

Stromal-immune cell interactions in health and disease

Abstracts

Selected short talks

A Role for Complement Receptors 1 and 2 in Follicular Dendritic Cell Organization in Germinal Centres

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Follicular dendritic cells (FDCs) are enigmatic stromal cells that reside within germinal centres (GCs). Here they capture and store immune complexes, via their Fc (FcRs) and complement receptors (CRs), promoting immune memory but also contributing to autoimmune disease. Complement receptor (CR)-1 and CR2 are expressed on B cells and FDCs. Cr2 knock out (KO) mice, lacking both receptors, have impaired antibody and GC responses.

Utilizing a novel ImageJ macro to analyse confocal microscopy images of spleen sections, we investigated how splenic FDCs in wild type (WT) and Cr2 KO mice behave over 14 days post sheep red blood cells (SRBC) immunization (i.v.). As expected, antibody and GC responses in Cr2 KO mice were impaired. Fewer FDCs were identified in Cr2 KO mice, and these exhibited differential localization and organization compared to WT mice. FDCs from WT but not Cr2 KO mice were actively dispersed in GCs. FDCs from Cr2 KO mice were more often found on follicles outside of the GCs and those within the GCs were closer to the periphery in comparison to WT FDCs.

Our results suggest poor FDC organization in GCs and failure to increase receptor expression after immunization may contribute to the inefficient immune responses observed in Cr2 KO mice. Interference of FDC-networks has been shown to reduce autoreactive-GC formation and auto-antibody titres. Hence the implication of these findings in autoimmune disease is of interest for future studies.

Generation of CD33 chimeric antigen receptor (CAR) invariant natural killer T (iNKT) cells for acute myeloid leukaemia (AML) immunotherapy

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Background: AML is the most common leukaemia in adults, with significant patient mortality, hence necessitating development of novel therapies. iNKT cells exhibit potent anti-tumour functions, which can be applied as “off-the-shelf” allogeneic cellular immunotherapy. Additionally, iNKT cells are a favourable cell platform over conventional CAR-T cells, due to extreme inherent cytotoxicity and lack of graft-versus-host-disease in an allogeneic setting. Pre-clinical studies and ongoing clinical trials have demonstrated effective CAR-iNKT cell responses against lymphoma and neuroblastoma with limited cytotoxicity. Our data suggests that human iNKT cells exhibit significant cytotoxicity against AML cells, hence we set out to develop CAR iNKT cells against CD33, a cell surface antigen highly expressed on AML cells, for more efficient killing.

Aims: Optimise a feasible and scalable pipeline for generating pure iNKT cells as a platform for human CD33 CAR-iNKT cells against AML.

Methods: CD1d tetramers were generated to identify iNKT-cells from healthy donor PBMCs, and MACS enrichment was optimised to generate pure iNKT cell populations. Ex vivo established iNKT cells were transduced by spinoculation with a 2nd-generation anti-CD33 CAR construct, and transduction efficiency was assessed by flow cytometry.

Results: Our data indicate that iNKT cell frequency is donor dependent, and directly correlates with purity of the enriched product. Established iNKT cells were successfully transduced with an efficiency of up to 92%.

Conclusions: Literature suggests this is the first time CD33 CAR-iNKT cells have been produced. This provides a proof-of-concept which can be investigated in our bespoke preclinical models of AML, to leverage their translational potential.

Targeting Cancer-Associated Fibroblasts in Head and Neck Cancer through MAGED4B Vaccination

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A myofibroblastic CAF (myoCAF)-rich stroma is associated with poor prognosis in multiple cancers, including head and neck cancer (HNSCC). In part, this is due to myoCAF suppressing anti-tumour immunity and contributing to immunotherapy resistance. Depleting myoCAF from tumours could potentiate immunotherapy response. However, a lack of specific myoCAF markers has limited this therapeutic strategy.

We identified cancer/testis antigen MAGED4B in the scRNA-seq HNSCC dataset and found its expression limited to myoCAFs and tumour cells. IHC analysis of HNSCC confirmed high MAGED4B expression in tumour cells (43/53) and myoCAF (25/53).

A MOC2 murine oral cancer model was developed to test the effect of MAGED4B-directed vaccination in HNSCC. MOC2 tumours were poorly differentiated and commonly metastasised to regional lymph nodes. Co-injection of MOC2 cells with TGF- β -treated murine tongue fibroblasts (myoMTF; 1:5 ratio) significantly increased tumour growth ($p=0.0015$) and α SMA expression ($p=0.0041$).

A pDOM DNA fusion vaccine against MAGED4B was tested in vivo on tumour-naïve mice and immunogenicity was confirmed by ex vivo IFN γ ELISpot. To generate tumours that express MAGED4B, MOC2 cells and MTF were retrovirally transduced with hMAGED4B. MOC2MAGED4B+ tumour growth was marginally hindered by the pDOM-MAGED4B vaccine ($p=0.0941$, vaccine/control tumour volume), but this sensitivity was lost in tumours with myoMTF co-injection. Further vaccine efficacy studies are ongoing.

Significant levels of myoCAF are present in around 50% of HNSCC, which has one of the lowest response rates to anti-PD1 immunotherapy (~15%). MAGED4B-directed vaccination has the potential to target both HNSCC cells and myoCAF simultaneously and has significant potential for combination immunotherapy in highly aggressive myoCAF-rich HNSCC.

Streamlining Data Exploration: HTML and Java Integration for Interactive TB Gene Expression Data Analysis

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Accessing and analysing vast amounts of data from biological databases is a common challenge. This is particularly evident in fields such as bioinformatics, where datasets, like Differential Gene Expression (DGE) data, can be exceedingly large and complex. For clinicians and Principal Investigators (PIs), navigating through such extensive datasets poses significant hurdles and can be time consuming.

To address this challenge, leveraging web-based technologies such as HTML (hypertext markup language), CSS (cascading style sheets), and JavaScript offers a promising solution. By employing these tools, scientific data can be presented in an interactive and user-friendly manner. HTML, in particular, emerges as a valuable format for presenting expression data due to its lightweight nature and compatibility with web browsers.

Using HTML, CSS, and JavaScript we developed an interactive interface to explore scientific data effortlessly. Through the development of individualized web-based applications, users can navigate through complex datasets with ease, visualizing and interacting with the data in real-time.

As a pilot, we used HTML and Java to present Tuberculosis-related gene expression data in a single light weight file where users can interactively explore gene expression and basic statistics without the need for raw data. The approach not only simplifies the process of data analysis but also facilitates collaboration and knowledge dissemination among researchers with diverse backgrounds. Furthermore, this approach can be adapted for various data analyses in the future, offering a versatile solution for interactive exploration across diverse datasets.

Immune modulation of the pre-metastatic niche in high-grade serous ovarian cancer via a stroma-Treg axis

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Metastatic spread of cancer requires a complex reprogramming of the local stromal and immune landscape, which ultimately promotes tumour seeding and growth. Yet, the molecular mechanisms that support this pre-metastatic niche remain largely unknown. Here, we investigated how a stromal-immune crosstalk supports early metastatic seeding of ovarian cancer (OC) cells to the omentum, the primary site of metastasis in patients.

Omental metastatic lesions were profiled by high-parameter flow cytometry and multiplex immunofluorescence imaging. We used a proximity-labelling system to interrogate the composition of early omental metastases. Drivers of tumour metastasis were investigated by *in silico* analysis of bulk RNAseq data at early timepoints, and single-cell RNAseq data from end-stage tumours.

Omental macrophages and stromal cells were identified to be in close contact to disseminating OC cells. Phenotypic analysis showed stromal proliferation and a shift in the composition of cancer-associated fibroblasts (CAFs) in early metastases, with a preferential expansion of MHC-II+ antigen-presenting (ap)CAFs. Upregulation of MCH-II in fibroblasts was shown to be IFN γ dependent. Transcriptionally, tumour-neighbouring stroma were enriched in epithelial-to-mesenchymal transition and TGF β signalling. We thus hypothesised that a crosstalk between apCAFs and regulatory T cells (Tregs) could be driving metastatic seeding. Depletion of Tregs *in vivo* completely abrogated metastasis and decreased the proportion of omental apCAFs.

We describe a novel MHC-II+ apCAF population that is up-regulated in the omental pre-metastatic niche and is in close contact with disseminating OC cells. Treg depletion alters CAF composition and abrogates OC metastasis, suggesting a possible crosstalk between these cell types

Development of a 3D in vitro Breast Cancer Organotypic Model of Obesity Associated Adipose Inflammation

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Obesity is an established risk factor for breast cancer in postmenopausal women. However, the underlying biological mechanisms of how obesity mediates breast cancer progression remains unclear, which may in part be due to a lack of suitable models. There is a paucity of breast tumour models which incorporates adipocytes, to replicate the complex tumour microenvironment associated with obesity. Therefore, our aim was to develop a 3D in vitro model that recapitulates the inflammatory environment associated with obesity in breast cancer patients.

To model the complexity of the breast tumour microenvironment, a 3D organotypic system was first developed containing breast tumour cells, myoepithelial cells, macrophages, and fibroblasts in a collagen type I matrix. To generate a novel model of obesity-associated breast cancer, hypertrophic adipocyte spheroids were generated and embedded within the collagen matrix. Adipocytes were treated with palmitic acid for 12 days to mimic caloric overload and induce a hypertrophic phenotype. Organotypic cultures containing adipocytes showed increased proliferation of breast tumour cells and the formation of large tumour islands. Infiltration of breast tumour cells and macrophages was observed within and surrounding adipocyte spheroids, replicating the adipose-inflamed border observed in obese breast cancer patients. Immunohistochemical staining of organotypic cultures revealed that macrophages co-localise with adipocytes, forming crown-like structures.

This organotypic system was then utilised as a drug testing platform. Organotypics were treated with paclitaxel, doxorubicin, tamoxifen or metformin for 48 hours and assessed for cell viability. Cultures containing hypertrophic-like adipocytes exhibited increased sensitivity to metformin and, conversely, resistance to paclitaxel, as compared to non-obese cultures.

This study proposes a 3D organotypic model which recapitulates the obese adipose environment in breast cancer patients. Furthermore, it provides a useful tool to interrogate aspects of obesity induced inflammation and to investigate the mechanisms underpinning obesity-related therapy resistance.

Poster presentations

P.01 Bioactive lipids mediating the resolution of skin inflammation

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Background: Psoriasis is a chronic inflammatory skin disease associated with triggering an inappropriate immune response. However, a critical factor driving chronic disease is a failure to resolve inflammation.

Bioactive lipid mediators play key roles in inflammation and are altered in psoriasis skin. However, the effect of many bioactive lipid species on inflammation remains unknown, particularly during resolution. We hypothesise that dietary alteration of lipid mediators may promote resolution.

Methods: Psoriasis-like skin inflammation was induced by topically treating ear skin with 20mg Aldara cream for 4 days, then allowing inflammation to resolve. Immune cell populations were analysed by flow cytometry, mass spectrometry was used to profile lipid species and dietary manipulation of lipids was performed by feeding mice a 60% fat (by kcal) diet for 10-12 weeks.

Results: In our model, psoriasis-like skin inflammation peaks at 4-6 days and resolves around 13 days post-initiation of treatment. In naïve skin, 18 COX-derived eicosanoid lipid species are detected by mass spectrometry. Flow cytometry can detect multiple immune cell types in naïve, inflamed and resolving skin and will be used to determine cells expressing receptors for bioactive lipids enriched in resolution. A high-fat diet alters immune cell populations in naïve ear and back skin, demonstrating a dietary lipid-immune cell link in skin.

Conclusions: We have developed a model system to allow measurement of lipid species in naïve, inflamed and resolving skin. This will allow the identification of lipid species with pro-resolution functions which could represent novel therapeutic targets in inflammatory skin disease.

P.02 Investigating Neutrophil-T cell interactions in the Lymph node

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Lymph nodes are an important organ in driving the adaptive immune response by uniting antigens, antigen presenting cells, and lymphocytes. Previous work has demonstrated that T cell interactions in the lymph node lead to different outcomes compared to in single cell suspensions, as a result of the spatial environment of lymph nodes, the presence of stromal cells, and movement of lymph. Other work has demonstrated that individual lymph nodes contain specialised immune cells with distinct phenotypes. We have three main aims to analyse cell interactions in human lymph nodes.

1. To understand the phenotypes of immune cell populations in human mesenteric lymph nodes. Groups include patients with inflammatory bowel disease (IBD) (ulcerative colitis and crohn's disease) and patients with colorectal cancer (non-draining lymph nodes without metastasis, which serve as healthy controls). We have optimised spectral flow cytometry panels which will stain for T cell surface phenotype, transcription factor expression and cytokine production; myeloid cells including neutrophils, dendritic cells and macrophages.
2. Develop a slice culture system for human mesenteric lymph nodes, based on the work of the Pompano group. We are currently optimising ex vivo lymph node slices to help us model neutrophil-T cell interactions within the lymph node. This involves slicing lymph nodes into 300-micrometre thick slices and culturing them in media for up to 48 hours.
3. Determine how slices from patients with IBD respond to antigenic or general inflammatory stimulus and how this differs to peripheral blood cells from the same patient in single cell suspensions. Readouts include flow cytometry, ELISA, RNA sequencing and spatial transcriptomics.

P.03 Fish immunity in a warming world

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Acute and chronic temperature changes pose significant challenges to ectotherms like fish. As water temperatures exhibit extreme temperature fluctuations, the impact on fish is likely to be significant. While temperature stress is known to cause immune dysregulation in fish, inconsistencies in experimental approach make it difficult to obtain a global view of the impacts of temperature stress on fish immunity. Many published studies use restricted sets of immune biomarkers, obtained at a restricted set of time points, thereby failing to provide the detailed spatiotemporal picture needed to fully understand whole organism immunity. Coupled with inconsistent definitions of 'acute' and 'chronic' exposure, a paucity of studies incorporating antigenic challenge and/or functional assays, gaining a comprehensive view of the extant literature in this arena is challenging.

We performed a systematic review of relevant peer-reviewed publications between Jan 2020 and June 2022. The Web of Science database was mined, filtered for relevant categories and studies not incorporating temperature as an independent variable were manually excluded. Data from the final 72 articles were extracted and compiled to include details of >20 variables including magnitude/directionality of immune biomarker change, method of measurement and details of any antigenic challenge. Species information from Fishbase were added. Downstream analysis revealed remarkably consistent impacts of temperature stress on fish species with transcript levels of key immune regulatory cytokines most obviously affected. These data provide a platform to understand the impact of the climate crisis on the immunological fitness of farmed and wild fish stocks.

P.04 Detecting and defining immunity to human cytomegalovirus

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Cytomegalovirus (CMV) is a ubiquitous member of the beta-herpesvirus family, infecting approximately 50% of adults worldwide. Infection is usually asymptomatic although CMV enters a life-long latent state with potential to reactivate. After allogeneic-haematopoietic-stem-cell transplant (allo-HSCT), CMV re-activation can cause morbidity and mortality. Prophylactic anti-CMV treatment in high-risk patients, CMV DNA monitoring and pre-emptive antiviral treatment in case of reactivation, are vital to avert such outcomes. T-cell mediated immunity is key in controlling CMV, and assays to measure T-cell CMV-specific immunity would improve personalised management post allo-HSCT. However, these assays are lacking in clinical practice.

We aim to evaluate QuantiFERON[®]-CMV, a commercial assay, for measuring CMV-specific T-cell immune reconstitution post-allo-HSCT. To corroborate results and provide additional information, parallel flow cytometric analyses were performed. Data are from a pilot study, which pre-empts longitudinal evaluation of QuantiFERON[®]-CMV and flow cytometry in allo-HSCT patients. Healthy volunteers were tested for the presence of anti-CMV IgG (an indicator of prior CMV infection), and for the presence/phenotype of CMV-specific T cells by QuantiFERON[®]-CMV and flow cytometry. CMV cellular immunity was only detected in the 6 of 11 individuals exhibiting a humoral response to CMV. Correlation between CMV reactivity detected by QuantiFERON[®]-CMV and flow cytometry was observed. CMV-specific CD8+ T cells were enriched for TEMRA phenotype and exhibited diverse patterns of CX3CR1 expression, a marker associated with cytolytic function. These data provide a platform to evaluate QuantiFERON[®]-CMV as a patient-management tool post allo-HSCT, and to explore CMV-reactive CD8+ T-cell frequency, phenotype and function during immune reconstitution.

P.05 Interrogating metabolic effects of the antimicrobial peptide cathelicidin on CD4+ T cells

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Cathelicidin is an antimicrobial peptide secreted by multiple cell types including neutrophils, epithelial cells, mast cells and macrophages. Studies indicate capacity for cathelicidin to alter metabolic activity of diverse cell types. Previous research from our lab has shown that cathelicidin drives Th17 development and protects these cells from apoptosis. As T cell metabolism has previously been linked to T cell differentiation, we hypothesise that the alterations in T cell fate following contact with cathelicidin may relate to changes in metabolism.

In this project, we investigated the metabolic pathways through which cathelicidin regulates T cell metabolism in murine splenic cell cultures.

Single cell suspensions of total splenocytes from C57BL6/JOlaHsd mice were cultured with anti-CD3 stimulation in the presence or absence of Th17 polarising conditions (TGF- β , IL-6, IL-23). The impact of cathelicidin in the presence or absence of metabolic pathway inhibitors was used to identify the effect on Th17 polarisation and cytokine production, and for metabolic capacity by techniques including extracellular flux analysis, mitotracker staining and SCENITH assay. We also assessed signalling through the PI3K/Akt/mTOR signalling pathway, by analysis of phospho-protein abundance.

Our findings confirm previous reports of cathelicidin promoting Th17 differentiation. Preliminary metabolic data indicate cathelicidin alters rates of oxygen consumption and extracellular acidification of Th17's. Both the mitochondrial mass and membrane potential is also shown to be significantly increased by cathelicidin in Th17 polarised cells. These data suggests that cathelicidin may exert effects on CD4+T cell differentiation and inflammatory activity via metabolic alteration.

P.06 Cancer-associated fibroblasts drive CXCL13 production in activated T cells via TGF-beta

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Tumour-reactive T cells producing the B-cell attractant chemokine CXCL13, in solid tumours, promote the development of tertiary lymphoid structures (TLS) and are associated with improved prognosis and responsiveness to checkpoint antibody immunotherapy (1). Cancer associated fibroblasts are the dominant stromal cell type in non-small cell lung cancer (NSCLC) where they co-localise with T cells and can influence T cell activation and exhaustion (2)(3). We questioned whether CAF directly promote CXCL13-production during T cell activation.

We characterised surface markers, cytokine production and transcription factor expression in CXCL13-producing T cells in NSCLC tumours and paired non-cancerous lung samples using flow cytometry. We then assessed the influence of human NSCLC-derived primary CAF lines on T cells from healthy donors and NSCLC patients during activation in vitro measuring CXCL13 production and expression of cell-surface markers and transcription factors by flow cytometry.

CAFs significantly increased the production of CXCL13 by both CD4+ and CD8+ T cells. CAF-induced CXCL13-producing cells lacked expression of CXCR5 and BCL6 and displayed a T peripheral helper cell phenotype. Furthermore, we demonstrate CXCL13 production by T cells is induced by TGF- β and limited by IL-2. CAF provide TGF- β during T cell activation and reduce availability of IL-2 both directly (by reducing the capacity for IL-2 production) and indirectly, by expanding a population of activated Treg. Inhibition of TGF- β signalling prevented both CAF-driven upregulation of CXCL13 and Treg expansion.

Promoting CXCL13 production represents a newly described immune-regulatory function of CAF with the potential to shape the immune infiltrate of the tumour microenvironment both by altering the effector-function of tumour infiltrating T-cells and their capacity to attract B cells and promote TLS formation. Pharmacologically targeting the mechanisms within the CAF-T cell activation pathway (+/- checkpoint antibody immunotherapy), will allow to assess rational drug combinations that can inform future clinical studies, and potentially identify an effective adjuvant treatment for NSCLC immunotherapy.

P.07 CAR-iNKT cell migration to tumor sites in multiple myeloma and primary CNS lymphoma

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Chimeric antigen receptor (CAR)-T cell immunotherapy has shown encouraging clinical remission rates for haematological malignancies, that are however followed by significantly high relapse rates especially for multiple myeloma patients. At the same time, CAR-T cell immunotherapy for solid tumors is less optimistic due to inefficient T-cell trafficking to tumor sites. Here we propose an alternative strategy that harnesses the powerful adaptive immune cell responses and overcomes the limitations of T cells. Invariant natural killer T (iNKT) cells are unique lymphocytes that express an invariant TCR (V α 24V β 11) that only recognises CD1d-presented glycolipid antigens and therefore are not HLA-restricted. CAR-iNKT cells exhibit highly potent anti-tumor activity by dual targeting CD1d-expressing cancer cells while also preventing graft-versus-host disease (GvHD). While comparing the migration mechanisms of T vs iNKT cells, we found that very late antigen-4 (VLA-4) integrin, regulating immune cell homing to the bone marrow and crossing of the blood brain barrier was homogenously highly expressed in resting and activated iNKT cells and less so in T cells. In static and dynamic adhesion, and transmigration assays of CAR-T and -iNKT cells, CAR-iNKT cells exhibited enhanced ability of firm adhesion to vascular cell adhesion molecule-1 (VCAM-1), ligand of VLA-4, and higher transmigration through the tight junctions of endothelial cells, mimicking the vasculature. Therefore, we propose an additional powerful feature of iNKT cells that facilitates homing to tumor sites and will be particularly useful when targeting multiple myeloma and brain lymphoma that we aim to study in vivo.

P.08 CD1c-autoreactive T-cells exhibit potent responses to Mycobacterium tuberculosis infection

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S.F., M.M., and S.M. designed research; S.F., M.M., J.G., D.G.B., D.B., S.S., R.S-K., P.T-S., A.L., R.S., L.T., A.W., L.D., P.E., and S.M. performed research; R.S-K., A.L., M.L., D.K.C., S.S., A.L., and P.E. contributed new reagents/analytic tools; S.F., M.M., J.G., D.G.B., L.T., A.W., M.L., L.D., D.K.C., A.L., S.S., L.T., A.L., P.E., and S.M. analyzed data; and S.M. wrote the paper, all authors edited and approved final version.

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains a major human pandemic. The lipid antigen presenting molecule CD1c presents mycobacterial lipid antigens to Mtb-lipid specific T-cells. However, autoreactive T-cells that recognise CD1c in complex with self-lipids are frequent in human blood, yet little is known about their role in TB. Here, we demonstrate CD1c expression in TB granulomas, but expression is limited which is suggestive of immune evasion by Mtb. CD1c-autoreactive T-cells exhibit weak autoreactive responses to CD1c+ APCs in the absence of foreign lipid antigens, and in response to Mtb infected CD1c+ APCs display strong activation, cytotoxic activity, and secrete substantial amounts of diverse cytokines. Overall, our data suggests that CD1c-autoreactive T-cells may have an underappreciated central role in host defence against Mtb infection