Fuelling the immune response II: UK immunometabolism 2022 Abstracts

PhD Bright Sparks: short talk – selected abstracts

A unique temporal requirement for the amino acid glutamine in type 1 conventional dendritic cells

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cDC1 are specialised in the presentation of antigens on MHCI to instigate CD8 Tcell responses. We have identified a novel requirement for the amino acid glutamine to support cDC1-MHCI presentation. Using quantitative proteomics, we show that naïve cDC1 have high expression of the amino acid transporters Slc1a5 and Slc7a5. We demonstrate that naïve cDC1 have high rates of amino acid uptake, mitochondrial membrane potential and constitutive mTORC1 activation in the absence of PRR stimulation. In line with this we observe that cDC1 have a high rate of protein synthesis (PS). We show that inhibition of glutaminolysis has a negligible effect on mitochondrial energy generation suggesting that glutamine is not a key substrate for ATP generation. However, acute glutamine deprivation induces severe ER-stress in cDC1 characterised by the accumulation of unfolded proteins in the ER and rapidly diminished PS rate. PS rate is intricately linked with the ability to mount MHCI-responses in naïve DC by maintaining a large pool of rapidly degraded peptides. Naïve cDC1 MHCI presentation of self-peptides is essential for the maintenance of CD8 T cell reactivity by tonic TCR signalling. Upon PRR activation, the requirement of high PS rate is uncoupled from MHCI presentation to maintain stable presentation of antigenic peptides. This study demonstrates a novel temporal requirement for glutamine in naïve cDC1 which is lost upon DC maturation. Glutamine appears essential to support proteostasis in naïve cDC1 when PS rate is coupled to MHCI peptide generation but is dispensable at later timepoints when these processes are uncoupled.

The influence of co-stimulatory domains on the metabolic regulation of chimeric antigen receptor (CAR) T-cell function

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CAR T-cell therapy (CD28 or 4-1BB co-stimulated) has shown remarkable clinical success in treating certain B-cell leukaemia's/lymphoma's, however, so far has not been achieved in the treatment of solid tumours. The challenging tumour microenvironment (TME), lacking in nutrients and oxygen, drives T-cell exhaustion/dysfunction, characterised by a loss of metabolic fitness and mitochondrial health. To counteract this, CARs deliver co-stimulation signals via CD28 or 4-1BB driving two distinct T cell functional and metabolic profiles. We have generated a parallel-CAR (pCAR) construct which efficiently delivers both CD28 and 4-1BB signalling in order to achieve improved T cell function in solid tumours. To investigate whether synergistic co-stimulation leads to enhanced metabolic fitness and thus T-cell function we iteratively stimulated pCAR/CAR T-cells, analysing T-cell phenotype, function and metabolic fitness by flowcytometry and flux analysis. We find that pCAR T-cells have a significantly higher expression of key nutrient transporters, contain more functional mitochondria and have an enhanced metabolic activity compared with conventional CAR T-cells. In line with this pCAR showed increased resistance to T-cell exhaustion. pCAR demonstrated sustained proliferation and cytokine release, resulting in increased anti-tumour activity both in vitro and in in vivo models. Our data shows that delivery of efficient synergistic CD28 and 4-1BB co-stimulation signalling in pCAR, leads to increased metabolic fitness, resulting in superior T-cell function compared to single co-stimulation. We here have a powerful model system to investigate how co-stimulation modulates T-cell metabolism with strong potential to become a novel therapeutic agent.

TNF-α drives naïve CD4+ T cell metabolic reprogramming upon activation

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Upon activation, T cells undergo substantial metabolic reprogramming to support their effector functions, largely driven by T cell receptor and CD28 signalling. Whether inflammatory cytokines further amplify this process is not well understood but could have implications in chronic inflammatory disease. TNF-α has been previously identified to act as a co-stimulatory signal in T cells, increasing proliferation and cytokine production. However, whether TNF α controls T cell metabolism has not been interrogated.

Here, purified naïve human CD4+ T cells were activated in the presence of TNF-α, or a neutralising anti-TNF-α antibody/relevant isotype control. Whilst increased exogenous TNF-α demonstrated little effect on T cell metabolism or function, blocking TNF-α signalling caused a reduction in the activation of naïve CD4+ T cells, alongside significant decreases in their rates of mitochondrial oxygen consumption and lactate production. Consistently, alterations in glucose metabolism in anti-TNF-α-treated cells were observed by stable isotope-based tracing, and pathway analysis of RNA-sequencing data identified a failure to upregulate key genes involved in oxidative phosphorylation. Interrogation of downstream signalling pathways identified that TNF-α drives these metabolic changes in naïve T cells through the PI3K/Akt/mTOR pathway, with the metabolic effects of TNF-α blockade blunted by Akt inhibition. Analysis of T cell differentiation under anti TNF α conditions highlighted a role for both TNF-α and Akt signalling in driving inflammatory Th1 and Th17 cell metabolism and function.

This work provides novel insight into the role of inflammatory cytokines in regulating immune cell metabolism and may aid future developments of anti-TNF-α biologics in the treatment of inflammatory disease.

Cellular iron restriction impairs T-cell metabolism and epigenetic regulation

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Iron-deficiency affects ~2 billion people, and ~2% of human genes encode iron-interacting proteins that operate in processes including mitochondrial metabolism, epigenetic regulation and DNA synthesis. Low iron profoundly impairs T-cell immune responses, but the mechanisms underlying this phenotype remain unclear.

Using in silico methods we predicted that T-cell iron content dramatically increases post-activation and that pathways including histone demethylation and oxidative phosphorylation may be particularly impaired by iron-deficiency. Consistent with the predicted importance of iron for T-cell chromatin remodelling, we observed that iron starvation impaired the removal of the repressive histone marker, H3K27me3, during in vitro CD4+ Th17 polarisation. Concurrently, iron limitation suppressed Th17 differentiation, RORγt and IL-17a expression. We next performed transcriptomic and proteomic surveys of iron depleted CD8+ T-cells to identify molecular consequences of iron scarcity. We observed alterations in metabolic gene sets including suppression of mTOR signalling pathways and enrichment of beta-oxidation proteins.

In parallel, to pinpoint crucial metabolic bottlenecks during iron-deficiency, we screened nutrients for their capacity to rescue T-cells during iron scarcity. Aspartate supplementation substantially ameliorated the inhibitory effects of iron-deficiency on T-cell proliferation and effector function. Further, T-cells lacking the dNTP degrading enzyme, SAMHD1, phenocopied aspartate supplemented iron starved T-cells suggesting that aspartate derived dNTPs may be limiting during iron starvation.

Our data indicate that low iron impairs key T-cell pathways, including nucleotide metabolism, but that specific interventions can overcome sensitivity to iron-deficiency. Our work provides metabolic and epigenetic mechanisms that may be the basis for the inhibition of T-cell responses by iron deprivation.

The source of dietary fat, rather than obesity, influences anti-tumour immunity

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Obesity increases the risk of many cancers and impairs immunosurveillance. However, little is known about whether the source of dietary fat affects tumour growth and anti-tumour immune responses. Here, we show that a high-fat diet (HFD) from animal sources (lard or butter), but not plant sources (palm, coconut, peanut oils) accelerates tumour growth, despite similar levels of obesity and adipose distribution. Animal-derived HFDs impaired NK and CD8 T cell metabolism by two distinct cell specific mechanisms leading to loss of IFNγ. The animal-derived butter versus plant-derived palm oil yielded the most different results and were used to investigate the mechanism further. Butter significantly accelerated tumour growth while palm-fed mice were completely protected from obesity-induced tumour growth despite equal obesity. Both HFDs caused a systemic metabolic switch to β-oxidation and impaired glucose handling regardless of fat source. However, at the metabolome level, critical differences were revealed which impacted tumor growth and immunity. Animal-derived HFDs resulted in incomplete fatty acid oxidation in key metabolic organs, causing an accumulation of circulating long chain acylcarnitines, particularly octadecanoyl-carnitine which completely impaired CD8 T cell metabolism and function. Furthermore, animal fat, but not plant fats

caused accumulation of intracellular lipids in NK cells, resulting in increased ROS, metabolic paralysis, and impaired IFNγ production. However, in plant-derived HFDs, NK cells activated antioxidant pathways involving Nrf1 and Nnt as a protective mechanism from lipotoxicity. Animal fat diets are therefore more harmful to anti-tumour immunity, which may inform the nutritional status of cancer patients to enhance traditional cancer therapies.

The Metabolic Basis of Postoperative T cell Immune-Suppression

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The immune response to surgery is characterised by broad postoperative immunosuppression of the adaptive arm and has been associated with increased postoperative morbidity. With age, reduced thymic output and aberrant homeostatic proliferation leads to a reduced lymphocyte count circulating in the periphery, leaving older individuals vulnerable to infection following surgical procedures. Metabolism has been implicated in these mechanisms, with lymphopenic individuals exhibiting mitochondrial dysfunction, increased apoptosis, and aberrant migration. Here we further characterise the T cell response to surgery and identify metabolic pathways associated with postoperative outcomes.

We recruited patients over the age of 55 undergoing major elective surgery, obtaining pre-operative, postoperative day (POD) 3 and POD7 blood samples. Flow-cytometry and single cell energetic metabolism by profiling translation inhibition (SCENITH) was used to phenotype and characterise perioperative T cell metabolism. These data were validated via bulk RNA sequencing and glutamine flux analysis.

Compared to baseline, surgical patients had a reduced frequency of CD3+ T cells at POD3, with CD4+:CD8+ ratios increasing by POD7. The metabolic T cell response to surgery was accompanied by increases in both glucose and glutamine dependence and a reduction in both mitochondrial and fatty acid oxidation dependence at POD3. By POD7, these changes reverted to levels comparable to baseline. Correlation of metabolic dependence values revealed an association between preoperative glutamine dependence and postoperative lymphocyte count.

These findings further characterise the mechanisms of postoperative immunosuppression and emphasise the importance of T cell metabolic fitness during the perioperative period.

PostDocs Bright Sparks: short talk – selected abstracts

Carbon source availability drives nutrient utilization in CD8+ T cells

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T cell function is highly influenced by metabolic state; however, how environmental nutrient availability impacts T cell metabolism and function remains poorly understood. Recent work using media formulations that mimic physiologic metabolite concentrations has revealed metabolic dependencies in cells not observed in conventional cell culture media. Here, we examined the impact of physiologic nutrient availability on the metabolism and function of CD8+ T cells. We report that the presence of physiologic carbon sources (PCS) in T cell media broadly impacts glucose utilization in CD8+ T cells and does so independent of transcriptional changes in T cell metabolic reprogramming. Stable isotope labeling (SIL) revealed that the presence of PCS reduced glucose contribution to the TCA cycle, and that a subset of PCS—acetate, beta-hydroxybutyrate (βOHB), and lactate—directly fuel the TCA cycle in proliferating CD8+ T cells. The addition of PCS was also sufficient to increase IFN-γ production by CD8+ T cells. We demonstrate that CD8+ T cells responding to Listeria infection preferentially consumed lactate over glucose as a TCA cycle fuel, with lactate enhancing both T cell bioenergetic capacity and contributing to biomass (i.e., pyrimidine nucleotide and lipid biosynthesis). Inhibiting lactate-dependent T cell metabolism in CD8+ T cells by silencing lactate dehydrogenase A (Ldha) impaired both CD8+ T cell metabolic homeostasis and proliferative expansion in vivo. Together, our data indicate that carbon source availability shapes T cell glucose metabolism and identify lactate as a bona fide fuel for bioenergetic and biosynthetic processes in effector T cells.

IL-17A drives weight loss and adipose tissue remodelling during chronic Trypanosoma brucei infection in a sex-dependent manner

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Trypanosoma brucei, the causative agent of Human African Trypanosomiasis, leads to cachexia and white adipose tissue (WAT) wasting, which may compromise the systemic immune response. However, the processes leading to these pathologies remain unclear. WAT is a critical metabolic organ, regulating not only systemic energy homeostasis but also fuelling the immune response to infection. Upon chronically infecting mice with T. brucei, we observed dramatic weight loss in males but not females, with significantly greater reduction in subcutaneous WAT (scWAT) mass in males compared with females. Bulk transcriptomic and pathway enrichment analyses of male and female scWAT revealed upregulation of T cell-associated transcripts in males only, including Cd4, Il17ra and Il17c. Additionally, we identified that circulating levels of a key regulator of WAT function, interleukin 17A (IL-17A), is elevated in the serum of mice and humans during chronic infection. To test whether IL-17A is important in the adipose tissue response to infection with T. brucei, we infected IL-17A knockout mice and found that the weight loss observed in males was abrogated. Unexpectedly, all major adipose tissue depots increased in size in naïve male IL-17A knockouts compared with their wild type counterparts, reinforcing the proposed role of IL-17A in regulating adipose tissue homeostasis. This work provides novel insights into the mechanisms underpinning weight loss and adipose-immune interactions during chronic infection. We propose that IL-17A links cellular and systemic immunometabolism and is a sex-specific driver of weight loss during chronic infection. This has critical implications for the stratification of treatment for infected patients.

Single cell analysis of SLC1A5 (ASCT2) activity into T cells using unnatural amino acids with click chemistry handles

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System level analysis of single cell data is rapidly transforming the field of immunometabolism. However, metabolic profiling by flow and mass flow is limited by the availability of robust monoclonal antibodies and fluorescently labelled nutrient analogues. We report here that in T cells, the unnatural amino acids homopropargylglycine (HPG) and azidohomoalanine (AHA), visualized through bioorthogonal reaction with azide and alkyne functionalized fluorophores after their uptake, behave like amino acids that are transported via SLC1A5 (ASCT2). HPG and AHA uptake by activated T cells was dependent on sodium and could be blocked by competitive inhibition using alanine, but not lysine or methylaminoisobutyric acid (MeAIB). AHA was as efficient as alanine and glutamine, the primary natural amino acids transported by SLC1A5, in blocking the uptake of 3H labelled glutamine in cytotoxic T lymphocytes. Moreover, like glutamine, HPG and AHA uptake was dramatically increased in activated T cells versus naïve T cells. Taken together, our findings provide an easy procedure to assess which cells support their function via SLC1A5 mediated uptake of amino acids in a sensitive single cell assay, wherein click functionalized reporter molecules can be introduced to match the preferred detection method.

The NDUFA4 family of mitochondrial proteins mediate electron transport chain remodeling during inflammation

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Mitochondria are highly dynamic organelles that respond to intra- and extra-cellular cues to fine-tune their function. This regulation underlies the functionality of immune cells in health and disease. One form of mitochondrial remodeling is the switching between alternative isoforms of respiratory chain subunits. NDUFA4 (otherwise called COXFA4) is an accessory subunit of the cytochrome c oxidase complex (CcO). Possible isoforms of this subunit exist within the genome, but their regulation and function are not well described. We have shown that the protein encoded by the little-studied gene C15orf48 is an isoform of the NDUFA4 subunit, which is expressed following inflammatory activation of macrophages. C15ORF48 protein then replaces NDUFA4 within the macrophage CcO complex. This switch is facilitated by a second product of the C15orf48 gene, microRNA-147b, which targets NDUFA4 mRNA. C15orf48 is strongly upregulated in inflammation-associated diseases, including rheumatoid arthritis and severe COVID-19, and macrophages from individuals bearing NDUFA4 mutations show elevated inflammatory cytokine and chemokine production. Preliminary investigation of differential interaction partners of the two proteins identified PARP1 as preferentially binding to NDUFA4, which may give insight into the role of this network in mitochondrial function. These results highlight potentially novel mechanisms of immune cell regulation that are highly relevant to inflammatory diseases in humans, where C15orf48 is frequently one of the most strongly upregulated genes. This work sets the scene for further investigation into the functions of these poorly understood mitochondrial proteins.

A glycogen-fuelled metabolic program controls early MAIT cell effector responses

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Mucosal Associated Invariant T (MAIT) cells are an subset of innate T cells which play a key role in host protection against bacterial and viral pathogens. MAIT cells recognise bacterial ligands presented by the MHC class-I like molecule MR1, but can also respond to inflammatory cytokines independent of their TCR. Upon activation can MAIT cells rapidly increase their production of cytokines (e.g. IFNy) and lytic molecules (e.g. Granzyme B). Most recently MAIT cells have been noted for their robust anti-cancer properties, with their relative abundance, unrestricted properties and potent effector function highlighting them as exciting potential candidates for immunotherapy in cancer. Our previous work has highlighted the importance of glucose metabolism for MAIT cell cytokine responses, however the timeframe for MAIT cell cytotoxicity (<2 hours) does not align with the time required to fully engage glycolysis (>6 hours). Therefore we investigated the metabolic requirements for early MAIT cell effector responses. We show that within 3 hours MAIT cells can rapidly increase their production of effector molecules such as IFNg and granzyme B, whilst potently killing target cancer cells. Furthermore, we show that these early effector responses are independent of glucose metabolism, which is critical for responses at 18 hours. Finally, we show that MAIT cells rapidly increase the machinery required for the breakdown of glycogen, and that inhibition of this metabolic programme limits MAIT cell cytotoxicity and early cytokine production. Collectively, our data details a novel metabolic program which supports MAIT cell "innateness".

ZFP36 and ZFP36L1 integrate transcriptome and metabolic reprograming of activated mouse CD4+ T cells

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Following stimulation, resting CD4+ T lymphocytes undergo rapid and dynamic changes in gene expression, accompanied by metabolic reprogramming. The ZFP36 family of RNA binding proteins act post-transcriptionally as negative regulators of gene expression. We combined quantification of mRNA transcription, stability, abundance and translation with identification of direct ZFP36-family target mRNAs and metabolic profiling to investigate the roles of these proteins in regulation of CD4+ T cell activation and differentiation. ZFP36 and ZFP36L1 were implicated in the control of genes driving anabolic processes, including direct targeting of numerous transcripts encoding rate-limiting enzymes and transcription factors. ZFP36 and ZFP36L1 limit glutaminolysis in activated T cells, and were further identified as limiting factors for the acquisition of the cytotoxic CD4+ T cell phenotype. Our data reveal potential roles for ZFP36 and ZFP36L1 in shaping the metabolism and differentiation of activated CD4+ T cells, through direct targeting of critical genes that drive these processes.

Short talk – selected abstracts

Salmonella subverts T cell activation during bacterial cancer therapy by inducing metabolic paralysis

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Bacterial cancer therapy (BCT) is a promising therapeutic for solid tumours. Salmonella enterica Typhimurium (STm) is well-studied among bacterial vectors due to advantages in genetic modification, metabolic adaptation, and motility. A longstanding paradox has been the redundancy of T cells for treatment efficacy; instead, STm BCT depends almost exclusively on innate phagocytes for tumour control. Here, we used TCR reporter mice (Nr4a3-Tocky-Ifng-YFP) and a colorectal cancer (CRC) model to interrogate T cell activity during BCT with an attenuated STm mutant. We found TILs produced IFN-γ predominantly in absence of recent TCR activity (IFN-γ+Timer-neg), exhibiting reduced polyfunctionality and TCR responsiveness. Modelling T-cell:tumour interactions with a tumour organoid platform revealed that soluble signals from the infected tumour could potently modulate TCR-driven T cell activation in a cell-intrinsic manner, but does not distrupt activation by cytokine cocktails. Investigating TCR signalling showed intact nuclear NFAT/NF-kB translocation, but severe disruption to the metabolic signalling networks required for full T cell activation. Our work shows for the first time that T cells are metabolically impaired during STm BCT—elucidating a decades-long enigma—and providing a fundamental target for augmenting treatment efficacy.

Glutamine metabolism is required for optimal IL-17 production by γδ T cells through maintenance of redox balance

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IL-17 is a proinflammatory cytokine that contributes to a wide range of immune responses, including host defence, autoimmunity, and tumour progression. γδ17 T cells are a small population of unconventional T cells, which emerge from the thymus developmentally pre-programmed to rapidly produce IL-17 in response to cytokine signals.

γδ17 T cells are characterised by high rates of oxidative metabolism, which is required for optimal IL-17 induction in response to cytokine activation. High rates of oxidative metabolism is associated with endogenous reactive oxygen species (ROS) production, and data from our lab and others has shown that γδ17 T cells are particularly susceptible to ROS-induced dysfunction compared to their IFN-γ-producing counterparts. Treatment of cells with increasing concentrations of H2O2 during cytokine activation dose-dependently reduced γδ17 T cell metabolic and functional capacity, including lipid uptake, mTORC1 signalling and IL-17A production.

Furthermore, we show that glutamine metabolism is required for optimal IL-17 induction by γδ17 T cells, through buffering of cellular ROS and maintenance of energy metabolism. Inhibition of glutaminase (GLS) resulted in increased cellular ROS and reduced IL-17 production by γδ17 T cells. Supplementation with N-acetyl cysteine during cytokine activation abrogated the effect of glutaminase inhibition on IL-17 production, suggesting glutamine metabolism is required for optimal ROS buffering in γδ17 T cells. Moreover, inhibition of glutaminase resulted in increased glycolytic profiles in γδ17 T cells, suggesting glutamine metabolism is also required to maintain energy flux through the mitochondria. Overall, these data show that glutamine metabolism is required for optimal γδ17 T cell function through maintenance of redox balance.

The oncometabolite succinate suppresses T cell anti-tumour function via inhibition of mitochondrial glucose oxidation

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Succinate dehydrogenase (SDH) loss-of-function mutations drive succinate accumulation in tumour microenvironments, for example in the neuroendocrine tumours pheochromocytoma (PC) and paraganglioma (PG). Control of innate immune cell activity by succinate is described, but effects on anti-tumour T cell function have not been interrogated. Here, exposure of human CD4+ and CD8+ T cells to tumour-associated succinate concentrations suppressed both degranulation and cytokine secretion, including of anti-tumour IFN-γ. Mechanistically, this was associated with T cell succinate uptake -partly mediated via the monocarboxylate transporter 1 (MCT1)-, inhibition of succinyl-CoA synthetase activity and impaired glucose flux through the tricarboxylic acid cycle. Consistently, pharmacological and genetic interventions restoring glucose oxidation rescued T cell function. To confirm this suppressive activity in vivo, we interrogated tumour RNA-seqencing data from patients with PC and PG, which revealed a profound suppression of IFN-γ-induced genes in SDH-deficient tumours compared to those with other mutations, supporting a role for succinate in modulating the anti-tumour immune response.

Glucagon like peptide-1 analogue therapy restores Natural Killer cell metabolism and cytokine production in people with obesity

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Obesity is associated with significant defects in Natural killer (NK) cells. People with obesity (PWO) have lower NK cell numbers which are less able to kill target cells and have deceased capacity to produce cytokines. This attenuated function is driven by a defective cellular metabolism which stunts the NK cell's ability to function correctly. GLP-1 is a naturally produced gut hormone involved in glucose homeostasis and satiety. Long acting analogues of GLP-1 are showing great effectiveness as both anti- diabetic and weight loss agents. In addition to the classical effects of GLP-1, it has been reported to have direct immunomodulatory activity. In this study we examined the effect of 6 months GLP-1 analogue therapy on NK cell function and metabolism in a cohort of PWO. We also investigated if GLP-1 related changes in NK cells were due to weight loss or direct. Like previous studies we found that PWO have reduced NK cell frequencies, paired with defective cellular metabolism and cytokine production. After 6 months of GLP-1 therapy we demonstrate improved IFNγ and Granzyme B production in PWO, which is paired with improved cellular metabolism (elevated CD98 expression, mTOR activity and HK-II levels). Interestingly, we show that these effects are independent of weight loss, and that GLP-1 can directly improve the metabolism and function of NK cells from PWO. In conclusion we demonstrate the GLP-1 therapy can restore NK cell functional responses in obesity independent of weight loss.

Metabolic stress induces tumour derived microRNA-21 to limit anti-tumour macrophage responses

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The role of the oncogenic yet anti-inflammatory microRNA-21 (miR-21) in the tumour microenvironment (TME) is not clearly defined, despite its up-regulation in many cancers. Since re-education of macrophages by tumour derived signals is a central step in cancer pathology, we investigated the role of miR-21 in this process. Up-regulation of miR-21 was confirmed in patient samples from primary and metastatic liver cancer, and this was associated with poorer prognosis and reduced immune infiltrate. Subsequently, it was found that co-culture of naïve human macrophages with the hepatocellular carcinoma derived cell line, HepG2, induces miR-21 expression, triggering macrophage reprogramming to an immuno-regulatory phenotype. Depletion of miR-21 in macrophages enhanced the induction of pro-inflammatory mediators such as TNF, boosting their anti-tumour capacity. Interestingly, up-regulation of mature miR-21 levels in co-cultured macrophages was not preceded by induction of the primary miR-21 transcript and was instead enriched in secreted exosomes from tumour cell lines. Another well recognised feature of tumours is their ability to utilise glucose to support the energy and biosynthetic demands of rapid proliferation. Previous work identified miR-21 as a key regulator of glycolysis in activated macrophages. Blocking exosome release led to an accumulation of tumour cell miR-21, significantly impairing glycolysis. Furthermore, metabolic stress in tumour cells impacted miR-21 expression in exosomes and associated up-regulation of miR-21 in macrophages. This suggests a model whereby the export of miR-21 has the dual benefit of allowing cancer cells to pursue uninhibited glycolysis while driving pro-tumour macrophage reprogramming.

Allergen exposure induces airway macrophage metabolic reprogramming

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Airway macrophages (AMs) are strategically located at the interface of the internal and external lung environment and form the first line of defence against invading pathogens. While AMs are key to maintaining immune tolerance, the mechanism by which AMs regulate lung homeostasis are poorly understood. Asthma is a chronic disease of the airways and most asthma patients are allergic to the aeroallergen house dust mite (HDM). Upon pro-inflammatory activation, macrophages undergo metabolic reprogramming from oxidative phosphorylation towards glycolysis, a switch required for many aspects of macrophage function. However, how aeroallergen affects the AM metabolic phenotype is unknown.

We measured metabolite levels in airway samples of a human allergen challenge model and murine models of HDM-induced allergic airway disease (AAD) by targeted GC-MS. Using qPCR and Seahorse extracellular flux assays, we analysed the metabolic phenotype of primary murine AMs following in vivo and ex vivo HDM stimulation.

Allergen challenge in mild asthmatics induced significant changes in sputum lactate and the TCA cycle metabolites itaconate, succinate, fumarate and malate. Similar alterations were seen in bronchoalveolar lavage of mice with AAD. Moreover, both AMs sorted from HDM-challenged mice and naïve AMs challenged ex vivo with HDM, developed a highly glycolytic phenotype, characterised by increased extracellular acidification and expression of glycolysis enzyme genes. Of note, the metabolic shift towards enhanced glycolysis was TLR4- and protease-independent.

Overall, our data show that inhaled allergen induces profound changes in metabolite levels of human and murine lungs and drives metabolic reprogramming of AMs towards glycolysis.

SLC7a5 amino acid transporter regulates metabolic responses in activated NK cells in vivo

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Natural Killer (NK) cells regulate nutrient uptake upon activation to meet the metabolic demands for antitumour and antiviral immunity. SLC7a5 is the predominant system L amino acid transporter expressed in activated NK cells. Previous work has shown that blocking amino acid uptake through Slc7a5 in NK cells in vitro disrupted metabolic pathways and NK cell effector function. In this study we have studied an NK cell specific Slc7a5 KO mouse. NK cells develop normally in this mouse and are found at normal numbers in naïve mice. These mice were then challenged with Poly(I:C), a double stranded RNA molecule, that acts as a simple mimic of RNA viral infection. The data reveal that in Poly I:C challenged mice SLC7a5 null NK cells have metabolic defects beyond reduced amino acid uptake. In particular, the levels of CD71 expression, the transferrin receptor, and of transferrin-iron uptake are reduced. Initial data suggests that this may be due to altered mTORC1/cMyc signalling. Further supporting metabolic deficiency in these cells is data showing that SLC7a5 KO NK cells have decreased mitochondrial mass and reduced IL15-induced proliferation. In terms of functionality, SLC7a5 KO NK cells have impaired production of IFN and reduced expression of key cytotoxic molecules granzyme B and perforin. SLC7a5 KO NK cells from In Poly(I:C) treated mice show significantly reduced cytoxicity towards tumour cells directly ex vivo.

Poster presentations

P.01 Reprogramming of bone-marrow myelopoiesis by β-glucan can rescue the immune-metabolic impairments induced by high-fat diet

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With the conceptualisation that innate immune cells possess memory-like properties, multiple stimuli have emerged which train myeloid cells in protective or pathogenic contexts. Inflammation associated with western diet have both been shown to drive myelopoiesis and enhance the inflammatory function of mature macrophages which underlie metabolic inflammation in cardiovascular disease and diabetes. Strategies which attenuate this pathogenic training hold promise for improved treatments for metabolic disorders. We examined if treatment with a fungal β-glucan, known to drive protective trained responses myeloid cells, would modulate immune function and induce a protective trained immunity phenotype. We demonstrated that the s.cerevisiae-derived whole glucan particle (WGP), a dietary fibre, can drive trained immunity responses in mouse bone marrow derived macrophages and via intraperitoneal injection of mice, by increasing myelopoiesis in bone marrow cells. Oral delivery of WGP drives myelopoiesis and alters the function of BMDMs from these mice. Mice fed a high-fat diet also increased myelopoiesis. Mature BMDMs from HFD fed mice had enhanced responses to restimulation with TLR ligands. However, when HFD was supplemented with WGP the BMDMs from these mice displayed attenuated responses to restimulation. In a dysbiosis-induced model of metabolic disease, whereby humanised microbiomes from obese-but-healthy/-diabetic patients were transplanted into mice fed a HFD, WGP supplementation reduced metabolic defects including decreased glucose tolerance and hepatic lipid levels, despite no changes in total weight gain. This data implies the WGP supplementation can alter the inflammatory phenotype of myeloid progenitors and mature progeny in a HFD context, which can impact disease progression. Further work is ongoing to identify the cellular substrates for both HFD training signals and reprogramming by WGP by examining the myeloid populations in the gut, liver and bone marrow compartments.

P.02 Human Mucosal Associated Invariant T cell proliferation is dependent on a MYC-SLC7A5-Glycolysis metabolic axis

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Mucosal Associated Invariant T (MAIT) cells are an abundant population of innate T cells which recognise bacterial ligands presented by the MHC class-I like molecule MR1. MAIT cells play a key role in host protection against bacterial and viral pathogens. Upon activation MAIT cells undergo

proliferative expansion and increased production of effector molecules such as cytokines. The molecular and metabolic mechanisms controlling MAIT cell effector functions are still emerging. In this study, we found that expression of the key metabolism regulator and transcription factor MYC is upregulated in MAIT cells upon immune stimulation. Using quantitative mass spectrometry, we identified the activation of two MYC controlled metabolic pathways; amino acid transport and glycolysis, both of which are critical for MAIT cell proliferation. Finally, we show that MYC expression in response to immune activation is diminished in MAIT cells isolated from people with obesity, resulting in defective MAIT cell proliferation and functional responses. Collectively our data details for the first time the importance of MYC regulated metabolism for MAIT cell proliferation, and provides additional insight into the molecular defects underpinning functional failings of MAIT cells in obesity.

P.03 Regulation of Glycolysis and the Pentose Phosphate Pathway in Mtb infected macrophage by the PFK-1 complex

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Increased glycolytic metabolism has emerged as a key process in mediating host defence against Mycobacterium tuberculosis (Mtb). However, little is known about the implications of host/pathogen interactions in this immune response. Previous work in our lab identified dampening of glycolysis during Mtb infection through sustained upregulation of anti-inflammatory microRNA-21 (miR-21). Subsequent targeting of mRNA encoding phosphofructokinase muscle (PFK-M) isoform at this committed and rate-limiting step of glycolysis by miR-21 results in diminished host responses, primarily through reduction in levels of pro-inflammatory IL-1b. However, abolition of glycolysis in Mtb-infected macrophages also alters levels of NO and ROS which could be linked to pentose-phosphate pathway activity. Recent work suggests that targeting PFK-1 isoform expression can alter the balance between glycolytic flux and PPP and we hypothesise that Mtb infection specifically alters PFK-1 isoform expression to reprogramme host metabolism in its favour through increased PPP. Gene expression and enzyme activity analysis reveals that infection with viable Mtb favors up-regulation of oxidative-PPP associated genes including G6PDH and 6PGD while repressing pro-glycolytic Pfk-m. Further work such as PPP inhibition aims to determine the importance of this anabolic pathway and associated processes in Mtb host defence, to determine if increased flux through PPP favors mycobacterial replication through by-passing oxidative metabolism and instead using PPP-derived NADPH to fuel de novo lipid/fatty acid synthesis which creates an environment permissive to Mtb replication.

P.04 Maternal immunometabolism adaptation in monocytes underpins functional changes during pregnancy

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Background: Pregnant women undergo metabolic and immunologic changes to ensure pregnancy success. Immunometabolism might provide a framework for understanding the immunologic changes of discrete maternal leukocyte subsets. Monocytes are known to undergo metabolic adaptation in response to different environmental cues and are likely key contributors to dynamically altered pro- and anti-inflammatory balance over pregnancy.

Methods: Monocytes were isolated with magnetic beads from peripheral blood of non-pregnant women (aged 18-40 years) and pregnant women at term (37+ weeks).

Results: NanoString© analysis revealed genes in various metabolic pathways were altered in monocytes from pregnant women: reductions in the pathway score for mTOR ($p=0.0004$) and NF-κB ($p=0.0004$); increases for AMPK ($p=0.0004$). Bioenergetic analysis revealed reduced maximal mitochondrial respiration ($p=0.0087$) with pregnancy; glycolysis was unaffected. Lower mitochondrial ($p=0.0024$; flow cytometry) and cardiolipin ($p=0.0357$; mass spectrometry) content was seen. As LPS-stimulated monocytes rely on glycolysis for their functional output, an alternative

PAMP that relies on OXPHOS was identified to unravel the functional consequences of perturbed mitochondrial function. Monocytes rely on OXPHOS when stimulated with MDP (increased basal respiration p=0.0162) and have reduced production of TNF in the presence of mitochondrial inhibitors (p=0.0146). IL-6 and TNF production in MDP-stimulated monocytes from pregnant women was reduced (p=0.0172; p=0.0068); the LPS-stimulated response was unchanged.

Conclusions: Pregnancy programmes late gestation monocytes by down-regulating their metabolic capabilities, especially oxidative phosphorylation. Understanding this natural plasticity in monocyte phenotype and function with pregnancy could yield new therapeutic approaches to metabolic and or immune-mediated diseases and reveal targets for improving pregnancy outcomes.

P.05 Understanding dendritic cell metabolism in the influenza infected mouse lung

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Dendritic cells (DCs) link the innate and adaptive immune response during anti-viral response. One molecular driver underpinning DC activation is an increase in metabolism to fuel effector functions but the metabolic profile of DCs during viral infection remains poorly defined.

We investigated which DC subsets take up influenza A virus (IAV) following infection. Flow cytometry analysis demonstrated that both CD103+ and CD11b+ DCs are positive for intracellular nucleoprotein 2- and 5-days following infection. This led us to examine the metabolic impact on bone marrow derived DCs, representative of CD11b+ cells, following in vitro exposure to IAV.

Using liquid chromatography mass spectroscopy (LCMS) we have found global metabolic changes 24 hours after exposure of DCs to IAV. This included a decrease in the cellular commitment to glycolysis and an increase in the metabolites associated with the TCA cycle and oxidative phosphorylation. Flow cytometry confirmed these data with elevated TCA metabolic enzyme activity including isocitrate dehydrogenase (IDH).

We also detected changes in molecules underpinning DC activation. DCs displayed a diversion of arginine to citrulline and toxic mediator nitric oxide (NO) as well as significant increase in the TCA cycle anti—inflammatory metabolite, itaconate. These changes may suggest that metabolic switching in DCs plays a role in initiating the immune response to viral infection.

These early changes to DCs may be important for the generation of immune memory. Future work will examine the 'metabolic rewiring' of lung DCs after IAV infection, providing mechanistic insight into the immunometabolism of the lung DCs.

P.06 Increasing the anti-cancer functions of NK-92 NK cells through targeting cellular metabolism

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NK cell-based immunotherapy has become a promising cancer treatment for cancer. One approach is the use of NK cell lines such as NK-92 as this overcomes many of the technical challenges associated with the use of human NK cells isolated from the blood. One of the major restrictions for the activity of NK cells against solid tumours are the conditions within the tumour microenvironment (TME) including adverse metabolic conditions. The Finlay lab has discovered that cellular metabolism is closely linked to NK cell anti-tumour functions, including NK cell cytotoxicity. However, little is known about the metabolic requirements for NK-92 to mediate anti-tumour cytotoxicity. This study investigated the metabolism of NK-92 cells and the importance for metabolic regulators, including mTORC1 and SREBP, in sustaining NK-92 cytotoxicity. Direct inhibition of metabolic pathways inhibited NK-92 cytotoxicity. While IL-2 signaling is required for NK92 cytotoxicity, the activity of the downstream signaling through mTORC1 and Srebp is dispensable. Interestingly, the oxidized cholesterol molecule 25-hydroxycholesterol (25-HC), a known inhibitor of SREBP activation,

potentially inhibited NK-92 cytotoxicity and mitochondrial metabolism, but this appears to be independent of SREBP. 25-HC can be made by tumour associated macrophages (TAMs) that express cholesterol-25-hydroxylase. These data argue that 25-HC in the TME would inhibit NK-92 anti-tumour activity.

Understanding NK92 cell metabolism will support designing better cell-based cancer therapeutic strategies in future. The data emerging from this project suggests that one strategy would be to engineer NK-92 cells to be resistant to the actions of 25-HC.

P.07 Mushroom digestion as a model to study gut-immune system cross-talk via Innate Immune Training

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Mushrooms have been used for thousands of years for their medicinal properties. The unique immune-modulatory nature of mushrooms is likely determined by distinct structural features of their cell wall, which contains high levels of β glucan polysaccharides. β glucans are known inducers of trained immunity, a phenomenon by which innate immune cells are metabolically and epigenetically altered following exposure to a primary stimulus, which boosts protective responses to infection.

Our study has shown that intact Agaricus Bisporus mushroom extracts have net anti-inflammatory properties in-vitro, which can mitigate pathogenic over-stimulation of the immune system, while crude lab-purified extracts containing β glucan can train macrophages. We are now refining this work by examining the immunomodulatory effects of mushroom extracts in the gut. We are performing in-vitro digestion experiments to reproduce jejunal samples of digested mushrooms, and characterising the dominant bio-molecular intermediates and metabolites of these samples using structural, biochemical and immune assays. We hypothesise that digestion releases β glucans from the mushroom cell wall, where they interact with local patrolling macrophages in the gut lining and induce training. To bolster our in-vitro work, we are performing feeding studies and oral gavage with mushrooms in mice, to look at known markers innate immune training.

This work aims to characterise the interaction between the mushroom cell wall and the gut during digestion to investigate the role of trained immunity as a medicinal property of mushrooms.

P.08 IL-4 Trained Macrophages Require OXPHOS to Enhance Protective Responses Against Mycobacteria

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Macrophages are innate immune cells that possess two highly adaptive capabilities: polarisation and innate memory. In the short-term, polarisation into the "classically activated" M1 macrophage enhances microbicidal and pro-inflammatory responses to an imminent challenge. In the long-term, innate training with stimuli such as β-glucans and the BCG vaccine causes enhancement of similar responses. Not only are the outcomes induced by these adaptive mechanisms very similar, but a key component of both is a metabolic switch towards heightened use of glycolysis as source of energy.

Macrophages can also polarise into an "alternatively activated" M2 phenotype, that can inhibit M1-driven inflammation and is associated with upregulated mitochondrial oxidative phosphorylation. Despite this metabolic profile and accompanying responses differing significantly from M1 polarisation and innate training, our results reveal that M2 polarisation induces protective innate memory. Secondary stimulation with toll-like receptor (TLR) agonists and killed mycobacteria, one week after M2 polarisation, revealed amplified production of the pro-inflammatory cytokines IL-6 and TNFα, as well as nitric oxide (NO) production. By contrast, secretion of the regulatory cytokine

IL-10 was not increased. Furthermore, M2-induced memory enhanced both uptake and killing of mycobacteria in vitro.

These M1-type responses were not accompanied by a metabolic switch towards enhanced glycolytic metabolism, instead oxidative phosphorylation remains upregulated and is further induced by secondary stimulation. This suggests that the hypothesised intrinsic links between heightened glycolytic metabolism with pro-inflammatory responses and innate training may not be applicable for all stimuli. These results suggest that M2-type macrophage activation counterintuitively induces beneficial antimicrobial innate training.

P.09 Hypoxia suppresses TCR signalling, activation and effector function of CD8+ T cells

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Microenvironmental hypoxia may drive immunosuppression and disease progression in cancer. Here, we aim to understand precisely how hypoxia influences CD8+ T cell anti-tumour capacity through analysis of key immune functions, cellular metabolism and signalling pathways. To do so, CD8+ T cells from human peripheral blood are pre-conditioned overnight in either normoxic (21% oxygen) or hypoxic (1% or 0.3% oxygen) conditions prior to anti-CD3/anti-CD28 stimulation and analysis. As early as 8 hours, IFN-gamma secretion by hypoxic CD8+ T cells is reduced and effects are sustained to 72 hours. Conversely, TNF-alpha secretion and cytotoxicity (granzyme-B release and CD107a externalisation) do not change, indicating specific rather than generalised effects. IFN-gamma suppression was not associated with nutrient restriction, since glucose concentrations in hypoxic culture media were not decreased but rather increased in hypoxic vs. normoxic cultures, potentially indicative of poorer activation. In agreement with this, expression of CD25 was also reduced in hypoxia, whilst CD69 increased. To further probe for defects in T cell activation we employed the Nr4a3-Tocky reporter system, which identified substantially delayed and suppressed activation of T cells in hypoxic conditions. Consistently, in human T cells we observed decreased phosphorylation of T cell receptor (TCR)-signalling proteins, nuclear NFAT translocation and expression of TCR-induced genes. Taken together the data indicate that hypoxia impairs T cell activation and certain downstream effector functions through direct effects on signalling pathways. Further work is needed to underpin the mechanisms driving this and define their relevance for CD8+ T cell suppression in the tumour microenvironment.

P.10 Repurposing canagliflozin for the treatment of T-cell mediated autoimmune disorders

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T-cell mediated autoimmunity is responsible for a range of chronic inflammatory conditions including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Unfortunately, current therapies are not always effective, leaving many patients with debilitating symptoms that severely restrict their quality of life, therefore highlighting the urgent requirement for novel treatments. It is known that aberrant T-cell metabolism underlies many autoimmune conditions, most of which have no cure. Gliflozins (canagliflozin and dapagliflozin) inhibit sodium-glucose transport leading to improved glycaemic control and are used to treat type 2 diabetes. Canagliflozin, but not dapagliflozin, has additional off-target effects inhibiting mitochondrial metabolism. This multi-targeted metabolic approach that canagliflozin offers is an attractive therapeutic option, highlighting the exciting potential of repurposing gliflozins to treat T-cell mediated autoimmune disorders. CD4+ T-cells were isolated from mice, peripheral blood of healthy donors and RA and SLE patients and exposed to gliflozins with/without cellular activation, and the effects on metabolism and function were determined using metabolomic, genomic and immunological methodologies. Here, canagliflozin has profound effects modulating human CD4+ T-cell metabolism by reducing glutamine entry into the TCA cycle and decreasing Myc and glycolysis pathway scores. Functionally, canagliflozin reduced activation marker expression (CD25, CD44 and CD69), cytokine secretion and blastogenesis in human T-cells and suppressed murine Th17 polarisation. Collectively, canagliflozin

has the ability to modulate CD4+ T-cell metabolism dictating downstream effector function and phenotype highlighting its potential as a treatment for autoimmunity.

P.11 Regulation of NK Cell Responses by Macrophage-Derived 25-Hydroxycholesterol

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Natural killer cells are cytotoxic lymphocytes with important anti-tumour and anti-viral functions. The Finlay lab has demonstrated that of 25-hydroxycholesterol (25-HC), and oxidised cholesterol metabolite, is a potent inhibitor of NK cell glucose metabolism and NK cell cytotoxicity (Assmann et al. 2017). An environment rich in 25-HC can occur during infection or within tumours due to the expression of the enzyme that makes 25-HC, cholesterol-25 hydroxylase (Ch25h), in macrophages. My research asks whether oxysterols like 25-HC produced and secreted by myeloid cells, such as Ch25h+ macrophages, can regulate the metabolism and function of NK cells in the local microenvironment.

To understand whether 25-HC generated by macrophages can inhibit NK cells responses during activation, NK cells were cultured with bone marrow derived macrophages (BMDM) (Ch25h-negative) or LPS-activated BMDM (Ch25h-positive) in the presence of activating cytokines, IL2/IL12. NK cells cultured with Ch25h-positive LPS-stimulated BMDM were smaller and expressed less granzyme B and IFN□ than those cultured in the presence of Ch25h-negative BMDM. This effect on NK cells was lost in the presence of Ch25h-knockout LPS-stimulated BMDM.

Does 25-HC similarly inhibit NK cells post activation? NK cells treated with IL2/IL12 for 20h and then exposed to 25-HC for a further 18 hours were viable and equivalent in size to control NK cells but had reduced perforin and interferon-γ expression alongside impaired cytotoxicity against tumour target cells.

These data suggest a new metabolic inhibitory pathway between macrophages and NK cells involving oxidised cholesterol metabolites that could have implications for anti-tumour immunity.

P.12 Placental macrophages from pregnant women with obesity have impaired function and metabolism

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Maternal obesity is associated with adverse obstetric outcomes, including spontaneous miscarriage, preeclampsia and increased infection risk for mother and baby. An altered immune-profile at the maternal-fetal interface has been observed in maternal obesity but the mechanisms involved remain enigmatic. Macrophages (Mϕs) are key contributors to inflammation and innate immunoregulatory function playing a significant role in the placenta. Mϕ metabolism regulates function in other tissue sites thus determining the metabolic and functional characteristics of placental macrophages (pMϕs) from women of differing body mass index (BMI) might provide insight into the adverse consequences of maternal obesity.

pMϕs were isolated from full-term placentas delivered by elective caesarean section. Functional and metabolic parameters of quiescent and lipopolysaccharide (LPS)-stimulated pMϕs from healthy weight (BMI=18.50–25kg/m^2) and obese (BMI≥35kg/m^2) women (n=4-10/group) were then determined. Metabolic gene expression analysis using Nanostring as well as ^{13}C-glucose tracing with gas chromatography-mass spectrometry revealed no differences between groups. However extracellular flux analysis demonstrated that pMϕs from women with obesity had significantly reduced oxidative phosphorylation and glycolytic parameters. These parameters included maximal respiration, spare respiratory capacity and maximal glycolysis (unstimulated p=0.0044, p=0.0243, p=0.0165; LPS-stimulated p=0.0016, p=0.0070, p=0.0010 respectively). Mitochondrial mass of pMϕs from women with obesity was also reduced (p=0.0519). pMϕs from women with obesity additionally displayed reduced phagocytic capacity (p=0.005) although their cytokine output was not altered.

Impaired phagocytic capacity of pMϕs from women with obesity may be linked to altered cellular metabolism. The extent to which this contributes to adverse outcomes associated with maternal obesity remains to be determined.

P.13 Rescue effect of previously synergistic drug combination Metformin and Diclofenac in T-Cell acute lymphoblastic Leukaemia cell lines

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T cell acute leukaemia (T-ALL) is an aggressive bone marrow-derived cancer causing the mass production of immature T cells preventing development of healthy cells, leaving these patients immunocompromised and vulnerable. Although 5-year survival rates are ≥85% in adults relapse rates are ~50 %. Moreover, treatment options are limited, and prognosis is poor.

Cancer displays metabolic flexibility to meet high energy demands required for rapid proliferation. Previous research has shown targeting metabolism has potential as a therapeutic avenue, without collateral cytotoxicity to healthy cells. Metformin an antidiabetic drug and Diclofenac an anti-inflammatory drug are both pre-approved with well-established safety profiles, potentially enabling fast-track repurposing. At physiological concentrations, these drugs display off-target affects inhibiting major metabolic pathways. Metformin is a mitochondrion inhibitor irreversibly binding to complex 1 of the electron transport chain, whilst Diclofenac inhibits the monocarboxylate transporters 1 and 4 (MCTs 1/4). MCTs are responsible for the transport of lactate influx (MCT1) and efflux (MCT4). Consequently, leading to the inhibition of glycolysis via intracellular lactate acidosis, subsequently inhibiting rate limiting enzymes of glycolysis. Thus, this combination targets two main energy generating pathways. Whilst research into this combination is limited it has shown promise in Gliomas and Acute Myeloid Leukaemia. T- ALL cell lines were subjected to various concentrations of drug combinations. Diclofenac alone induced cell death in the cell line panel. However, in combination with Metformin, we observed a concentration-dependant rescue effect. However, the metabolic pathways which orchestrate this effect are yet to be determined. This was explored using various techniques.

P.14 Long-chain bioactive ceramide C24 reduces Foxp3+ CD4+ Treg lymphocyte differentiation leading to inflammatory sequelae of obesity

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Dysregulated immune responses have been shown to induce and escalate obesity-induced inflammatory microenvironment (OIIM). OIIM is associated with multiple obesity-linked diseases including cardiovascular disease, type II diabetes and autoimmune diseases. (Hruby&Hu, 2016). Recent studies have highlighted the critical immunomodulatory potential of distinct bioactive lipids during the establishment of OIIM. Ceramides are a group of bioactive sphingolipids which may play a key role in promoting the OIIM due to their modulatory effects on T lymphocytes (Chaurasia et al., 2019). Contrarily, N-3 polyunsaturated fatty acids (omega-3) shown to have an anti-inflammatory action to counteract the obesity-induced inflammatory response. Omega-3 can induce this anti-inflammatory response via potentially blocking the corresponding receptors of ceramides and modulating the differentiation and motility of T lymphocytes (Mildenberger et al., 2017 & Cucchi et al., 2019)

Using a 3-week western diet murine model, we have identified an elevation of specific ceramide candidates, within the plasma, lymph nodes, spleen and fat using ultrahigh performance chromatography coupled with mass spectrometry. In relation to the inflammatory response, we see a significant reduction in regulatory T cell (Treg) population within the spleen by flow cytometry. In addition, Western diet supplementation with 10% omega-3 was shown to significantly boost the Treg population in spleen and mesenteric lymph nodes compared to western diet group, and also shown to reduce elevated ceramide levels to basal levels.

We therefore hypothesised that specific ceramides, elevated under a western diet, may drive T lymphocytes towards a pro-inflammatory cell fate by reducing Foxp3+ Treg population and that omega-3 supplementation could counter balance this response. To investigate the direct role ceramides may play on the CD4+ T cell response, an in vitro CD4+ T cell differentiation model was utilised. Murine or human naïve CD4+ T cells were differentiated into regulatory T cells in the presence of candidate ceramides. Flow cytometry and ELISA analysis of this in vitro model confirmed that C24 ceramide impaired Treg differentiation. Such an effect could potentially contribute to pro-inflammatory effects of ceramides in vivo.

P.15 The 'ins and outs' of biological Iron and Plasmacytoid Dendritic Cell (pDC) interferon responses

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Plasmacytoid dendritic cells (pDC) are a subset of DC which are implicated in antiviral immunity through their robust production of type 1 interferon and high expression of the endosomal TLRs. However, their metabolism has been poorly characterised and only studied using pDC differentiated from bone marrow in vitro, which are not fully representative of pDC in vivo. High resolution proteomic analysis of pDC isolated directly from the spleen of mice has uncovered new insights into the metabolic configuration adopted by pDC and revealed an iron signature to these cells.

It's well known that biological iron is an important immune mediator in other immune cells (NK/T-cells) to sustain proliferation and metabolism. We hypothesise that iron may be important for pDC function. We have shown that pDC express the highest level of CD71 in comparison to all other immune cell subsets in the spleen. We've also shown that pDCs constantly recycle iron-bound transferrin both while resting but also when activated with the TLR9 viral agonist CpGA. Moreover, we've shown that free iron availability is important for sustaining CpGA induced IFNa production in pDC over the short-term. Finally, we see that pDC possess an iron storage phenotype, expressing high levels of ferritin, potentially to buffer against conditions of low serum iron availability, as in certain viral infections (E.G. COVID-19), allowing pDC to continue to secrete IFNa.

This is the first time the importance of iron for pDC interferon responses has been described, highlighting a new role for biological iron in the immune system.

P.16 Circadian disruption alters the inflammatory response of lung fibroblasts: Implications for respiratory disease

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Fibroblasts are stromal cells abundant throughout tissues including the lung and are integral effector cells that coordinate the immune response. Circadian or 24-hour rhythms in the lung play a crucial role in mediating immune cell function through control of intracellular metabolic pathways. However, the role of circadian regulation of metabolism in the fibroblast immune response has yet to be investigated.
We find that upon IL-1β stimulation, lung fibroblasts lacking the core circadian clock protein BMAL1 (Bmal1-/ -fibroblasts) differentially express chemokine and growth factor genes in comparison to Bmal1+/+ fibroblasts. Bmal1-/- fibroblasts have significantly higher glycolysis, and inhibition of glycolysis reduces chemokine gene expression. We observe increased immune cell migration to IL-1β stimulated Bmal1-/- fibroblasts in comparison to Bmal1+/+ fibroblasts. Mice that are intranasally stimulated with LPS at two times of day show differences in immune cell recruitment, with increased neutrophil and inflammatory monocytes recruitment at the start of the active phase in mice. We

also have evidence to suggest that mice lacking Bmal1-/- have altered immune responses to intranasal LPS compared to Bmal1+/+, and our in vitro data suggests that this is due to the relationship between the circadian clock and metabolism regulation of the fibroblast immune response.
These results lay the foundation for new therapeutic targets or interventions for the treatment of inflammatory lung diseases that display circadian variation in symptoms including asthma, COPD, and infections, all of which worsen in circadian disrupted individuals such as shift-workers.

P.17 A tale of two cytokines; circadian and metabolic responses to IL-1β versus TNFα in chondrocytes and relevance for osteoarthritis

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Osteoarthritis (OA) is a common joint disease characterised by loss of cartilage. Chronic low-grade inflammation, mediated by cytokines IL-1β and TNFα, along with disruption of the circadian clock drive this pathology. Chondrocytes are the constituent cell type in cartilage, and under homeostasis synthesise crucial proteins for the stability and strength of cartilage extracellular matrix. With inflammation and circadian disruption, chondrocytes switch from synthesising anabolic factors like Collagen II to synthesising catabolic factors such as MMP13 – leading to cartilage destruction. However, whether metabolic reprogramming in chondrocytes leads to this catabolic phenotype is unclear.

We found that IL-1β but not TNFα led to complete disruption of the chondrocyte circadian clock prompting us to explore the intracellular metabolic differences between these cytokines. Both cytokines induced glycolysis and suppressed mitochondrial respiration. Quantitative proteomics was also carried out to profile the chondrocyte proteome in response to IL-1β and TNFα, showing differences in the amino acid transporter SLC7a2 which transports arginine. We have also showed differences in the metabolic enzyme Arginase2 which converts arginine to ornithine. Arginine uptake by chondrocytes was selectively increased with IL-1β, not TNFα, using radiolabelled amino acid uptake assays. Strikingly, IL-1β-induced Mmp13 production could be suppressed by preventing arginine uptake through siRNA silencing of Slc7a2.

Collectively, this data shows that cytokines prevalent in the pathogenesis of OA cause specific alterations on the chondrocyte circadian clock and in parallel drive distinct metabolic features. Understanding these metabolic features may provide new therapeutic pathways for OA, a disease in which no disease-modifying treatments exist.

P.18 Exploring immunomodulatory effects from a metabolic model associated with treatment response in people with rheumatoid arthritis

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Rheumatoid arthritis (RA) is a degenerative disease involving an inappropriate immune response targeting tissue within the synovial joint. If left untreated/poorly managed, RA can lead to crippling disability and even early death in severe disease. The first-line treatment for patients is methotrexate, but up to 40% of patients do not respond to it, leading to a worse outcome where an early and aggressive treatment is needed to avoid long-term damage of the joints. Predicting who

will respond to methotrexate is therefore of great value, and so there is an urgent need to identify novel biomarkers to predict patient responses and direct optimal treatment. The metabolome of patients with early RA was generated from the Targeting Synovitis in Early Rheumatoid Arthritis (TaSER) trial involving plasma samples analysed using a liquid chromatography mass spectrometry platform. This provided the opportunity to develop a metabolomic profile associated with patients' responses to treatment that may inform the mechanisms by which inflammation is resolved with successful treatment. A supervised machine learning approach was employed to generate a model that was associated with the response to treatment. In addition, the putatively identified metabolites from the model, including L-tryptophan, itaconate and trimethylamine N-oxide, were investigated for their potential immunomodulatory roles using an in vitro approach, exploring their effects on inflammatory processes in macrophages which are reported to be important drivers of inflammation in RA.

P.19 Molecular clock synchronization limits inflammasome activation and reduces IL-1β release in a Bmal1-dependent manner

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The molecular clock is the timekeeping system within cells that aligns cellular physiology to daily changes in the external environment and is principally regulated by the master circadian protein, BMAL1. Macrophages are instrumental cells of the innate immune system, and their inflammatory function is carefully orchestrated by the molecular clock. Macrophages are also the primary producers of IL-1β, a potent pro-inflammatory cytokine that plays a central role in inflammatory disease. Maturation of IL-1β requires the formation of multiprotein complexes called inflammasomes, which release IL-1β through an inflammatory form of cell death termed pyroptosis. Numerous inflammatory diseases driven by IL-1β exhibit time-of-day variation in symptom and disease severity. However, we have yet to fully understand the molecular clock mechanisms that govern inflammation.

We find that the NLRP3 and AIM2 inflammasome are under the control of the molecular clock, whereby clock synchronization reduces inflammasome-mediated IL-1β release in a Bmal1-dependent manner. Moreover, production of gasdermin-D, the pore-forming mediator of pyroptosis, is clock-regulated and enhanced with Bmal1-/-deletion. Bmal1-/- macrophages also exhibit distinct mitochondrial morphology, marked by dysfunctional mitochondrial fission and reactive oxygen species production, known drivers of inflammasome activation. These findings uncover a novel mechanism by which the molecular clock exerts a protective effect over inflammation by limiting inflammasome activation and preventing excessive IL-1β release. Future work will aim to understand the importance of clock-controlled inflammasome induction in respiratory diseases and rheumatoid arthritis for therapeutic potential.

P.20 Recognition of a yeast beta-glucan particle by Dectin-1 triggers immunometabolic signalling required for immune training

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Fungal beta-glucans are major drivers of trained immunity which increases long-term protection against secondary infections. Heterogeneity in beta-glucan source, structure and solubility alters interaction with the phagocytic receptor Dectin-1 and could impact strategies to improve trained immunity in humans. Using a panel of diverse beta-glucans we describe the ability of a specific yeast-derived whole-glucan particle (WGP) to reprogramme metabolism and thereby drive trained immunity in human monocyte-derived macrophages in-vitro and mice bone-marrow in-vivo.

Presentation of non-soluble, non-aggregated WGPs led to the formation of the Dectin-1 phagocytic synapse with subsequent lysosomal mTOR activation, glycolysis and epigenetic rewiring. Intraperitoneal or oral administration of WGP drove bone-marrow myelopoiesis and improved mature macrophage responses, pointing to therapeutic and food-based strategies to drive immune training. Thus, the investment of a cell in a trained response relies on specific recognition of beta-glucans presented on intact microbial particles through stimulation of the Dectin-1 phagocytic response.

P.21 Exposure to glioma cells-conditioned media activates Nrf2 in mouse and human macrophages

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Gliomas are the most aggressive forms of adult brain cancers with very high mortality rates. Due to a lack of therapeutic options, the overall prognosis remains extremely poor. Intriguingly, around 30% of the high-grade glioma tumour mass is comprised of macrophages, including peripheral infiltrating macrophages and brain-resident microglia. This myeloid population contributes to the pro-tumorigenic, immune-suppressive glioma microenvironment, but the underlying mechanism(s) is poorly understood. RNAseq data indicate that the expression levels of multiple targets of transcription factor Nrf2 are elevated in the microglial population of IDH-wildtype gliomas. Nrf2 is the key regulator of cellular redox homeostasis and a suppressor of pro-inflammatory responses. We hypothesised that Nrf2 activation in the glioma-associated myeloid population is potentially due to reciprocal cross-talk with the tumour cells within the microenvironment.
To test our hypothesis, a cell culture model was established where RAW264.7 macrophages were treated with conditioned media from spheroid cultures of early-passage murine glioma. This treatment increased the Nrf2-target gene expression in macrophages. Moreover, a similar pattern of Nrf2 activation was observed in parallel utilising conditioned media from human glioma cell lines and THP1-derived macrophages. Interestingly, the levels of Immune-Responsive Gene 1 (Irg1) also increased, and RNAi-mediated Nrf2 depletion prevented this increase. This agrees with proteomic analyses of bone-marrow-derived macrophages linking differential Nrf2 activity status to the protein abundance of Irg1 which potentially contributes to the immunosuppressive effects. Together, our findings suggest that glioma-secreted factors contribute to Nrf2 activation in macrophages, which in turn may promote an immunosuppressive microenvironment.

P.22 RIG-I induced reactive oxygen species drive metabolic changes that modulate the antiviral immune response in human hepatocytes

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Hepatocytes mediate diverse activities including processing of gut-derived products and regulation of whole body metabolism. They also play a major role in mediating systemic inflammation through the production of acute phase proteins and complement components, and responding locally to infection by hepatropic viruses via pattern recognition receptors, such as RIG-I and TLR3. Immune cells alter their metabolism to meet the metabolic demands of an induced immune response and reactive oxygen species (ROS), generated through a metabolic switch, have been identified as a key link between the immune response and the metabolic state of the cell. We sought to determine whether hepatocytes also use this process to mediate anti-viral immunity. Using two hepatoma cell lines (HepG2 and Huh7), treated with polyI:C, a viral analogue, we explored the interplay between the metabolic state of hepatocytes and their innate antiviral immune activation. In concomitance with increased gene expression and protein levels of type I and type III interferons due to RIG-I

activation, we observed a reduction in the oxidative phosphorylation and mitochondrial membrane potential. Using MitoSOX as ROS-fluorescent probe, we found that mitochondrial reactive oxygen species levels are increased during RIG-I dependent antiviral response. Furthermore, modulating ROS levels through scavengers (N-acetyl cysteine and reduced glutathione) and oxidant agents (rotenone and antimycin), we were able to regulate the amplitude of the antiviral response, decreasing or enhancing the response respectively. Our results highlight the key role of mitochondrial ROS in shaping the antiviral immune responsiveness of human hepatocytes.

P.23 Obesity limits dendritic cell production of type 1 interferon

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Obesity is a global health concern, with over 600 million people worldwide living with obesity. It is well established that obesity is associated with increased risk of chronic inflammatory co-morbidities. Obesity is also linked to increased susceptibility to infection, and diminished vaccination efficacy.

A critical first step of host protection or successful vaccination against a pathogen is the capture and presentation of antigen by Dendritic cells (DCs). DCs are professional antigen presenting cells (APCs) which capture, process antigens and present their antigen load to waiting T cells, thus, launching the adaptive immune response. Additionally, DCs provide critical signals which instruct and shape the immune response. Our group has previously reported that DCs from PWO display defective cytokine production, potentially resulting in less effective host responses.

Immune responses require significant energy and bio-intermediates, provided by intrinsic metabolic pathways. The type of metabolic pathway activated within the cell can also determine the type of immune response elicited. We propose that defective metabolism underpins the altered DC functionality reported in obesity.

Here we show that bone marrow-derived DCs from mice fed a high fat diet (HFD) have decreased cytokine production compared to standard diet (SD) mice. Using metabolic inhibitors, we assessed the metabolic pathways required to produce these cytokines, namely TNFα and IFNβ. Finally, we demonstrate dysregulated cellular metabolism in DCs from HFD animals. Collectively, our data details defective DC responses in obesity, which may compromise successful immunity that is expected following infection with or vaccination against pathogens.

P.24 Stunted Glycolysis prevents NET formation in neonatal neutrophils

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Background
Neutrophils are rapid first responders, specialised in the killing of pathogens. Multiple defects exhibited by human neonatal neutrophils contribute to severe infection and sepsis. One key killing mechanism, virtually absent in umbilical cord blood (UBC) neutrophils, is neutrophil extracellular traps (NET)s which depends on metabolic function. As little is known about the metabolic function of neonatal neutrophils, we have investigated the role of cellular metabolic parameters in the failure of UBC neutrophils to produce NETs.
Methods
Neutrophils from adult peripheral blood or UBC were isolated using magnetic-bead based cell sorting. NET formation of isolated cells was determined by fluorescence microscopy of DAPI stained cells treated for 3 hours with or without PMA (50 nM) +/- 2-deoxy-glucose (2DG). Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured using extracellular flux analysis. Mitochondrial content and morphology were examined using fluorescence microscopy and flow cytometry.
Results

Unlike adults, no NET formation was observed in neonates treated with PMA and NET formation in adults was completely abrogated by the addition of 2DG (n = 5/group). Neonatal neutrophils exhibited significantly lower maximal ECAR (p=0.016) and OCR (p=0.016) on treatment with PMA, whilst pre-treatment with 2DG prevented any increase in ECAR and OCR for both groups (n = 4-5/group). Mitochondrial content and morphology did not differ between adults and neonates (n= 4/group)
Conclusion
These data suggest that defects in NET production by neonatal neutrophils may arise as result of an inability to sufficiently upregulate glycolysis upon stimulation.

P.25 The Role of Germinal Centre Hypoxia and Metabolic Regulation in an Active T Follicular Helper Cell Response

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Germinal centre (GC) formation occurs in secondary lymphoid organs in response to antigenic stimuli. GC reactions are essential for production of high affinity-matured, class-switched antibodies, and the activation of GC B cells requires assistance from T follicular helper (Tfh) cells, that provide co-stimulation and cytokines. However, the factors that regulate Tfh cells and direct their influence on antibody production remain poorly characterised. Previous studies using model antigens have shown that active GCs contain regions of hypoxia, which is associated with increased glycolytic metabolism and may play a role in controlling Tfh cell activation. We aim to understand if GCs arising during infection are also hypoxic, and how hypoxic and metabolic regulation differs in GCs of type 1 and type 2 polarised immune responses.

We have collected activated lymph nodes from mice infected with either the intestinal helminth Heligmosomoides polygyrus or the respiratory virus Influenza A, and measured hypoxia and glycolytic and lipid metabolism. Our data suggest distinct metabolic profiles of Tfh cells in Th1 and Th2 mediated infection. We propose that the extent of hypoxia in the microstructure of the germinal centre fuels the metabolic shift that supports Tfh activation, regulating B cell maturation and antibody production. Mechanistic insight into the immunometabolism of Tfh cells, and the signals that control these pathways, may provide new therapeutic targets for controlling antibody production.

P.26 People living with T2D have a dysfunctional immunometabolic profile defined by an increased number of senescent T-cells

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Background – Type 2 diabetes is characterised by an abnormal immune response, leading to the loss of insulin sensitivity and a reduction of insulin. This increases circulating blood sugar, causing inflammation, and exacerbating diabetic symptoms. This inflammatory environment may increase the presence of two subsets of late-stage T-cells, known as EMRA, which accumulate in T2D.

Materials and Methods – PBMC's and serum were isolated from whole blood samples. Both diabetic and healthy controls were recruited through the ongoing SENgen study. Data was obtained through flow-cytometry, electron microscopy and qPCR.

Results – EMRA's were significantly increased in the T-cell compartment in T2D. A unique population of KLRG-1 negative EMRA cells was significantly increased, this group had reduced cytotoxicity, which was further reduced in the presence of TGF-β. The mitochondrial network was, rounded, fissed and had a decreased mass. This phenotype could be replicated with TGF-β, resulting in the

upregulation of mitochondrial fission markers DNM1L, Fis1 and Mff. T2D EMRA's had elevated expression of superoxide gene SOD2 and NOX4 and a decrease in cell survival gene PRK2D. The SASP produced by EMRAs had an enhanced inflammatory profile in the T2D group. SCENITH showed an alteration in T-cell metabolism, with changes to glycolysis, mitochondrial dependence, FAO and glutaminolysis being observed in T2D.

Conclusion – EMRA T-cells are a heterogeneous population that are highly dysfunctional and rapidly accumulate in T2D participants. EMRA cells have an altered metabolism, gene expression and mitochondrial network that arise through increased inflammation and high glucose levels.

P.27 Targeting cholesterol metabolism as a novel immune checkpoint in viral infections and cancer

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Metabolic targets may provide novel strategies to complement existing classical checkpoints in order to boost the highly exhausted T cells directed against viruses and tumours. Recent studies demonstrated that inhibition of acyl-coenzyme A:cholesterol acyltransferases (ACAT) can both enhance murine anti-tumour CD8+ T cell efficacy and mediate a direct antitumour effect. We showed that reduced formation of membrane lipid microdomains is a feature of PD-1hi exhausted T cells. We therefore investigated the potential for rescue of exhausted human virus-and tumour-specific T cell responses by modulation of cholesterol metabolism and lipid microdomain formation. We found that ACAT inhibition could enhance the expansion of functional virus-specific T cells from donors with chronic hepatitis B virus (HBV-) or acute SARS-CoV-2 infection. ACAT inhibition also boosted hepatocellular carcinoma (HCC)-specific T cell responses directly isolated from human liver and liver tumour lesions in the majority of patients. Responding T cells showed increased lipid microdomain formation, reduced lipid droplets, enhanced T cell receptor (TCR) signaling and TCR-independent bioenergetic reprogramming. ACAT inhibition had a complementary effect with other immunotherapies, with increased responsiveness to PD-1 blockade and enhanced functional avidity of T cells genetically engineered to recognize HBV and tumour cells.

Taken together, modulating cholesterol metabolism by inhibition of ACAT is a promising therapeutic target, enhancing immune responses in acute and chronic infection and in cancer.

P.28 Metformin restores immune and metabolic dysregulation in the inflammatory skin disease hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is a chronic inflammatory disease affecting ~1% of the population. It is characterised by painful nodules and lesions in intertriginous areas of skin. The disease is under-recognised and under-diagnosed, and current treatment options are inadequate. Thus, a better understanding of HS pathogenesis is needed to identify new therapeutic targets. To date, the role of metabolic pathways in HS has not been investigated

We therefore analysed the immune and metabolic pathways in skin and blood of HS patients compared with controls using RT-qPCR, flow cytometry and seahorse flux analysis. Furthermore, we determined the effects of the anti-diabetic drug metformin, which is known to modulate metabolism, in ex vivo skin explant cultures and in blood from patients treated with the drug.

We found that HS PBMC exhibited increased protein levels of activation markers in several lymphocyte populations. In HS skin, the same markers were elevated in non-immune cells as well as

lymphocytes. Analysis of gene expression showed an increase in glycolytic enzymes and inflammatory cytokines in both skin and PBMC of HS patients. In the skin explant culture media, we found increased levels of several inflammatory cytokines, which were decreased following ex vivo treatment with metformin. Finally, glycolytic markers and cytokines were reduced in the blood of patients treated with metformin.

Our findings demonstrate increased inflammation and metabolic activity in HS, which can be modulated using metformin, highlighting metabolism as a potential new therapeutic target in HS.

P.29 Quantification of Germinal Centre Hypoxia Offers Insight into their Altered Immunometabolism during Disease States

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The formation of germinal centres (GCs) in secondary lymphoid organs is a key adaptive immune response to pathogenic infection. Antibodies produced by GC B cells have undergone affinity maturation against their target antigen and have been class-switched for increased efficacy. Such GC B cell maturation is impossible without co-stimulation signals and cytokines from T follicular helper (Tfh) cells. Dysregulation of this process leads to autoantibody production and the development of autoimmune conditions, such as rheumatoid arthritis (RA). Interestingly, the rapid cellular expansion associated with GC reactions results in regions of increased hypoxia, switching cells from oxidative phosphorylation to glycolytic metabolism. Understanding the impact this metabolic switch has on GC formation and maturation may help decode the processes behind development of protective versus pathogenic antibodies.

Our research has focused on Heligmosomoides polygyrus and Influenza A as model infections in mice to explore the GC distribution of hypoxia in a Th2- versus a Th1-mediated response through immunofluorescence imaging of lymph nodes taken from infected and naïve mice. In addition, we are exploiting the Collagen Induced Arthritis (CIA) model to determine whether GC hypoxia in inflammatory arthritis reflects a Th1, Th2, or unique pattern. Quantification of imaging and qPCR of lymphoid tissues, by helping to corroborate our findings, should develop our mechanistic insight into GC immunometabolism and further our understanding on how to control and modulate antibody production in RA and infection.

P.30 The effects of AMPK modulation on neutrophil ROS and NET production in bronchiectasis

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Introduction: Bronchiectasis is a disease of permanent bronchial dilation. Patients have neutrophilic inflammation in the airways. There is a need for therapies that can reverse neutrophil dysfunction in bronchiectasis. The energy sensor kinase, AMPK has been shown to modulate neutrophil functions. This study aims to characterise the effects of AMPK modulation on neutrophil reactive oxygen species (ROS) and neutrophil extracellular trap (NET) production.
Methods: Neutrophils were isolated from whole blood taken from age-matched controls and bronchiectasis patients. Neutrophils were pre-treated with an AMPK activator(A769662) and/or AMPK inhibitors(Resistin,SBI-0206965), before stimulation by LPS for ROS kinetics and NET assay.
Results: 11 matched controls and 18 bronchiectasis patients were recruited. LPS increases ROS production, but the response was not significantly different between disease groups. In bronchiectasis, A769662($p=0.0011$) significantly reduced ROS production rate following LPS stimulation, and co-treatment with the endogenous AMPK inhibitor resistin($p=0.0127$) did not block this effect. In controls, SBI-0206965($p=0.0077$) pre-treatment increases ROS production rate when LPS-stimulated. When pooled together, A769662($p=0.0003$) decreases ROS production rate, while SBI-0206965($p=0.0112$) increases ROS production rate following LPS stimulation.
There was no difference in spontaneous NETosis, and LPS and PMA stimulated NETosis between bronchiectasis and controls. Resistin($p=0.0171$) pre-treatment significantly increases NETs release in

response to LPS.
Conclusions: We conclude that peripheral neutrophils of bronchiectasis patients and matched controls are not different in NETs and ROS production. Specific AMPK modulators has a direct effect on ROS production, but not NETosis, suggesting that AMPK activation has the potential to reduce oxidative stress but not NETosis in bronchiectasis.

P.31 Lung stem cells upregulate lipid transport metabolism during early IAV infection

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Seasonal influenza viruses infect 5-10% of the UK population each year and, though most people suffer mild symptoms, influenza is a significant cause of severe illness and mortality. The virus also has pandemic potential, further increasing the likelihood of severe disease. Current strategies of controlling influenza with vaccines and treating it with antivirals have limited efficacy, and new approaches are urgently needed. We hypothesise that accelerating the repair of damaged tissue after infection could be an effective therapeutic in both mild and severe influenza. In the lung, epithelial repair is carried out by lung basal cells, and we show that at times of peak infection and peak repair, basal cells proliferate and increase in total number. Analysis of single cell transcriptomics of IAV infected lung tissue shows that basal cell activation is accompanied by an increase in lipid transport and localisation, and an increase in amino acid metabolism driven by SLC3A2. This suggests that the activation of basal cells during infection could be driven by an early induction of metabolic pathways - an indirect result of the inflammatory environment, or a direct result of virus interaction. We suggest that this can affect repair, leading to faulty differentiation and dysplastic repair. By understanding how basal cells respond to infection, we hope ultimately to be able to tailor treatments to improve tissue repair and reduce influenza morbidity.

P.32 IL-4 induces changes in Cholesterol Biosynthesis in pleural and peritoneal cavity macrophages

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Cholesterol biosynthesis is a stringently regulated process with numerous rate-limiting steps. Increases in cholesterol biosynthesis are usually observed following a decrease in available cholesterol in the form of exogenous lipo-proteins. The serous cavities are a nutrient-rich site, however, the availability of lipo-proteins within these sites is unclear. Bulk RNAseq analysis of pleural large cavity macrophages (LCM) highlighted a striking upregulation of the cholesterol biosynthesis pathway at day 4 following in vivo delivery of IL-4c.

In this work we investigated the temporal dynamics of IL-4 dependent cholesterol biosynthesis within BMM, peritoneal and pleural LCM in mice.

Utilising qPCR analysis of sorted serous LCM over a time course (D1, D2, D4) of IL-4c exposure we characterised the cholesterol biosynthesis pathway. At D1 following IL-4c, expression of the rate limiting enzyme Hmgcs is increased. In contrast, in vitro treatment of BMM with IL-4 did not lead to activation of the pathway. Further assessment of LCMs revealed that Sqle the second rate limiting enzyme does not increase until day 4 of IL-4c treatment, which we confirmed by flow cytometry. In addition to Sqle, Fdps, Lss, Fdft, Fdps and Arv are also increased by day 4 post IL-4c. Increased Arv, a gene involved in cholesterol export led us to determine levels of cholesterol in serous fluid; increased levels of cholesterol were detected following IL-4c exposure.

Taken together, this data suggests that IL-4 exposure increases the levels of master regulators of the cholesterol pathway as well as stimulates increased cholesterol translocation and release into the cavity.

P.33 Differential exosome profiles impact on T cell metabolism following COVID-19 infection

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Introduction
As important mediators of intercellular communication alterations in exosomes have been reported in various diseases, including in cancer and viral infections. Here we explore the profile of recovered COVID-19 patient-derived exosomes and their possible impact on the T cell mediated immune response.

Methods
Our cohort included healthy donors (pre and post vaccination) and donors recovered from mild or severe COVID-19. Exosome origin was established by staining exosomes for more than 30 cell-specific surface markers by flow cytometry. For functional experiments, pre-activated human T cells were co-cultured with 5ug patient-derived exosome on two consecutive days, before assessment of metabolic changes by SCENITH and flow cytometry.

Results
Exosome profiles related to disease severity, indicating that different subsets of cells were contributing to the immune response in mild and severe infection and vaccination. Donor-derived exosomes influenced the metabolic profile of T cells in vitro, with exosomes derived from donors recovered from mild disease overall inducing suppression of glycolysis, while those from donors recovered from severe disease allowed for increased T cell activation and corresponding metabolic reprogramming.

Conclusions
We find that exosomes in the circulation sustain distinct profiles both post vaccination and following an immune response, making them interesting candidates as biomarkers. Furthermore we show that these donor derived exosomes influence T cell metabolism, indicating their contribution in shaping anti-viral immunity.

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P.34 Inflammation induced innate antibodies recognising OxPLs may regulate macrophage metabolism within the pleural cavity

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The influence of IL-4 on macrophage metabolism has been the focus of many studies of bone-marrow derived and peritoneal macrophages. In contrast, the influence of in vivo exposure to IL-4 on the metabolic profile of pleural cavity macrophages has not been determined. IgM is the first antibody produced during an immune response, the secreted form is pentameric and often polyclonal. Within the pleural cavity, natural antibodies are secreted by innate-like B-cells residing within FALCs of the pericardium & mediastinum. Whether locally produced IgM auto-antibodies that recognise lipoproteins have a role in the normal function of macrophages in contexts other than atherosclerosis is under investigated. In the setting of acute pristane induced lupus, helminth infection and IL-4c exposure we detect IgM antibodies recognizing mixed populations (OxLDL/OxPAPC) and defined (POVPC) lipid species within pleural fluid. This data suggests the presence of multiple lipid species within the pleural cavity during inflammation, lipidomic analysis is underway to characterize the pleural lipid profile.

Using sIgM deficient mice, we show for the first time IL-4c dependent increases in oxygen consumption & spare respiratory capacity within Pleural LCM and find this is reduced in the absence of IgM. Furthermore, we detect a significant deficit in IL-4c expanded pleural LCM number when

compared to IgM sufficient mice and this defect can be partially rescued by intra-pleural delivery of E06 an anti-oxPL IgM.

Taken together these data reveal an in vivo mechanism by which lipid regulation of macrophage metabolism is modified by innate like antibodies within the pleural cavity.

P.35 The V141L variant in IFNAR1 alters the whole blood immunometabolic response to stimulation and is associated with resistance to viral infection in humans

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The single nucleotide polymorphism (SNP) rs2257167 in the IFNAR1 gene encoding the receptor for type I interferon (IFN-I) results in an amino substitution (V141L; IFNAR1^{L141}) that is associated with increased susceptibility to multiple autoimmune diseases. The functional impact of this SNP on IFN-I binding, signalling and interferon regulated gene (IRG) induction are unknown. Here, we analysed transcriptomic and genomic data from two independent healthy cohorts to assess the functional consequences of this SNP. In both cohorts, we found that IFNAR1^{L141} is an expression quantitative trail loci (eQTL) for IFNAR1 and increases its expression in whole blood. In line with increased IFNAR1 expression, IFNAR1^{L141} donors had an increased IFN-I gene signature and increased expression of several IRGs following whole blood stimulation with LPS, polyIC and the live influenza A virus. Interestingly, IFNAR1^{L141} donors also had increased expression of several metabolic genes including CD36, PTGS2 and BST1 following stimulation with LPS, polyIC and IAV. To study the effects of IFNAR1^{L141} in infection we used VirScan to profile the history of viral exposure in one of our cohorts. We found that donors carrying the risk allele were more protected against CMV and HSV-1 infection, indicating that IFNAR1^{L141} is protective in an infection setting. Additional *in vitro* work is underway to comprehensively assess the differences in metabolic profiles induced by IFNAR1^{L141}.

P.36 A Novel Method to Profile T Cell Metabolism

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Cellular metabolism is now known to be a key driver of T cell phenotype, fate, and function. For this reason, harnessing metabolism is now seen as a strategy to improve the antitumor efficacy of adoptive T cell therapies. The Agilent Seahorse XF T cell Metabolic Profiling (XF TCMP) kit is a new solution that, combined with the Seahorse XF analyzer, allows for the measurement of basal bioenergetic metabolism, metabolic poise, and respiratory capacity in T cells. This assay utilizes an improved uncoupler (BAM15), which reduces the need for optimization and improves the robustness of uncoupled respiration measurements in human and mouse T cells and also in other immune cells like NK cells. The XF TCMP kit supports two workflows: the XF T Cell Persistence assay and the XF T Cell Fitness Assay. In this study, the XF T Cell Persistence assay was used to evaluate the metabolic phenotype of T cells expanded in different cell culture formulations, demonstrating that cell culture medium composition can impact metabolic parameters associated with persistence. The XF T Cell Fitness assay was used to evaluate the role of fatty acid oxidation in nutrient-restricted conditions, in both acutely and chronically activated human T cells. The data demonstrate that chronically activated cells were more sensitive to metabolic perturbation and may have reduced metabolic fitness under nutrient restriction. Taken together, these assays can contribute to improving T cell therapy design and optimizing product development.