFNs on the generation of CD8⁺ TRMs is both via stromal and bone marrow-derived cells. Our data reveal how the axis controlling type I IFN induction instructs and regulates CD8⁺ TRM cell responses to RSV infection.

Putative intestinal-derived resident memory T cells can be identified in human blood (ex-Trm) and are altered in Crohn's disease

Beverley Rodger, Inva Hoti, Eve Hornsby, Hannah Gordon, Amy Lewis, Andrew Silver, James Lindsay, Andrew Stagg

Queen Mary University of London, UK

Tissue-resident memory T-cells (Trm) persist in tissues and can contribute to inflammation. Trm can re-enter the circulation (ex-Trm), giving rise to new effector and Trm populations. Human skin-derived ex-Trm can be identified based on co-expression of the residency marker CD103 and cutaneous-leukocyte antigen (CLA), a skin-tropism marker. Based on this, we hypothesised that intestinal-derived ex-Trm are CD103⁺a4b7⁺ and are altered in patients with inflammatory bowel disease (IBD).

PBMCs and colonic cells were obtained from healthy volunteers and IBD patients (Crohn's disease or ulcerative colitis), and analysed by multi-colour flow cytometry and single-cell RNA sequencing (scRNAseq).

Over 80% of colonic αT-cells were CD69+ Trm in health and IBD. Unlike CD4+ Trm, CD8+ Trm comprised CD103+ and CD103- subsets, with CD69+CD103- cells significantly reduced in IBD. Trm from inflamed colonic tissue were more stem-like, with increased expression of tissue-egress molecules. Putative gut-derived ex-Trm were identified amongst TCRα+CD45RA- blood cells as a b7++CD103+ population, indicative of cells expressing both αb7 and CD103(αE)b7 integrins. Gut-derived and skin-derived (CLA+CD103+) ex-Trm shared a characteristic phenotype. Gut ex-Trm were significantly reduced in patients with Crohn's disease but not ulcerative colitis. scRNA-seq analysis of gut ex-Trm revealed increased expression of cytotoxicity-associated genes in Crohn's disease.

Inflamed IBD intestinal tissue is associated with altered CD8+ Trm distribution and a stem-like Trm phenotype. Gut-derived ex-Trm are reduced in Crohn's disease; the recruitment of ex-Trm could explain the patchy nature of Crohn's disease inflammation. The maintenance of residence and generation of ex-Trm may provide new therapeutic targets.

Viral-associated COPD exacerbations drive ILC subset plasticity and an ILC1-favoured microenvironment

Kyle T. Mincham, Garance F. M. Meyer, Nicoletta Bruno, Aran Singanayagam, Robert J. Snelgrove
Imperial College London, UK

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory disease of the lungs and 3rd leading cause of death worldwide. Cigarette smoking remains the most definitive risk factor for disease onset. Patients with COPD are prone to viral-driven exacerbations. Innate lymphoid cells (ILCs) are early effector cells, with fundamental roles in tissue homeostasis and immunity, but whose dysfunction is increasingly implicated in chronic lung disease pathology. ILCs are classified into 3 major subsets, ILC1, ILC2 and ILC3. However, ILCs exhibit dramatic phenotypic and functional plasticity in response to the local inflammatory milieu.

To recapitulate early COPD developmental processes and end-stage emphysematous disease, mouse models of acute/chronic cigarette smoke or elastase exposure respectively were employed. Cigarette smoke and elastase-treated mice were subsequently exposed to Poly(I:C) or live rhinovirus (RV1B) respectively. Full-spectrum flow cytometry was utilised for high-resolution ILC evaluation, to accurately capture the true heterogeneity of ILCs.

Total ILCs were elevated in the lung and airways in both models, with a heightened propensity for ILC1 expansion at the expense of ILC2s. Poly(I:C)-induced respiratory challenge was characterised by further evidence of ILC2-to-ILC1 transition, with lung gene expression analysis revealing a microenvironment supportive of enhanced ILC1 responsiveness. Moreover, high-dimensional unbiased analysis of spectral cytometry datasets revealed the true plasticity of ILCs following RV1B-induced exacerbation of elastase treated mice, with the expansion of an ILC2-to-ILC1 transitional subset within the lungs.

Overall, exaggerated ILC1 responsiveness represents a prominent feature of COPD-associated pulmonary inflammation, which may be enhanced by an ILC2-to-ILC1 switch following acute viral exacerbation.

Macrophage-secreted DNase prevents neutrophil extracellular trap mediated impairment of efferocytosis and promotes atherosclerosis progression.

Umesh Kumar Dhawan¹, Hanna Englert², Manikandan Subramanian¹

¹Centre for Biochemical Pharmacology, William Harvey Research Institute, Queen Mary University of London, UK, ² Institute for Clinical Chemistry and Laboratory Medicine, Laboratory of Molecular Inflammation, University Medical Centre Hamburg, Germany

Neutrophil extracellular traps (NETs) are potent damage-associated molecular patterns that elicit robust inflammation and immune cell activation and are associated with the progression of several chronic inflammatory diseases such as atherosclerosis. We recently showed that NETs in systemic circulation are cleared by release of DNase1 and DNase1L3 following active sensing by liver and intestine respectively. However, how NETs generated within an atherosclerotic plaque are cleared locally is not understood. Using a mouse model of hematopoietic cell-specific knockout of DNase1/DNase1L3, we demonstrate a critical role for lesional macrophage-secreted DNase1/DNase1L3 in the clearance of NETs within atherosclerotic plaques. Additionally, we show that lipid-mediated endoplasmic reticulum stress in macrophages dysregulates the release of NET-induced DNase in a PERK-ATF4 dependent pathway with consequent impairment in clearance of lesional NETs. From a pathological perspective, we show that the accumulated NETs activate a HMGB1-TLR4-ADAM17 dependent signalling cascade that culminates in cleavage and shedding of the efferocytosis receptor Merck with consequent impairment in clearance of apoptotic cells resulting in exacerbated atherosclerotic plaque progression.

Adventitial mast cell activation causes pericyte loss and vascular remodelling during early life asthma

Régis Joulia, Franz Puttur, Lewis Entwistle, Helen Stölting, Anastasia Voitovich, Simone Walker, Laura Yates, Segal Saglani, Clare Lloyd

NHLI, Imperial College London, London, UK

Mast cells (MCs) are crucial effector cells during tissue immune responses and central to allergic airways diseases (AAD) in adults, however their importance during early life remains to be investigated.

AAD was induced in 7-day-old pups by repeated exposure to house dust mite (HDM) over 3 weeks. Precision cut lung slices (PCLS) were employed to investigate the localisation and activation of MCs during early life AAD.

We observed that neonatal lungs exhibited two MC populations with an abundant presence of connective type (m-MCP6+) MCs and a minor mucosal (m-MCP1+) MC population. These cells were strongly associated with large airways and blood vessels in adventitial regions. The distribution of MCs changed during AAD with an increased number of mucosal MCs but no difference in connective MCs. However, only connective MCs were highly activated, indicated by the presence of extracellular MC granules. Interestingly, we showed that MC granules bind to structural cells surrounding blood vessels called pericytes. Upon interaction with MC granules, pericytes contract their cell protrusions leading to destabilisation of the pericyte/endothelial cell interaction, ultimately leading to vascular loss. Finally, we employed spatial transcriptomic in endobronchial biopsies of children with asthma

and defined enrichment in MC protease genes in vessel rich areas suggesting change in the lung vasculature.

In summary, our data provide a detailed mapping of the organisation of MCs during early life. Moreover, AAD leads to activation of connective type MCs and ultimately vascular loss in adventitial areas. The latter suggests short- and long-term detrimental effects on lung function.

CD14 marks tissue CD8+T-cells instructed by myeloid cells and modulated by LPS

Laura J. Pallett¹, Leo Swadling¹, Mariana Diniz¹, Alexander A. Maini², Marius Schwabenland³, Adria Dalmau Gasull³, Jessica Davies¹, Stephanie Kucykowicz¹, Jessica K. Skelton⁴, Niclas Thomas¹, Nathalie M. Schmidt¹, Oliver E. Amin¹, Upkar S. Gill⁵, Kerstin A. Stegmann¹, Alice R. Burton¹, Emily Stephenson⁶, Gary Reynolds⁶, Matt Whelan¹, Jenifer Sanchez⁷, Roel de Maeyer², Clare Thakker¹, Kornelija Suveizdyte¹, Imran Uddin¹, Ana M. Ortega-Prieto⁴, Charlotte Grant⁸, Farid Froghi⁸, Giuseppe Fusai⁸, Sabela Lens⁹, Sofia Pérez-del-Pulgar⁹, Walid Al-Akkad¹⁰, Giuseppe Mazza¹⁰, Mahdad Noursadeghi¹, Arne Akbar², Patrick T. F. Kennedy⁵, Brian R. Davidson^{8,10}, Marco Prinz³, Benjamin M. Chain¹, Muzlifah Haniffa⁶, Derek W. Gilroy², Marcus Dorner⁴, Bertram Bengsch³, Anna Schurich⁷ & Mala K. Maini¹

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Background and Aims: The liver is bathed in bacterial products, including lipopolysaccharide transported from the intestinal portal vasculature, but is able to maintain a state of tolerance that is then exploited by persistent pathogens and tumours. The cellular basis mediating this tolerance, yet allowing a switch to immunity or immunopathology, needs to be better understood for successful immunotherapy of liver diseases.

Method: We analysed the phenotype and function of CD14-expressing CD8 T cells directly ex vivo from resected/explanted human liver and explored their derivation, functionality, expansion and LPS-responsiveness in multiple in vitro and in vivo models.

Results: Here we show that a variable proportion of CD8+ T cells compartmentalized in the human liver co-stain for CD14 and other prototypic myeloid membrane proteins and sit in close proximity to CD14^{high} myeloid cells in the liver. CD14⁺CD8⁺ T cells exhibit increased turnover, activation and constitutive immunomodulatory features with high homeostatic IL-10 and IL-2 production ex vivo, and enhanced antiviral/anti-tumour effector function after TCR engagement. Whereas stimulation via CD14 by bacterial lipopolysaccharide not only increases the frequency of CD14⁺CD8⁺ T cells in vitro and in vivo, but skews their function towards the production of chemotactic and regenerative cytokines. This CD14⁺CD8⁺ T cell profile seen ex vivo in tissues, can be recapitulated by the acquisition of membrane proteins—including the lipopolysaccharide receptor complex—from mononuclear phagocytes, resulting in augmented tumour killing by TCR-redirected T cells in vitro.

Conclusion: A proportion of CD8 T cells compartmentalised in the liver express CD14/TLR4/MD2, recapitulated in vitro by membrane acquisition from mononuclear phagocytes. Thus, bacterial products in the gut-liver axis and tissue stromal factors can fine tune liver immunity by driving myeloid instruction of CD8+ T cells with immunomodulatory ability.

Flask talks abstracts

Tumour-infiltrating double negative B cells give rise to regulatory plasma cells in response to Toll-like receptor 7 signals in renal cell carcinoma

Zara Baig¹, Hannah Bradford¹, Chris Piper¹, Thomas Mitchell², Hans Stauss¹, Maxine Tran³, Claudia Mauri¹

¹ Institute of Immunity and Transplantation, Pears Building, Royal Free Hospital, University College London, UK, ² Wellcome Trust Sanger Institute, Wellcome Genome Campus, UK, ³ Department of Surgical Biotechnology, Royal Free Hospital, University College London, UK

In renal cell carcinoma (RCC), high B cell infiltration and regulatory B cells (Bregs) have been linked to worse prognosis, while B cell-rich tertiary lymphoid structures have been associated with improved response to immunotherapy. To better delineate the pro-tumour versus anti-tumour functions of B cells in RCC, we combined single cell RNA-sequencing with spectral flow cytometry to comprehensively characterise the B cell landscape in RCC and matching background kidney, tumour margin and blood. Our analysis revealed 11 B cell clusters with a significant expansion in CD21+CD11c- double negative (DN) 1, CD21-CD11c- DN3 B cells and plasma cells in the tumour compared to background kidney and blood. Differential gene expression analysis revealed significant enrichment for the Toll-like receptor (TLR) signalling pathway specifically in DN1 B cells and in vitro TLR7 and TLR9 stimulation resulted in DN1 expansion and differentiation into IL-10+ DN regulatory B cells and IL-10+ regulatory plasma cells. This was supported by trajectory analysis which confirmed that DN1 are the precursor of plasma cells in the tumour only. IL-10+ B cells and IL-10+ plasma cells were located at the edges of tertiary lymphoid structures (TLS), suggesting extrafollicular differentiation. Our findings propose that DN1 tumour B cells may respond to innate TLR stimulation at the TLS by upregulating IL-10 and undergoing extrafollicular differentiation into regulatory plasma cells. The depletion of DN1 B cells or inhibition of the TLR7 and TLR9 pathways in RCC may be an attractive therapy to prevent Breg polarisation and alleviate suppression at tertiary lymphoid structures.

Simultaneous CD38 and PD-1 blockade eliminates tissue-resident memory T cells in ART1+ murine non-small cell lung cancer

Ricardo M. Sainz, Erik Wennerberg

The Institute of Cancer Research, UK

Immune checkpoint inhibitors (ICI) targeting PD-1/PD-L1 have been shown to promote anti-tumour immunity in melanoma and non-small cell lung cancer (NSCLC). Unfortunately, for NSCLC patients the anti-tumour responses are transient. CD38 is an ectoenzyme that catabolises extracellular NAD into precursors for immunosuppressive adenosine generation. CD38 blockade has been clinically tested in combination with PD-1 inhibitors in NSCLC patients based on the rationale that reduced

adenosine generation could potentiate the ICI effect. However, this combination has consistently failed in NSCLC patients. Recently, tumour expression of ADP-ribosyltransferase-1 (ART1) was characterised as an additional immune resistance mechanism in NSCLC. ART1 uses free NAD as a substrate to mono-ADP-ribosylate the P2X7 receptor primarily on tissue-resident memory T cells (TRM) leading to their elimination by NAD-induced-cell death (NICD). We hypothesise that, in ART1-expressing tumours, T cell-expressed CD38 plays a cytoprotective role by catabolizing free NAD, thus counteracting ART1-mediated NICD of TRMs. This could potentially explain the lack of synergy between anti-CD38 and anti-PD-1 treatments in NSCLC patients. C57BL/6J mice were inoculated intravenously with KP1-WT (low ART1 expression) or KP1-ART1OE murine lung adenocarcinoma cells with constitutive ART1 overexpression. Both groups of mice received systemic anti-CD38 and/or anti-PD-1. In the KP1-ART1OE group, CD8+ TRM and CD4+ TRM were 1.8 times and 2.0 times lower in aPD-1+aCD38 treatment vs aPD-1 treatment alone ($p < 0.05$) respectively. No significant differences in TRMs were observed in the KP1-WT group. Our data indicate that CD38 plays dual roles in the immune regulation of T cells where its cytoprotective effects dominate in ART1-expressing tumours.

Exploring Novel Approaches for Developing Immunotherapeutic Interventions in a Mouse Model of Colorectal Cancer

Jake Scott¹, Mr. James Geary¹, Dr. Sarah Hulin-Curtis¹, Professor Owen Sansom², Professor Andrew Godkin¹ Professor Awen Gallimore¹

¹ Cardiff University, UK, ² University of Glasgow, UK

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in the developed world. FoxP3+ regulatory T cells (Tregs) are increased in frequency in CRC, whilst there is some evidence that Tregs may limit cancer growth by controlling inflammation in the early stages, there is a body of evidence indicating that they inhibit T cell responses to cancer antigens, helping cancers evade immune attack. In a clinical study carried out by our lab, it was found that treatment of patients with cyclophosphamide (CY) selectively depleted Tregs and correlated with improved T cell (IFN- γ) responses to the cancer associated antigen 5T4. Additionally, vaccination with a modified vaccinia Ankara Virus expressing 5T4 (MVA-5T4) also induced robust anti-5T4-IFN- γ T cell responses.

To address unanswered questions arising from these findings and the limitations associated with pre-clinical models of CRC, it was first necessary to establish successful colonoscopy-guided implantation of CRC cells into the colonic submucosa of mice. Administration of low-dose metronomic CY depleted Tregs resulting in regression of MC-38 tumours by T cell dependent mechanisms and partial regression of KPN-organoid derived tumours. In parallel, building on the identification of a novel cancer associated antigen in patients with CRC (hDNAJB7), produced a recombinant adenoviral (rAd) vector expressing hDNAJB7. The immunogenicity of the rAd-hDNAJB7 vector was demonstrated through homologous prime/boost immunisation of mice and a single immunodominant, MHC class I H2-Db restricted epitope was identified. Future experiments will evaluate the anti-tumour effects of vaccination as a monotherapy or in combination with low-dose CY.

Efferocytes Release Extracellular Vesicles to Promote Inflammation Resolution via Prosaposin-GPR37 signalling

Purbasha Bhattacharya ^{1,2}, Umesh Kumar Dhawan ³, Mohammed Tayab Hussain ³, Praveen Singh ^{1,2}, Karran Kiran Bhagat ³, Aarushi Singhal ³, Shani Austin-Williams ³, Shantanu Sengupta ^{1,2}, Manikandan Subramanian ^{2,3}

¹ CSIR – Institute of Genomics and Integrative Biology, India; ² Academy of Scientific and Innovative Research (AcSIR), India; ³ William Harvey Research Institute, Faculty of Medicine and Dentistry, Queen Mary University of London, UK

The phagocytic clearance of dying cells by macrophages using a specialised process called efferocytosis is critical for successful resolution of inflammation. Efferocytes release several soluble mediators to effect intercellular communication and resolve inflammation following tissue injury. However, whether efferocytes release extracellular vesicles (EV) such as exosomes and their role in the resolution of inflammation is unknown and represents a critical knowledge gap. Herein, we demonstrate that efferocytes release EVs (Effero-EVs) which skews macrophages towards a pro-resolving M2 phenotype. Importantly, Effero-EVs increase the efferocytosis efficiency of naïve macrophages in a feed-forward mechanism both in vitro and in several murine models of inflammation in vivo. Mechanistically, we show that Effero-EVs are enriched in prosaposin (PSAP) which interacts with GPR37 on naïve macrophages to increase the expression of the efferocytosis receptor Tim4 via an ERK-AP1 signalling cascade. Using siRNA-mediated knockdown and chemical inhibitors of specific GPCR signalling, we validated the relevance of the identified pathway in vivo. From a translational perspective, we demonstrate that Effero-EVs have therapeutic potential since the administration of Effero EVs to a murine model of advanced atherosclerosis led to increased lesional macrophage efferocytosis efficiency, decreased plaque necrosis, and decreased vascular inflammation suggesting an overall improvement in atherosclerotic plaque stability. These data demonstrate that Effero-EVs play a critical role in inflammation resolution in vivo and can be developed as a therapeutic agent for the management of atherosclerosis and other chronic inflammatory diseases.

Utilising Vitamin D3 to enhance antigen-specific immunity in the skin and lung of older adults

Emma S Chambers

Blizard Institute, Queen Mary, University of London, UK

Introduction: The UK has an ageing population. Ageing is associated with increased morbidity and mortality from infections, decreased vaccine efficacy and increased systemic inflammation termed inflammageing. Vitamin D insufficiency is more common in older adults and has been associated with frailty and increased inflammation. Vitamin D insufficiency has been linked with increased incidences of lung infections such as COVID-19 - which older adults experience most morbidity and mortality. In addition, vitamin D insufficiency is associated with reduced antigen-recall responses in the skin of older adults. Collectively this data suggests that vitamin D supplementation may be a way to enhance immunity in older adults.

Objective: The aim of this work was to determine if Vitamin D3 supplementation could boost cutaneous and lung-specific antigen-specific immunity in older adults.

Methods: Two human studies were undertaken. Firstly, a clinical study to determine if Vitamin D3 supplementation could boost antigen-specific immunity in the skin of older adults (≥65 years). Secondly, a clinical trial to determine if Vitamin D3 supplementation could reduce COVID-19 breakthrough infection and enhance COVID-19 vaccination immunogenicity.

Results: We showed that Vitamin D3 supplementation significantly increased cutaneous antigen-specific immunity in older adults. This enhancement was associated with reduced inflammatory cytokine production from inflammatory monocytes recruited to the skin. Conversely, Vitamin D3 supplementation did not reduce COVID-19 breakthrough infection and did not enhance COVID-19 vaccine immunogenicity.

Conclusions: Vitamin D3 supplementation significantly enhanced cutaneous but not lung antigen-specific immunity – suggesting a skin-tropic role for Vitamin D3.

Overzealous degradation of bioactive collagen fragment Pro-Gly-Pro by leukotriene A4 hydrolase (LTA4H) perpetuates fibrosis in Idiopathic Pulmonary Fibrosis (IPF)

Kornelija Suveizdytė, Dhiren F. Patel, Chloe J. Pyle, Patricia P. Ogger, Nicoletta Bruno, Kyle T. Mincham¹, Philip L. Molyneaux¹, Toby M. Maher¹, Adam J. Byrne, Robert J. Snelgrove

Imperial College London, UK

Introduction: Proline-Glycine-Proline (PGP) is a collagen-derived tripeptide liberated during inflammation, capable of promoting both neutrophil recruitment and airway epithelial cell proliferation. During acute, self-resolving inflammation, PGP is readily degraded by LTA4H to limit its bioavailability. A failure to efficiently degrade PGP results in chronic neutrophilia and pathological airway epithelial remodelling in chronic lung diseases (CLD) such as asthma, chronic obstructive pulmonary disease. However, the PGP-LTA4H axis has not been investigated in IPF – a CLD characterised by aberrant alveolar epithelial repair and ensuing fibrosis.

Methods: PGP-LTA4H axis was characterised in bronchoalveolar lavage of 69 IPF and 17 control individuals. The role of PGP in alveolar epithelial repair was interrogated by scratch assays and its role in driving the pathology was interrogated in a mouse model of bleomycin fibrosis utilising *Ita4h*^{-/-} animals, and administration of exogenous PGP.

Results: Contrary to other CLDs, PGP was undetectable in IPF patients and in bleomycin treated mice owing to its overzealous degradation by high levels of LTA4H. In addition, PGP was also demonstrated to promote alveolar epithelial repair *in vitro*. Studies in bleomycin treated *Ita4h*^{-/-} mice suggested that LTA4H functioned to limit neutrophilic inflammation early after bleomycin treatment but enabled aberrant epithelial repair during the fibrotic phase. Accordingly, administration of PGP concomitantly with bleomycin treatment to wild type mice resulted in augmented neutrophilia. Conversely, delayed treatment with PGP during the fibrotic phase ameliorated lung fibrosis.

Conclusions: PGP promotes alveolar epithelial repair, whilst overzealous degradation of PGP by LTA4H potentially underlies aberrant epithelial repair and fibrosis in IPF patients.

The role of innate signaling pathways on initial responsiveness to house dust mite exposure and their link to development of allergic airway inflammation

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Atopic asthma arises following development of hypersensitivity to particular inhaled antigens, whereby subsequent challenges cause characteristic allergic airway inflammation (AAI) in the lung. Toll-like receptor (TLR) and RIG-I-like receptor (RLR)-signalling pathways are synchronous pathways that contribute to expression and regulation of type I interferons, amongst other cytokines and chemokines. MyD88/TRIF and MAVS are key adaptor proteins for downstream signalling of the TLR- and RLR pathways, respectively. Using adult mice (wildtype (wt), Mavs^{-/-} or Myd88/Trif^{-/-}) challenged with 25µg of house dust mite (HDM; *D. pteronyssinus* extract), we studied the role of innate signalling pathways on HDM sensitisation (single challenge) and subsequent AAI development (multiple challenges). Additionally, cultured alveolar macrophages (mex-AMs) were exposed to HDM and their cytokine release was measured. In all experimental models, HDM-challenged Myd88/Trif^{-/-} mice or mex-AMs did not show responsiveness to HDM. Alternatively, in the model of allergen sensitisation, Mavs^{-/-} mice demonstrated similar responsiveness to HDM as wt mice. However, in the AAI model, Mavs^{-/-} HDM challenged mice exhibited lower Th2/Th17 responses but higher IgE responses than wt mice. Furthermore, Mavs^{-/-} mice showed enrichment of alveolar macrophage populations after multiple HDM challenges. These results demonstrate that MyD88/TRIF dependent TLR-signalling is essential for macrophage responsiveness and AAI induction after HDM challenge. However, also the RLR signalling pathways have a notable role in altering macrophage, Th17/Th2 and B cell responses following allergen challenge.

Decoding host-microbiome interactions that regulate cancer immunity. Killing cancer with a gut instinct

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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, we report the serendipitous finding that mice with enhanced vitamin D (vitD) tissue 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 gut 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.

Poster abstracts

Neonatal skin Tregs regulate melanocyte function via PPAR γ pathway

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Background: In adult skin, regulatory T cells (Tregs) modulate the behaviour of hair follicle stem cells. However, a role for early life skin-seeding Tregs in facilitating tissue homeostasis is unknown.

Methods: To assess neonatal Treg function, we utilised Foxp3-DTR transgenic mice to undergo three depletion regimens during postnatal days of life (PD): early Treg depletion on P6, P8 (EDT), late Treg depletion on P10, P12 (LDT), and full Treg depletion on P6-P12 (FDT). Skin histopathology was assessed, and immunophenotyping performed via flow cytometry. Whole skin bulk RNA-sequencing and in situ transcriptomics were performed. Functional rescue studies were performed by administration of small molecule modulators of the PPAR γ pathway.

Results: Normal skin pigmentation failed to develop in EDT and FDT animals, but not LDT. We did not observe any increase in immune cell numbers after EDT regimen. Whole skin bulk RNA-seq was performed immediately after the depletion regimens. Melanocyte marker genes, such as Dct, and PPAR γ target genes were downregulated only after EDT and FDT. In situ transcriptomics showed downregulation of PPAR γ target genes in the hair follicles, where melanocytes reside. Small molecule PPAR γ agonists rescue pigmentation in Treg-depleted mice. Finally, scRNA-seq data of human melanocytes showed differential expression associated with developmental stage and diseased states.

Conclusions: We report that P6-P8 represents a critical window for Treg-melanocyte axis that defines the development of pigment-producing melanocytes. We identify the PPAR γ pathway as a target of early life Tregs in exerting their function on regulation and development of melanocytes in skin.

Too big to fail: How do lung progenitors repair the lung after influenza virus infection?

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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 restored. This recovery correlates with increased activation and proliferation of progenitor cells. Using differential expression and pathway analysis, we found lung basal cells activation and proliferation is fuelled by a switch from lipid metabolism to high energy yield oxidative phosphorylation. 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.

Lung antigen presenting cell-CD⁴ T cell interactions are required for the optimal generation of influenza virus specific memory CD⁴ T cells

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Influenza A virus (IAV) infection drives the formation of mucosal memory CD4 T cells that can protect the host from re-infection. An indepth understanding of the signals involved in the formation of these cells is required for us to design vaccines that can direct the generation of protective memory cells. Following IAV infection of mice, lung IAV-specific memory CD4 T cells persist in airway-proximal clusters. Clusters, also containing B cells, dendritic cells, and macrophages, reduce in size from day 10 to 40 post-infection. Despite this reduction, the proportion of lung IAV specific CD4 memory T cells within clusters increases, suggesting clusters support memory cell maintenance. Blockade of T cell receptor-peptide MHC signals by intranasal delivery of anti-MHCII at days 6 and 12 did not reduce the number of lung memory CD4 T cells but did reduce the overall size of clusters and the number of CD4 T cells within clusters. Anti-MHCII treatment also reduced the ability of immunodominant IAV-specific CD4 T cells to produce the anti-viral cytokine, interferon-gamma. In contrast, analysis of a polyclonal population of IAV specific CD4 T cells revealed no effect. Immunodominant IAV specific CD4 T cells expressed higher levels of PD1 and ICOS than the polyclonal population. These data indicate that immunodominant T cells may be more dependent on T cell: APC interactions than other IAV-specific CD4 T cells. Together these data suggest that interactions between lung APCs and CD4 T cells influence the quality of memory CD4 T cells generated by infection.

Understanding Dynamic Immune Responses within ³D in vitro Human Skin Models

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Dynamic communication between tissue resident cells and circulating immune cells, such as monocytes, orchestrates the skin's responses to infection and plays a role in tissue repair and regeneration. However, the factors that drive monocyte recruitment into human skin during inflammatory events and the specific signals that direct monocyte fate are poorly understood.

Current 3D in vitro models recapitulate the basic structure of skin by fabricating dermal and epidermal like layers but do not effectively model the complex and dynamic interactions between the tissue and the immune system. The aim of this research is to gain new mechanistic insights into inflammatory responses within human skin, through the development of a novel immune-responsive in vitro model. Using 3D bioprinting technology, we fabricated a microfluidic channel (500 μm diameter) within a fibrin based dermal compartment. To mimic the vasculature, human umbilical vein endothelial cells (HUVECs) were used to line the microchannel. NTERT keratinocytes were seeded on top to create an epidermal layer and isolated CD14⁺ monocytes were injected into the microchannel. Specific activation of the inflammasome in the epidermal layer, with lipopolysaccharide and nigericin, stimulated significant ($p = 0.0454$, $N = 3$) monocyte migration from the microchannel to the epidermal layer after 24 hours compared to untreated controls. These findings demonstrate that the developed microfluidic skin model has immune responsive capabilities and could be used to investigate human specific inflammatory responses within the skin. On-going studies aim to investigate inflammatory responses in the developed in vitro microfluidic model using single cell transcriptomics.

Therapeutic STm Δ aroA preferentially invade proliferating cells in colorectal cancer

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Attenuated *Salmonella typhimurium* (STm) home to and colonise tumours, where it induces tumour regression via mechanisms including metabolic competition, reducing tumour stemness and inducing an immune response. In this study, we aimed to further understand the mechanism by which attenuated STm specifically target tumour stem cells. Using a range of invasion machinery-deficient STm, coupled with pharmacological inhibitors, we identify type-3 secretion system component SipB engagement with host membrane cholesterol as essential for intracellular invasion of STm. Previous studies have shown that cell-surface cholesterol is maximal during mitosis, and we show that STm preferentially invade ki67⁺ cells and blockade of proliferation abolishes STm invasion. While SipB-deficient STm cannot invade intracellularly, we show that SipB-deficient STm can colonise polyps in *Apc^{min}/+* mice when delivered by oral gavage and thus still have the potential to affect tumour metabolic landscape and immune infiltration. Bulk RNA-sequencing of organoids infected with STm Δ aroA or STm Δ aroA in combination with a cholesterol sequestering compound allowed comparison of the effect of extracellular and intracellular bacteria on tumours in vitro. Intracellular infection of mouse tumour organoids upregulated a range of chemokines and innate pathways, whereas extracellular bacteria led to enrichment of genes involved in metabolic pathways, which may drive differential immune responses in vivo. We propose that preferential invasion of fast-dividing cancer stem cells by *Salmonella* via SipB—cholesterol interaction diminishes the stem cell pool within the tumour, as well as altered chemokine production. We next aim to identify to what extent intracellular invasion is needed for therapeutic efficacy.

Type I interferons induced upon RSV infection change the lung microenvironment and impair seeding of lung metastatic breast cancer cells

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Breast cancer is the most common cancer in women. Despite the high survival rate, invasive breast cancer is the cause of 7% of all cancer deaths, with the lungs being a common site of metastases. The lungs are also susceptible to lower respiratory tract infections, the third leading cause of death globally. Acute viral respiratory infections induce transitional changes in the lung microenvironment that could impact metastasis initiation and growth. However, the interplay between these diseases remains understudied. Here, we used the primary murine MMTV-PyMT breast cancer cells in an experimental lung metastasis model, to investigate the effect of RSV infection on metastasis initiation. We show that RSV infection, a day prior to tumour cell injection, reduces the number, but not the size, of metastatic nodules, in a type I interferon dependent manner. Since the size distribution of the lung metastasis was similar in all groups, we studied the effects of RSV infection or intranasal administration of recombinant IFN- during tumour cell extravasation and seeding by in vivo imaging of luciferase-expressing MMTV-PyMT. Lower levels of luciferase activity in the lungs of both experimental groups were observed as early as 3.5h post cell injection, suggesting an impairment in metastasis initiation. Interestingly, reduced tumour cell proliferation were detected in 3D cultures of epithelial cells from RSV-infected or IFN-α treated mice. Altogether, our results show that type I IFNs induced by RSV infection reprogram the lung microenvironment and consequently interfere with the ability of the tumour cells to successfully initiate metastatic colonisation.

The induction of IL-10⁻ producing B regulatory cells leads to immunotolerance recovery with grass pollen subcutaneous immunotherapy

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IL-10⁺ regulatory B cells (Bregs) play essential roles in tolerance maintenance to sensitised allergens in allergic rhinitis (AR). We hypothesise the frequency of IL-10⁺ Bregs is lower in seasonal and perennial AR and restored following allergen immunotherapy (AIT). Moreover, we hypothesise IL-10⁺ Bregs restore immune tolerance to the sensitising allergen through inhibiting allergen-specific Th2A cell responses.

PBMCs were collected from non-atopic controls (NAC; n=18), grass pollen allergic (GPA; n=18), house dust mite allergic (HDMA; n=16), cat allergic (CA; n=16) and GP-subcutaneous immunotherapy (GP-SCIT; n=16) subjects. PBMCs were stimulated with CpG/CD40L and IL-10⁺ Breg induction was quantified by flow cytometry and unbiased clustering tools, viSNE and FlowSOM. IL-10 levels were measured using ELISA. PBMCs were stimulated with anti-CD3/CD28 alongside recombinant IL-10 and proliferation of Th2A cells were quantified by flow cytometry.

Significant dysregulation in the proportion of five IL-10+ Breg subsets was observed in GPA, HDMA and CA subjects (all $p < 0.05$). GP-SCIT subjects demonstrated increased IL-10+ Breg frequencies compared to GPA individuals. Allergic individuals demonstrated decreased provision of IL-10 compared to NAC ($p < 0.01$; HDMA). IL-10+ Bregs were significantly increased in TIM-1 and IgG4 expression compared to IL-10- B cells ($p < 0.05$). FlowSOM revealed a specific IL-10+ metacluster of dysregulated B-cells found in both seasonal and perennial allergic patients compared to NAC ($p < 0.05$). Recombinant IL-10 modulated the proliferation of Th2A cells, which was neutralised by anti-IL-10.

IL-10+ Bregs are dysregulated during allergic inflammation, with similar Breg subset aberrations underlying perennial and seasonal allergies. Moreover, GP-SCIT restores the frequency of IL-10+ Breg frequencies.

Senescent cytotoxic CD4⁺ T cells cause skin pathology in human cutaneous leishmaniasis

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Cytotoxic activity is one of the main signatures causing immunopathogenesis in human cutaneous leishmaniasis (CL). In this work, we demonstrate that CD4⁺ T expressing granzyme B and CD107a accumulate in lesions of CL patients. This population demonstrated increased expression of activating NK receptors (NKG2D and NKG2C), while their ligands (MICAB and HLA-E) were upregulated in lesional macrophages and fibroblasts. Interestingly, isolated CD4⁺ T cells from the lesional site demonstrated a great ability to kill K562 target cells, suggesting that they may mediate skin pathology in a nonspecific manner through the interaction of NK cell receptors and their ligands. Phenotypic analyzes indicate that cytotoxic lesional CD4 T cells (CTL-CD4) are represented by resident and migrating populations with the predominance of effector (CD27-CD45RA-) and TEMRA (CD27-CD45RA+) subsets. Both subsets presented increased expression of cutaneous lymphocyte antigen and therefore, higher skin-homing ability. Interestingly, EMRA CD4⁺ T cells showed higher granzyme b and CD107a expression, representing the main cytotoxic population in CL lesions. The predominance of senescent CTL- CD4⁺ cells was confirmed by IF analysis that reveals an accumulation of p16⁺ cells producing granzyme, which positively correlated with the lesion size observed in CL patients. Collectively our results provide the first evidence that senescent CD4⁺ T cells with cytotoxic features may participate in the skin pathology of human cutaneous leishmaniasis.

A putative piglet model of environmental enteric dysfunction in infants: an exploration of immune development and gut barrier function

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Malnutrition appears to contribute to the development of environmental enteric dysfunction (EED) which affects ~25% of children under 5 in LMIC. It is associated with inflammation, malabsorption, stunted growth and increased risk of infection. Pigs share many features of human immunology, gastrointestinal physiology, metabolism, microbiology, and diet, and develop many characteristics of EED when weaned early and abruptly, although recover within 2 weeks. To explore the hypothesis that EED characteristics are maintained if early weaned piglets are subjected to malnutrition, and thus generate a valuable model of EED, litter-matched piglets were subjected to early (21d) abrupt weaning and maintained on either 50% (n=6) or 100% (n=6) of the feed required for optimal growth for 56 days. 4-colour fluorescence immunohistology was used to quantify the expression of immune-associated and intestinal barrier-associated protein expression in the proximal and distal jejunum, and colon. Malnutrition resulted in significant increases ($p < 0.001$) in epithelial CD45 expression at all locations along the intestinal tract. Interestingly, there were also significant differences ($p < 0.001$) in CD45 expression between the proximal and distal jejunal, and colonic epithelium in both control and malnourished piglets. In the underlying lamina propria, there were differences in CD45, CD172, MHCII DR and capillary endothelium marker expression where direction and significance ($p < 0.05$ - $p < 0.001$) were highly dependent on intestinal location. Expression of barrier function-associated proteins was also depend on location. Reliable strategies to combat EED remain elusive, in part due to a paucity in knowledge of disease ethology. However, our putative EED piglet model could help to address this.

Long-term lung inflammation after SARS-CoV-2 infection of mice

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A subset of patients suffering from COVID-19, a disease caused by SARS-CoV-2, can develop persistent multi-organ dysfunction and inflammation, despite viral clearance. Post-acute sequelae of COVID-19 (PASC), also known as long COVID, can manifest in a variety of different symptoms including dyspnoea and chronic lung disease. However, the mechanisms behind the possible immunopathology of respiratory PASC symptoms remain poorly understood due to the lack of appropriate animal models. Here, we used a novel genetic cross of 129 mice and C57BL/6-K18-hACE2 mice to study the long-term effects of SARS-CoV-2 infection within the lungs. These transgenic mice, in which the expression of human ACE2 (hACE2) receptor is driven by the cytokeratin-18 promoter (K18) in epithelial cells, were infected with a low dose of the ancestral strain of SARS-CoV-2. We observed that while complete viral clearance and full recovery from weight loss occurred around day 8 post infection, the presence of prolonged inflammation in the lungs and airways persisted up to day 28 post infection. In parallel, we show that the long-term inflammation was associated with the recruitment of CD4+ and CD8+ T cells to the lung. These data suggest a potential mechanism linking T cell driven inflammation to respiratory PASC manifestations

Investigating the developmental dynamics of TRM cells across organs using a novel Ki67 fate reporter mouse strain

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By utilising novel Ki67 fate reporting mice, we are able to permanently label proliferating cells, and their progeny, at a given time point, and track these cells over time. With a specific focus on tissue-resident memory T cells (TRM), we compare the temporal dynamics of memory subsets in the skin, lungs, and gut, in contrast to T cell populations in both primary and secondary lymphoid organs. By harnessing the power of quantitative immunological approaches, we can leverage this system to shed light on the origin, persistence, destiny, and overall dynamics of various T cell memory subsets.

The Evolution of a Transplantable Tumour

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One of the hallmarks of cancer is the ability to evade the immune system. Remarkably, there are non-human cancers that can transmit across individuals irrespective of the histocompatibility barrier. A well-known example is the Canine Transmissible Venereal Tumour (CTVT), which is a contagious mammalian clonal allograft.

To understand how a mammalian cancer might evolve the ability to escape allogeneic recognition, we have passaged a genetically well-characterized mouse melanoma cell line from syngeneic mice (C57BL/6) into progressively more allogeneic mouse crosses and eventually into fully haplotype mismatched BALB/c mice. At each passage, we have performed flow cytometry analysis on dissociated tumours to characterize the host intratumoral immune infiltrate and performed RNA-seq analysis on the isolated tumour cells to obtain a more comprehensive view of the evolution of these tumours.

Multiple passaging rounds resulted in a stepwise adaptation of the tumours to evade allogeneic recognition, eventually permitting their tolerance in fully mismatched mice. Flow cytometry showed greater immune infiltration in the earlier passages, including CD8+, CD4+ T cells and NK cells which, however, were unable to reject the tumour. In the later passages, the immune infiltrate was significantly reduced, except for MHC II+ CD11c+ Dendritic cells and F4/80+ CD11b+ Macrophages, which increased. RNAseq data on isolated tumour cells demonstrated changes of gene expression during passaging characterized by progressively stronger anti-viral signature. These data demonstrate that our experimental model can successfully evolve tumours that bypass the histocompatibility barrier. Our model will provide new mechanistic insights into allograft tolerance that may be used to blunt organ transplant rejection.

Tissue-specific dysregulation of the immune system in Crohn's disease

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Crohn's disease (CD) is a type of inflammatory bowel disease (IBD) characterised by chronic inflammation in the gastrointestinal tract. The biological mechanisms underlying CD pathology are not yet fully understood, but evidence suggests a complex interplay between genetic, immune, and environmental factors. Here, we performed single-cell RNA-seq (scRNA-seq) on 45 CD patients with matched terminal ileum biopsies and blood samples. By integrating both tissues, we observed substantial heterogeneity across both the innate and the adaptive immune populations. For instance, we were able to characterise tissue-resident T cells in the terminal ileum, and compared to blood samples, B cells from the terminal ileum had downregulation of MHC II-related genes. Similarly, we were able to characterise a plasmacytoid-like subset of dendritic cells (DCs) that has been previously hypothesised to infiltrate the gut of CD patients and enhance inflammation. At the single cell level, this population was characterised by high levels of expression from TCF4, PTPRE, IRF8, and BCL11A. Altogether, our findings provide a detailed characterization of the tissue-specific and systemic transcriptional states that are present in the immune system of CD patients.

Stable neonatal natural Bregs contribute to immune regulation in adulthood.

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Regulatory B (Breg) cells, are known to restrain immune responses associated with autoimmune diseases and are induced in response to inflammatory signals to dampen excessive inflammation. A relatively large population of natural Bregs has been previously described in neonates. The stability of this population throughout adult life is unknown. By genetic fate mapping or adoptive reconstitution of bone marrow depleted mice we have shown that neonatal derived natural Bregs can be found in multiple tissue-organs in adult mice. Although natural Bregs in adults transiently down-regulate IL-10 expression, this "latent" Breg cells retained their memory and robustly re-expressed IL-10 and re-gained their suppressive function in response to inflammatory stimuli. In addition to IL-10, neonatal latent Bregs express, similarly to induced Bregs, an immune suppressive signature which includes aryl-hydrocarbon receptor (Ahr) and lymphocyte activation gene 3 (Lag3). We also showed that unlike induced Bregs, neonatal Bregs develop independently from gut microbiota. This study demonstrates for the first time the existence of stable neonatal natural Bregs that develop at early stage of development versus the induced Bregs population, which develops in response to inflammatory stimuli in the periphery of adults.

Deciphering the tissue B cell landscape

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B cells are pivotal players in the body's immune response. Principally studied for their role in the humoral antibody response, their antibody-dependent roles are also increasingly being recognized e.g. the production of pro / regulatory cytokines. These multifaceted immune cells are well recognized players in disease where they can be either protective or pathogenic. Largely researched in the infection and autoimmune setting, their key role in tumorigenesis is also becoming apparent. Whereas the studies of B cells in humans has been largely restricted to peripheral blood, very little is known about the molecular and functional characteristic of B cells resident in different organs. To bridge this gap, we have taken advantage of the wealth of public scRNAseq datasets of human tissues and began to molecularly characterize the tissue resident B cell subsets in health compared to colon cancer. We report a decrease in IgA plasma cells mirrored by an increase in IgG plasma cells, memory B cells and naïve B cells in tumour tissue compared to the healthy counterpart. Gene expression analysis also reveals putative functional differences between the different subsets in health and cancer. Computational cell communication methods indicate a crosstalk between B cells and colon epithelial cells, which is suggested to be more pronounced in the tumor and colitis setting based on gene expression results.

Collectively, these results highlight unique changes in colon tissue B cells and further reveal differences in these subsets in the cancer and colitis setting. Future work will aim to add additional tissues to this analysis to create a comprehensive overview of tissue B cells in health and disease. Ultimately, understanding the tissue specific adaptations and responses of B cells within tissues could lead to the development of more targeted and effective therapeutics.

