Every cell is an immune cell: early drivers of immune responses

Abstracts

Selected short talks

Activated hepatic fibroblasts promote the retention and functional impairment of tissue-resident CD8+T-cells in the human liver

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The function and phenotype of liver compartmentalised T-cells - tissue-resident T-cells (TRM) - have been well-defined in hepatotropic infections and cancer. We have defined a population of highly functional IL-2-producing intrahepatic CD69+CD103+CD8+TRM that contribute to control of hepatitis B infection. However, whether CD8+TRM are involved in tissue damage and repair (fibrosis) in the liver remains unclear.

We are increasingly aware that tissue stroma can orchestrate local immunity. We have previously shown that activated hepatic stellate cells (HSCs; via their ability to produce IL-15/TGF□) dictate the derivation of CD8+TRM. This is now supported by an increased retention of CD8+T-cells with a TRM phenotype (CD69+CD103+) in livers of individuals with highly activated HSCs in vivo - those with advanced fibrosis. Notably, ex vivo CD8+TRM from individuals with fibrosis, express more of the co-inhibitory receptors, PD-1 and Tigit, produce less pro-inflammatory cytokines (IFN□/TNF□), and have a reduced degranulation capacity upon short-term TCR stimulation. In vitro co-culture of activated HSCs and CD8+TRM from healthy, non-fibrotic individuals recapitulates the functional impairment seen in fibrosis. Significantly we demonstrate increased expression of the PD-1 ligand, PD-L1, on activated HSC as fibrosis progresses, suggesting a potential axis for the functional impairment of CD8+TRM in fibrosis.

Our data show that HSCs are key players in modulating local liver TRM responses, with the potential capacity to drive TRM exhaustion. Understanding the crosstalk between TRM and their surrounding microenvironment will likely reveal novel pathways, such as checkpoint blockade, to reinvigorate the function of essential antiviral/anti-tumour immune sentinels in the liver.

Pulsatile activation of CAR-T cells enhances IL-9 release independent of exposure to Th9 polarizing cytokines

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Chimeric Antigen Receptor (CAR) T-cell (CAR-T) therapy is a modern therapeutic modality for specialized targeting of cancer cells, providing rapidly increasing prognosis times. Despite notable successes, particularly against B-cell malignancies like B-cell acute lymphoblastic leukemia (B-ALL), CAR-T therapy exhibits limited efficacy against both hematological malignancies and solid tumors. This limitation is partly attributed to the rapid exhaustion of CAR-T cells in response to antigen persistence, pro-inflammatory cytokines in the tumour microenvironment and maintained tonic signalling. T-helper type 9 cells (Th9) represent a recent addition to the T-helper subtypes and are commonly found in areas with prolonged antigen exposure, such as sites of chronic inflammation and helminth invasion. Recent research has identified Th9-like CAR-T cells (T9-CARs) as possessing enhanced longevity and effectiveness in killing tumors. However, formulating T9-CARs presents challenges such as the limited number of unique conserved phenotypes and the necessity for exposure to polarizing cytokines. Here, photoactivatable anti-CD19 CAR-Ts (OptoCARs) were exposed to CD19-bearing B-cells and periodically inactivated to show that pulsed CAR-T activation, when compared to constant, substantially distorts both the OptoCAR-T transcriptome and cytokine profile. Notably, we observed a substantial increase in IL-9 expression at both the transcript and protein levels with longer recovery times between activation. CD25 and Foxo1 also showed an increase in expression with pulsed OptoCAR activation, suggesting intermittent inactivation of CAR-Ts encourages differentiation into a more stem-like phenotype, potentially akin to T9-CARs, with proposed increased persistence, proliferation and anti-tumour ability in vivo.

Intestinal helminth and latent tuberculosis co-infection drives cytotoxic effector Vδ1+ T cells with shared T cell receptor repertoires

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The immune response to Mycobacterium tuberculosis (Mtb) infection limits progressive disease and frequently drives a state of asymptomatic latent infection. This response involves the concerted efforts of both conventional and unconventional T cell populations. Human γδ T cells expressing a semi-invariant Vγ9+Vδ2+ T cell receptor (TCR) provide a rapid innate immune response to Mtb infection. However, the utility of the γδ T cell repertoire in long-term control of latent TB infection (LTBI) is unclear. Here, we assessed the γδ T cell immune repertoire in a Kenyan population with Interferon-γ release assay (IGRA)+ latent TB infection (LTBI+) or local contacts with IGRA- blood tests (LTBI-). We found that γδ T cell frequencies in the blood were broadly increased in Kenyan individuals independent of LTBI status. Interestingly, Vδ1+ T cells predominated Vγ9+Vδ2+ T cells to become the most prevalent γδ T cell population in the blood, in contrast to western populations. Moreover, this increase of Vδ1+ T cells in Kenyan individuals was solely attributed to the expansion of CD27- cells with a highly cytotoxic phenotype (Granzyme A+ Granzyme B+ Perforin+). Vγ4+ transcripts were significantly increased in these individuals, with the greatest expansion in LTBI+ individuals. Finally, we observed Vδ1-CDR3 sequence sharing only in LTBI+ individuals with intestinal helminth coinfection. Together, these data indicate that the immune response in LTBI encompasses the expansion of highly cytotoxic Vγ4+Vδ1+ T cells, with a repertoire directed specifically towards helminth coinfection.

Spatio-temporal analysis of Trypanosoma cruzi infection and host response dynamics in experimental models of digestive Chagas disease treatment.

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Digestive Chagas disease (DCD) is an enteric neuropathy caused by infection with the protozoan parasite Trypanosoma cruzi (American trypanosomes). There is a lack of evidence on the mechanism of pathogenesis and rationales for treatment. We used a female C3H/HeN mouse model that recapitulates key clinical manifestations to study how host-parasite interactions shape DCD pathology and to evaluate the impact of treatment with the front-line anti-parasitic drug benznidazole. The host response in infected colon tissue was dominated by upregulation of genes linked to type 1 cellular inflammation with evidence of tempering via multiple immuno-regulatory/inhibitory pathways. Curative anti-parasitic treatment at 6 weeks post-infection resulted in sustained recovery of gastrointestinal transit function, whereas treatment failure led to infection relapse and gradual return of DCD symptoms. Neuro/immune gene expression patterns shifted from chronic inflammation to a tissue repair profile after cure, accompanied by increased cellular proliferation, glial cell marker expression and recovery of neuronal density in the myenteric plexus. Delaying treatment until 24 weeks post-infection led to partial reversal of DCD, suggesting the accumulation of permanent tissue damage over the course of chronic infection. Our study shows that murine DCD pathogenesis is sustained by chronic T. cruzi infection-driven inflammation rather than an inevitable consequence of acute stage denervation. The risk that irreversible enteric neuromuscular tissue damage and dysfunction will develop highlights the importance of prompt diagnosis and treatment. These findings support the concept of treating asymptomatic T. cruzi infected individuals with benznidazole to prevent DCD development.

Don’t forget about platelets! How antibodies can drive platelet responses to Salmonella

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Invasive non-typhoidal Salmonella is responsible for >75,000 deaths/year and >500,000 cases/year globally. 75% of these cases occur in Sub-Saharan Africa, an increasing number of which are from multi-drug resistant strains. Elucidating mechanisms involved in pathogenicity could help identify novel pathways to help control these devastating bloodstream infections. Platelet interactions with bacteria can lead to thrombus formation, which could be beneficial for host control of infection (immunothrombosis), or harmful through uncontrolled inflammation and organ damage (thromboinflammation). Whether Salmonella can cause platelet activation is unknown. We therefore assessed aggregation in the platelet rich plasma of 26 healthy donors to Salmonella Typhimurium using light transmission aggregometry.

Instead of the all-or-nothing aggregation responses typically seen to bacterial stimuli, aggregation responses to Salmonella varied fully across the spectrum from no response to full aggregation, with the length of time platelets took to aggregate (lag time) also varying between donors. Swapping platelets and plasma between strong and weak responders revealed plasma to be the source of the variation between donors. Inhibitor studies showed the FcγRIIA receptor is necessary for aggregation, and depleting Salmonella-binding antibodies from plasma abolished aggregation responses. Correlating levels of Salmonella-specific total IgG, IgG1, IgG2, IgG3 and IgG4 to platelet responses revealed total IgG levels correlated with platelet aggregation levels – donors with higher antibody levels had both stronger platelet responses and shorter lag times. We therefore conclude that IgG antibodies are responsible for donor variation in platelet aggregation responses to Salmonella, which could have implications on whether the response is immunothrombotic or thromboinflammatory.

Alveolar epithelial cells internalise and clear Aspergillus fumigatus via Surfactant Protein D opsonisation

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Aspergillus fumigatus (Af) affects over 3,000,000 individuals annually, with invasive aspergillosis having mortality rates of over 50%. Alveolar epithelial cells (AECs) have instant, extensive, and likely prolonged contact with Af upon inhalation. We recently demonstrated that AECs provide a potent means of antifungal defence against Af in vivo, and that dysfunctional epithelial antifungal activity in at-risk patients may provide a safe haven for Af to reside intracellularly. Relatively little is known about the factors controlling Af uptake and clearance by AECs and the dependency of these processes on fungal germination. We locked Af into specific morphotypes using fluorescent auxotrophic pyrG- strains, evaluated internalisation using imaging flow cytometry, and determined that swollen conidia are 2-fold more readily internalised than resting conidia. Using a combination of fluorescent lectins and cell wall mutants, we determined that surface mannans likely dictates fungal uptake and clearance by AECs, with mannose and the mannose-binding lectin Concanavalin A reducing (by 88%) and abolishing (100%) Af internalisation, respectively. Through the evaluation of multiple receptors, we have identified Surfactant Protein D (SP-D), a secreted collectin highly expressed by AECs and with known mannose binding affinity, as a key driver in these interactions. When SP-D is knocked out, there is a 68% decrease in Af internalisation. Our work is now focused on systematically evaluating the role of SP-D in Af-AEC interactions and its importance in disease. Understanding how AECs contribute to antifungal clearance could provide novel avenues for the prevention and treatment of fungal diseases.

Poster presentations

P.01 **Effect of GM-CSF on the immune response of human monocytes against C. albicans**

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During fungal infections, granulocyte-macrophage colony-stimulating factor (GM-CSF) promotes the survival, adhesion, and migration of immune cells such as neutrophils, macrophages, and monocytes. GM-CSF enhances phagocytosis, cytokine secretion, and reactive oxygen species production, all of which improve antifungal activity. This study investigates how GM-CSF influences the interaction between human inflammatory monocytes and C. albicans. We hypothesise that exploring GM-CSF-driven changes in monocytes during C. albicans infection will reveal novel protective mechanisms, shedding light on the potential of GM-CSF as a therapeutic agent against fungi. Markers of alternative macrophage activation, CD206 and CCL22, are increased in GM-CSF-treated monocytes alongside Dectin-1, CD11b, and MHCII, indicating a more nuanced effect of GM-CSF on immunity beyond the promotion of inflammation. In response to infection with C. albicans, GM-CSF-treated monocytes, compared to macrophage-colony stimulating factor (M-CSF)-treated monocytes, increased TNF-α, IL-6, IL-1β, and CCL22 secretion despite similar phagocytic activity (flow cytometry and ImageStreamX) and fungal viability (CFU). Interestingly, hypha growth is more effectively controlled by GM-CSF monocytes, indicating enhanced anti-fungal activity and differential phagosome maturation. These results are linked with a drastic reduction of surface CD206 after C. albicans uptake in GM-CSF monocytes, which would be consistent with enhanced CD206 shedding. Further studies are needed to examine the precise mechanisms leading to reduced hypha growth and CD206 reduction as a means to better define GM-CSF anti-fungal properties.

P.02 **A novel Aspergillus fumigatus dual-reporter strain to investigate the transcriptional signatures driving effective fungal clearance by alveolar epithelial cells**

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Aspergillus fumigatus causes over 3,000,000 infections, the majority of which can be attributed to the inhalation of its spores. The size of A. fumigatus spores suggests that their interactions with alveolar epithelial cells (AECs) are likely frequent and prolonged. We previously demonstrated that AECs can efficiently internalise and kill A. fumigatus in vitro and during murine infections, and that these processes are impaired in human COPD-derived AECs in vitro, suggesting that defective internalisation and killing could facilitate susceptibility to fungal disease. However, relatively little is known about the molecular and transcriptional basis of these processes and the tools needed to investigate intracellular fungal viability are limited. To investigate A. fumigatus-AEC interactions in depth, we developed and optimised a novel dual-reporter strain of A. fumigatus (A1160+tdT, mGL). This strain constitutively expresses the tdTomato fluorophore (tdT), but only expresses the mGreenLantern fluorophore (mGL) under the control of a xylose-inducible promoter. Thus, following exogenous supplementation of xylose, metabolically active A1160+tdT, mGL (tdT+, mGL+) and inactive A1160+tdT, mGL (tdT+, mGL-) can be identified, quantified and sorted. Using this novel reporter, AECs containing intracellular A. fumigatus were fluorescence activated cell sorting (FACS) sorted based on infection outcome and their transcriptional profile was investigated using RNA-seq. This revealed a transcriptional profile which is specific to AECs that have taken up A. fumigatus, a different expression pattern based on the viability of the spores, and ultimately showing that cellular metabolism may be driving effective fungal clearance.

P.03 **Lag3 and PD-L1 cumulatively govern CD4+ T cell activation during checkpoint immunotherapy**

Lozan Sheriff and David Bending

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Combination immunotherapies incorporating PD-1/PD-L1 pathway blockade are potent treatments for some cancers. However, how the individual agents interact to alter T cell activation remains poorly understood, particularly for CD4+ T cells. Here, we reveal how Lag3 and PD-L1 co-blockade modulates CD4+ T cell activation. Combination therapy (CT) drove additive and synergistic changes in CD4+ T cells, resulting in increased TCR signal duration, elevated ICOS and CCR6 expression, and a T follicular helper (Tfh) cell transcriptional bias. During a polyclonal immune response, CT enhanced Tfh cell development and ICOS expression, which was largely dependent on PD-L1 – but not Lag3 – pathway inhibition. CD4+ T cell transcriptional changes in CT-treated mice intersected with those observed in CD4+ T cells from CT-treated melanoma patients, with notable increases in ICOS and CCR6. The PD-L1 and Lag3 pathways, therefore, intersect to govern fundamental aspects of CD4+ T cell activation and we identify biomarkers for CT monitoring.

P.04 **Characterizing the expression of chemokine receptors in CD4+CD28null T cells from patients with inflammatory bowel disease**

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Rationale:
Inflammatory bowel disease (IBD) collectively describes chronic inflammation within the gastrointestinal tract. Previous studies show that CD4+ T cell migration to the gut contributes to IBD progression. Chemokines and their receptors have important roles directing cell movement and this pathway is a current therapeutic target in IBD treatment. CD4+CD28null (CD28null) T lymphocytes are specialised cells that expand in a subset of patient with inflammatory diseases. The aim of this project was to characterise the expression of chemokine receptors in CD28null T cells which may regulate their migration and function in IBD.

Methods:
Fresh whole blood from IBD patients was used to quantify the expression of chemokine receptors in CD28null and CD4+CD28+ (CD28+) T cells using flow cytometry. Transwell chemotaxis assays using sorted CD4+ T cells from IBD patients was performed to assess the role of chemokine receptors in cell migration. Flow cytometry was used to assess the effector functions (production of inflammatory cytokines, cytolytic molecules and degranulation) of CD28null T cells following chemokine stimulation.

Results:
Chemokine receptor expression was significantly higher in CD28null T cells compared to CD28+ T cells. Preliminary data suggest that CD28null T cells have a greater chemotactic index towards chemokines compared to CD28+ T cells. Ongoing experiments are investigating the effector T cell function in response to chemokine stimulation.

Conclusions:
CX3CR1 is preferentially expressed by CD28null T cells. CD4+ T cells from IBD patients with an expansion of CD28null T cells migrate towards chemokines. Current work is evaluating whether chemokine stimulation alters the effector functions of CD28null T cells.

P.05 **Does GDF11 affect the immunomodulatory properties of older and younger donor human bone marrow mesenchymal stromal cells?**

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With an ageing population, cell-based therapies aimed at reducing both inflammation as well as promoting tissue repair without cytotoxic side effects is a current key goal for clinical therapy. Human mesenchymal stromal cells (hMSC) are a key progenitor for this approach. Unfortunately, older donor MSCs (65+) typically have lower therapeutic potential, and when expanded in vitro, can become senescent, further reducing their functional properties. Therefore, new methods of expanding hMSCs whilst retaining their functional properties remains a key scientific goal. A promising candidate for improving hMSC function during laboratory expansion is growth factor differentiation 11 (GDF11). GDF11 gradually naturally declines as we age, and restoring levels of this protein has been thought to be one strategy for combating the ageing process. However, conflicting reports in the field disagree about its potential to rejuvenate hMSC from older donors.

To address the potential of GDF11 to improve the therapeutic potential of hMSC, bone marrow derived hMSC from younger (19-28) and older (65-85) donors were cultured with different doses or durations of GDF11 supplementation. hMSC immunomodulatory potential were evaluated through the ability to suppress T cell proliferation, modulate regulatory T cell maturation or the upregulation of immunomodulatory proteins (galectin-9 and PDL-1). In this study there were no significant effects observed on improved immunomodulatory capacity of either younger or older donor hMSC suggesting that supplementation with GDF11 does not alter these important functions. However, GDF11 treatment does alter other hMSC physiology processes and therefore the full effects of GDF11 remain to be elucidated.

P.06 **Contribution of CPA-LPS to the recognition of Pseudomonas aeruginosa (PA) by human dendritic cells**

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Understanding the mechanisms underlying the recognition of PA by the immune system is crucial for developing effective therapeutic strategies. The study investigates the role of CPA-LPS (common polysaccharide antigen lipopolysaccharide) in the recognition of PA by human dendritic cells (huDCs). Lectin binding assays were performed using fixed planktonic PAO1 wild-type (wt) and LPS mutant strains (PAO1-C CPA+/OSA+, ΔwbpM CPA+/OSA-, Δrmd CPA-/OSA+, ΔwbpL CPA-/OSA-) incubated with Fc-DC-SIGN. To assess huDC-PA interaction, huDCs were co-cultured with planktonic PAO1wt and LPS mutants at an MOI of 1 for 2 and 4 hours. Viability and cytokine production (TNF-α, IL-6, and IL-1β) were evaluated. The findings indicate that DC-SIGN binding to PA is CPA-LPS dependent. huDCs did not significantly affect bacterial cell numbers at 2 and 4 hours post-infection (hpi). Furthermore, LPS glycosylation enhanced TNF-α and IL-6 production by huDCs in response to PA without having a significant effect on IL-1β levels. The impact of CPA-LPS on cytokine production appears to be DC-SIGN-independent, as similar cytokine responses were observed in CPA+/OSA- and CPA-/OSA+ strains. The contribution of DC-SIGN is probably less relevant in the presence of high LPS doses. Future work will focus on the pro-inflammatory properties of purified LPS from PA wt and mutants, elucidating the role of DC-SIGN at low LPS doses. Antibiotic susceptibility assays will be conducted to assess the potential impact of CPA-LPS on PA virulence and treatment efficacy. This study advances knowledge of CPA-LPS and DC-SIGN interactions, guiding future research and therapeutic development.

P.07 **Optimising the Use of Degradable Microcarriers in Stirred Tank Bioreactors for the Production of Immunomodulatory hMSCs**

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Human mesenchymal stromal cell (hMSC)-based therapies possess substantial potential for both immuno-suppressive and tissue regeneration properties. However, current clinical trials require a large number of cells (~100 million hMSCs per dose) for an effective therapy. To meet this demand, this rare population of tissue progenitors requires expansion in the laboratory. Using traditional planar expansion methodologies, the long-term culture and passaging necessary to generate these cell numbers often leads to hMSC senescence, loss of crucial immunomodulatory properties, and relies on rigid substrates that tend to promote spontaneous differentiation into osteogenic lineages. Additionally, these methods are labour-intensive and are limited by surface area-to-volume ratios during culture, restricting their production at large scale.

Stirred tank bioreactors (STR) combined with microcarriers substrates facilitate greater cell culture densities and control over physicochemical parameters necessary for optimal cell growth. However, current cell harvest methodologies from microcarriers post expansion subject hMSCs to intense mechanical and proteolytic enzymatic stress for cell detachment. This results in low recovery and reduced cell viability with a loss of immunomodulatory properties.

This study optimises STR bioprocessing methodologies using degradable microcarriers that promote the expansion of naïve, immunomodulatory hMSCs as well as reducing harvesting time. We observe an up to 15-fold expansion of cells with 91-99% recovery and high viability from STR systems. We demonstrate that these cells continue to proliferate in subsequent cultures and maintain their lineage differentiation potential. Importantly, hMSCs from STR cultures retained a naïve, stem like phenotype, that could still suppress the proliferation of T-cells, promote the generation of regulatory T-cells, and undergo T-cell induced apoptosis. We illustrate the potential of this bioprocessing approach to generate large numbers of therapeutically active progenitors required for cell banks and clinical therapy. Our results provide a simplified pipeline to address some of the main downstream challenges in the large-scale expansion and isolation of therapeutically viable hMSCs.

P.08 **Regulation of CD4+ T cell Il10 transcription and TCR signal strength in tolerance and cancer**

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Interferon (IFN)γ, mostly derived from natural killer cells and adaptive lymphocytes, promotes inflammation and induces target destruction. IFNγ drives activation of CD8+ T cells and Antigen Presenting Cells (APCs), generating an increasingly potent immune response via self-perpetuating IFNγ signalling and increasing Major Histocompatibility Complex class II expression which activates CD4+ T cells via their T cell receptor (TCR). This response is counteracted by negative immune regulators, limiting damage to self by excessive immune responses via Regulatory T cells (Tregs) such as Type 1 Regulatory T cells (Tr1 cells). These induce negative regulation through, for example, PD-1, CTLA-4 and TIGIT cell surface expression but also through immunosuppressive cytokine interleukin-10.

Previous data showed increasing dose of self-peptide sufficiently and rapidly induces increasing Il10 transcription and strong TCR signalling in a tolerogenic TCR model. So, we investigated the IL-10:TCR signalling strength relationship.

In a tolerising environment, a high dose of self-peptide induced a Tr1-like phenotype in Il10+ CD4+ T cells within 24 hours, and that Ifng preceded Il10 transcription by around 4 hours. Through APCs, anti-IFNγ antibodies significantly reduced CD4+ Il10 transcription, strong TCR signalling, and Tr1-like markers, which was additive with anti-IL-27. Anti-IFNγ increased tumour burden on mice, reducing survival, and Il10 and TCR.strong markers, an effect that was neutralised by combination anti-PD-L1 treatment. Notably in cancer models, Il10 transcription predominantly arose from Foxp3+ Tregs.

These data demonstrate the importance of IFNγ and TCR signalling strength for modulating IL10 expression in distinct subsets of Tregs under different immune environments.

P.09 **Inflammatory bowel disease remodels circulating and tissue-resident populations of γδ T cells**

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Human γδ T cells constitute only a small proportion of all the circulating T lymphocytes in the blood but are but are enriched at mucosal barriers. Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD) is thought to involve a dysregulated response from the mucosal immune system to the intestinal microbiota resulting in chronic inflammation of the gastrointestinal tract. Here, we investigated the dynamics of human paediatric γδ T cell subsets in the intestinal tissue and circulation at the early stages of IBD. Using immunophenotyping and T cell receptor (TCR) repertoire profiling we found that children at the first diagnosis of IBD displayed clonal populations of Vδ1+ TCRs in the blood compared to age and gender matched healthy children. Moreover, circulating Vδ1+ T cells in IBD children had shifted their phenotype from a CD27+ naïve-like population to a cytotoxic CD27neg effector population. We then explored the relationship of circulating and intestinal resident populations of Vδ1+ T cells. Firstly, we found that expanded Vδ1+ T cell clonotypes in circulation, duodenum, terminal ileum and rectum contained unique tissue-location dependent TCR repertoires. Secondly, chronic Crohn's disease associated inflammation drove the remodelling of terminal ileum resident Vδ1+ T cells. Our study indicates that tissue-resident Vδ1+ T cells in discrete niches of the intestine display unique TCR repertoires. Moreover, the onset of IBD has a major impact on intestinal and circulating populations of Vδ1+ T cells. These findings show that the γδ T cell repertoire is highly responsive to the early stages of chronic intestinal inflammation.

P.10 **Nicotinamide Riboside Kinase 1 augments cytoplasmic NAD/H upon CD4+ T cell activation, controlling NADP/H synthesis, reactive oxygen species abundance, inflammatory function and survival**

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CD4+ T cell function is underpinned by metabolic reprogramming upon activation. Increased glycolysis provides biosynthetic precursors for clonal expansion and promotes cytokine expression. In parallel, elevated mitochondrial oxidative phosphorylation (OXPHOS) generates heightened ATP and reactive oxygen species (ROS). ROS disseminate and signal, promoting T cell differentiation, but must be mitigated to prevent oxidative damage. Nicotinamide adenine dinucleotide (NAD/H) is an essential redox cofactor for glycolysis and mitochondrial OXPHOS. It is also phosphorylated to NADP/H, which regulates ROS levels. NAD/H abundance increases in line with CD4+ T cell metabolism upon activation, but synthetic pathways are not fully characterised.

In this study, we interrogated expression and activity of nicotinamide riboside kinase 1 (NRK1) in CD4+ T cells, which phosphorylates nicotinamide riboside (NR), directing it into the NAD salvage pathway. We identified this increases upon cell stimulation, driven by TCR and CD28 signalling. NRK1 non-redundantly contributes to NAD/H abundance in these cells but suppresses their activation and function. Consistently, NRK1-deficient CD4+ T cells have a hyper-inflammatory phenotype, expressing high levels of effector cytokines, which occurs alongside impaired viability.

Mechanistically, this is linked to NRK1 redistribution to the cytoplasm upon CD4+ T cell activation, where it locally elevates NAD/H levels. This supports glycolysis, but more profoundly impacts cytoplasmic NADP/H generation, thereby determining ROS abundance and nuclear NFAT translocation. During invasive fungal infection, NRK1 activity critically maintains effector CD4+ T cell frequencies within affected tissues, confirming that regulation of immune cell metabolism at the subcellular level determines whole organism immune responses.