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Mucosal associated invariant T cell deficiency and functional impairment in obstructive sleep apnoea: implications for cancer co-morbidity

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Obstructive sleep apnea syndrome (OSAS) is characterised by repeated stopping and starting of breathing during sleep due to throat muscle relaxation. Epidemiological studies demonstrate systemic immune alterations and increased risk of cancer and cardiovascular disease in people with OSAS. OSAS is more common in people with obesity (70% of people with OSAS have obesity) yet remains an independent risk factor for these complications. Early, consistent treatment with CPAP may prevent some complications, but the knowledge gaps in the mechanism of OSAS are preventing more effective treatments. Mucosal Associated Invariant T (MAIT) cells are an innate immune cell population known to be altered in obesity. MAIT cells have been linked to the development of auto-inflammatory conditions including metabolic disease. Furthermore, MAIT cells are dysregulated in cancers and the IL-17 producing subset can promote tumour development. Despite being linked to OSAS by these confounding factors, this is the first study of MAIT cells in OSAS. In this study we demonstrate a reduction in MAIT cell number in people with OSAS that is independent of BMI, and that negatively correlates with OSAS severity. Further, we show a shift in MAIT subpopulations with implications for their in vivo functionality including cytokine production and altered cellular metabolism. In conclusion, we show for the first-time significant alterations in MAIT cells in the setting of OSAS. MAIT cell frequencies may represent a novel marker of severity and/or a therapeutic target. This study confirms the importance of the immunometabolic aspect of OSAS, highlighting opportunities for treatments of OSAS.

Serum proteome analysis of innate antiviral mediators in patients with advanced liver disease awaiting transplantation

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Individuals with a chronic liver disease (CLD) have an elevated risk of severe SARS-CoV-2 infection with 30-day mortality rate almost twice that of the general population. The biological basis for poor COVID-19 outcome in this population is not well understood. Patients with CLD are known to display several physiological defects associated with reduced hepatocyte function including reduced levels of antiviral proteins and dysregulated inflammatory response. In this study we aimed to define the role that these defects play in COVID-19. Serum samples from patients with CLD (n=58) were analysed using mass spectrometry, SARS-CoV-2 pseudoparticle assays, and multiplex bead arrays to quantify inflammatory and acute phase proteins. Principal component analysis serum proteome identified distinct clustering for samples with lower MELD-Na scores (5-19) versus higher MELD-Na scores (>20), as well as clustering based on underlying disease aetiology. Analysis of proteins differentially regulated in individuals with high MELD-Na scores identified significant dysregulation of systemic inflammation and coagulation. Patients with high MELD-Na scores had increased IL-6, and reduced prothrombin levels. To assess the impact of these alterations in serum proteome on viral entry and replication, serum samples were tested in SARS-CoV-2 pseudoparticle assays or viral infection assays. There was no difference in viral entry or viral infection with serum from low MELD-Na and high MELD-Na patients. Patients with end-stage liver disease awaiting liver transplantation display an altered serum proteome. This is associated with dysregulated systemic inflammation and coagulation but not a defect in systemic antiviral mediators capable of influencing SARS-CoV-2 entry and replication.

Investigating the role of IL-22 in CNS regeneration

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Multiple sclerosis (MS) is an immune-mediated, demyelinating disease of the central nervous system (CNS) which causes progressive disability in patients, in part due to a failure of remyelination. CD4+ T cells, although involved in MS pathogenesis, are also required for CNS remyelination. IL-22, a CD4+ T cell-secreted cytokine, has shown protective effects in the CNS and regenerative properties in other tissues. IL-22 binding protein (IL-22BP) is an MS risk gene and IL-22BP-/- mice show less severe EAE, with IL-22 over-expression demonstrating protection in EAE. Therefore, I hypothesise that IL-22 has regenerative effects in demyelinating diseases. To investigate the effects of IL-22 in vitro, mixed glial cells, oligodendrocyte progenitor cells (OPC), and microglia were treated with recombinant IL-22. Recombinant IL-22 had no significant effect on proliferation or differentiation of OPCs nor on microglial phagocytosis. Recombinant IL-22 also did not significantly affect developmental myelination in ex vivo organotypic brain slice cultures. To study the role of IL-22 during remyelination in vivo, focal demyelinated lesions were induced in murine spinal cords using lysolecithin. IL22RA1 was expressed within demyelinating lesions, predominantly by microglia, at 5 days post-lesion. However, this receptor did not co-localise with IL-10Rβ, the partnering signalling component, which was expressed by astrocytes. Although IL22RA1 was expressed following CNS demyelination at a key time-point of myelin phagocytosis and OPC differentiation, this did not accurately reflect expression of the functional IL-22 receptor complex. This may explain why recombinant IL-22 had no effect on OPCs in vitro or in ex vivo models of developmental myelination.

Priming is a fundamental mechanism of inflammasome regulation in myeloid cells

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The NLRP3 inflammasome is an immunological sensor that detects multiple microbial- and host-derived signals. Activation is a two-step process, requiring an initial priming stimulus followed by a second activation signal, but how different priming stimuli affect NLRP3 signalling is unclear. While priming is closely associated with NLRP3, the impact of priming on other inflammasomes such as NLRP1 has not been characterised. Caspase-recruitment domain-only proteins (COPs) and pyrin domain-only proteins (POPs) regulate inflammasome activation, but our knowledge of their contribution to inflammasome priming is incomplete. Using primary mouse and human macrophages and human induced pluripotent stem cell (iPSC)-derived macrophages, we investigated the effectiveness of different priming stimuli on inflammasome activation and COP/POP expression. The role of type I interferon (IFN) signalling was characterised using the JAK inhibitor Tofacitinib and Ifnar1-/- macrophages. Bacterial and viral stimuli induced transcription-dependent and -independent priming of NLRP3, respectively, but all stimuli triggered formation of ASC specks, indicating inflammasome formation. In contrast, long-term LPS priming limited NLRP3 and NLRP1 activation which was dependent on type I IFN signalling. Microbial stimuli and IFN signalling also increased COP/POP expression, suggesting COPs and POPs potentially act in a negative feedback mechanism to prevent excessive inflammasome activity. In addition, priming with microbial and sterile stimuli triggered NLRP3 inflammasome activation in iPSC-derived macrophages, similar to observations in HMDM, indicating that they are a useful model to study inflammasome regulation. These findings highlight the importance of priming in inflammasome regulation and the role of IFN signalling, which drives tolerance to long-term LPS priming.

The anti-inflammatory effects of helminth-derived peptides in a model of endotoxin-induced acute lung injury

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Helminth excretory-secretory products, including helminth defence molecules (HDMs), are potent immunomodulatory compounds released throughout the course of infection. A HDM from Fasciola hepatica (FhHDM-1) and its C-terminal derivative (FhHDM-1.C2) exhibit potent immunomodulatory properties in models of autoimmune and inflammatory disease, offering a potential avenue for the development of novel therapeutics. The aim of this study was to investigate the effects of FhHDM-1 and its derivative peptide in models of acute lung inflammation. FhHDM-1 and FhHDM-1.C2 inhibited Escherichia coli and Pseudomonas aeruginosa LPS-induced TNF-α release in murine macrophages. In vivo, prophylactic and therapeutic administration of both FhHDM-1 and FhHDM-1.C2 significantly decreased total cells and polymorphonuclear leukocytes in the bronchoalveolar lavage fluid (BALF). Levels of pro-inflammatory cytokines IL-6 and CXCL1/KC were also reduced in BALF. Current work is investigating intracellular targets of the FhHDM peptides within the lung and our research suggests a potential role for AMP-activated protein kinase (AMPK). Overall, our findings indicate that FhHDM-1 and FhHDM-1.C2 limit LPS-induced acute lung inflammation and may represent a novel biotherapeutic for neutrophil-dominated lung disease.

Nanomodulation of microRNAs in macrophages

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Macrophages are key effector cells with a multifaceted role in the innate immune response, through their role as phagocytes and by the production of cytokines, chemokines and expression of co-stimulatory molecules when activated. Macrophage activation occurs along a spectrum, and macrophages are capable of both potentiating inflammation, classified as an 'M1' like response, or suppressing inflammation and promoting tissue repair and regeneration in an 'M2' like state. Manipulation of this polarisation capacity has the potential to be clinically useful in diseases where macrophages are key drivers of pathogenicity, including but not limited to Sepsis, Multiple Sclerosis, and Rheumatoid Arthritis. MicroRNA's have become increasingly established as key regulators of cellular processes, including pathways that drive inflammation. Of particular interest is mir-155, whose expression is high in the 'M1' and suppressed in an IL-10 driven 'M2' phenotype. Here we investigate the therapeutic potential of miR-155 anti-miRNA oligonucleotides (AMO) on limiting the macrophage inflammatory response and its delivery to macrophages by formation of nanoparticles through complexation with star shaped polypeptides. We show the formation of stable complexes between AMO-155 and star shaped polypeptides, and using flow cytometry show uptake of these particles preferentially to M1 stimulated bone marrow derived macrophages (BMDM). Additionally, we show that when incubated with cells of the peritoneal exudate, a mixed immune cell population, these particles are preferentially uptaken by macrophages. Overall we show a promising delivery mechanism for microRNA based therapeutics to macrophages, the efficacy of which will be examined in future in vivo models.

Non-Invasive classification of macrophage polarisation by 2P-FLIM and machine learning

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Classically activated macrophages (M1) may be characterized through their use of aerobic glycolysis as an energy source, whereas alternatively activated macrophages (M2) show a greater usage of oxidative phosphorylation (OxPhos). Microenvironmental cues can promote polarization of macrophages through metabolic reprograming, therefore impacting function. As a result, there is a growing interest in real time non-invasive monitoring of macrophage metabolism. FLIM is a real-time non-invasive imaging technique of important metabolic co-factors such as free/protein-bound nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD). However, a FLIM based metabolic analysis of macrophage polarization and immunomodulation has yet to be clearly established. M1 and M2 activation of primary human macrophages was achieved using IFNγ and IL-4 respectively and verified by PCR and ELISA. Extracellular acidification (ECAR) and oxygen consumption (OCR) ratios were measured after polarization, validating the metabolic profile of M1 and M2 macrophages. FLIM was performed continuously in large field-of-view images of individual polarized macrophages also challenged metabolically using small molecules. We uncover photonic FLIM variables that are pronounced under the action of carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) that stratify the phenotype of polarised human macrophages, observed using Uniform Manifold Approximation and Projection (UMAP) clustering. This stratification and photonic variables emanating from a FLIM approach, served as the basis for machine learning models. Applying a random forest model, identified three three key FLIM parameters and achieved a ROC AUC value of 0.944 when classifying human macrophages. Our results can help establish FLIM as an alternative to endpoint metabolic methods.

Type I IFNs and IL-18 synergistically enhance IFNg production in NK cells by upregulating cMyc-dependent iron metabolism

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Type I interferons are important for early and fast activation of NK cells. However, how IFN signaling exert its effect is only partially understood. We found, that IFNs in combination with IL-18, but not IL-12/IL-2, very effectively induced production of IFNg and Granzyme B in NK cells as early as 4h post-stimulation. This enhanced functionality was sustained after longer stimulations of 18h. NK cell cytotoxicity was also elevated after the stimulation with IL-18 and IFNs in comparison to IL-18 or IFNs alone. The adoptive transfer of 18h IL-18 + IFNbeta stimulated NK cells more effectively reduced the growth of established B16 melanomas, which was accompanied by an increased tumour infiltration of activated NK cells. In the first hours after stimulation with IFNs+IL-18 cMyc and pS6 signalling was increased, following by an enhanced upregulation of transferrin receptor (CD71) and transferrin-iron uptake at later time points. Recent work from our lab has demonstrated the importance of iron for the metabolism and function of NK cells in anti-viral responses. These metabolic changes were dependent on mTOR pathway as rapamycin inhibited the increase of IFNg, CD71 and transferrin uptake by IFNs. In summary, our data suggest that type I interferons cooperates with IL18, but not IL2/IL12, to enhance NK cell function through promoting cMyc signaling and iron-dependent metabolism.

Enrichment of a novel, pathogenic macrophage population with distinct transcriptional and metabolic signatures in rheumatoid arthritis synovial tissue

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Synovial-tissue macrophages significantly contribute to Rheumatoid Arthritis, yet the precise nature/function of macrophage subsets within the inflamed joint remains unexplored. RA, Arthralgia and healthy-control synovial-tissue biopsies and synovial fluid analysed via flow-cytometry, CD206+CD163+ and CD206-CD163- macrophages sorted from RA synovial-tissue by FACSAria sorter; RNAseq, FLIM analysis and heathy-fibroblast experiments performed. A spectrum of macrophage activation states exists within the inflamed synovium. Multidimensional single-cell analysis identifies enrichment of CD206+CD163+ synovial-tissue macrophages co-expressing CD40 in RA synovial-tissue compared to fluid ($p<0.05$), associated with increased disease-activity and treatment response. CD206+CD163+ macrophages are present in healthy synovial-tissue, however, co-expression of CD40 is completely absent ($p<0.05$). In contrast, protective CX3CR1-expressing macrophages in healthy synovium are depleted in RA, a phenotype that begins to disrupt prior to clinical manifestations of disease. RNA-seq analysis indicates that CD206+CD163+ macrophages are transcriptionally distinct from synovial-tissue CD206-CD163- and RA polarised-macrophages, with unique tissue-resident gene signatures. Differing metabolic demands between CD206+CD163+/CD206-CD163- subsets demonstrated by NAD(P)H FLIM analysis. Functionally CD206+CD163+ macrophages produce pro-inflammatory mediators (reversed by CD40-signalling inhibition) and induce a pathogenic phenotype in healthy synovial-fibroblasts, thus further contributing to the local inflammatory response. We have identified a novel transitional population of tissue-resident macrophages in the RA synovium that become activated early in RA disease and correlate with disease activity and treatment response. Uncovering the molecular patterns and cues that transform this immunoregulatory macrophage population into a dysfunctional inflammatory activation state may provide opportunities to reinstate joint homeostasis in RA patients.

Inhibition of anti-viral TLR3 responses by itaconate in idiopathic pulmonary fibrosis patients and implications for disease progression

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Idiopathic pulmonary fibrosis (IPF) is a fibrotic lung disease of unknown aetiology. It is a fatal condition with a mean survival of less than 3 years. Currently, there is an unmet clinical need for the development of novel biomarkers and therapeutics in IPF. IPF disease progression has been associated with viral and bacterial infections. We previously demonstrated that TLR3-IRF3 induced anti-viral responses had a protective effect in primary IPF lung fibroblasts. Furthermore, defective TLR3 function accelerated disease progression in IPF patients and was linked to higher mortality rates. The metabolite itaconate has been identified as a potent immunomodulator with anti-bacterial and anti-viral properties. The role of itaconate during viral infection in IPF is unknown. Here, we investigated the effect of a synthetic analogue of itaconate, 4-OI, on TLR3 function in primary lung fibroblasts from IPF patients. We demonstrated that poly(I:C) treatment induced IRG-1 transcription, the gene responsible for itaconate synthesis, in IPF lung fibroblasts. In addition, 4-OI treatment directly induced expression of the antioxidant proteins, NRF2 and HMOX-1 in IPF fibroblasts. In contrast, 4-OI treatment decreased TLR3-induced expression of anti-viral IFN-β, RANTES and RIG-I in IPF lung fibroblasts. These effects were associated with a concomitant reduction in TLR3 protein expression in IPF fibroblasts following poly(I:C) treatment. In this study, we demonstrated for the first time that itaconate (4-OI) has the ability to modulate TLR3 function in IPF lung fibroblasts. These findings warrant further investigation to elucidate the specific role of itaconate during viral infection and in disease progression in IPF.

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 generated NK cell specific SLC7a5 KO mice. The development, frequency and absolute numbers of NK cells appear to be normal in naïve KO mice. These mice were then challenged with Poly(I:C), a double stranded RNA analog, to mimic 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 (transferrin receptor) and the uptake of transferrin-iron are reduced. Initial data suggests that this may be due to altered mTORC1/ cMyc signaling. Further data supporting metabolic deficiency in these cells include SLC7a5 null NK cells having decreased mitochondrial mass and reduced IL-15 induced proliferation. In terms of functionality, SLC7a5 null NK cells have impaired production of Interferon gamma and reduced expression of key cytotoxic molecules such as granzyme B and perforin. Culturing SLC7a5 null NK cells from Poly (I:C) challenged mice show significantly reduced cytotoxicity against tumour cells ex vivo. This work has important implications for NK cells within the tumour microenvironment where conditions may impair SLC7a5 expression and/ or the availability of amino acids.

Cytosolic dsRNA improves neonatal innate immune responses to adjuvants currently in use in paediatric vaccines

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Pattern recognition receptors (PRRs) of the innate immune system represent the critical front-line defence against pathogens, and new vaccine formulations target these PRR pathways to boost vaccine responses, through activation of cellular/Th1 immunity. The majority of paediatric vaccines contain ALUM or MPLA as adjuvants to encourage immune activation. Evidence suggests that elements of the innate immune system, currently being targeted for vaccine adjuvanticity do not fully develop until puberty and it is likely that effective adjuvants for the neonatal and paediatric populations are being overlooked due to modelling of responses in adult systems. We recently reported that the activity of the cytosolic nucleic acid (CNA) sensing family of PRRs, is strong in cord blood and peripheral blood of young children. This study investigates the function of CNA sensors in subsets of neonatal immune cells and shows that myeloid cells from cord blood can be activated to express T cell co-stimulatory markers, and also produce Th1 promoting cytokines. CD80 and CD86 were consistently upregulated in response to cytosolic Poly(I:C) stimulation in all cell types examined and CNA activation induced robust Type I IFN and low levels of TNFα in monocytes, MDMs and moDCs. We have compared CNA activation to adjuvants currently in use (MPLA/ALUM), alone or in combination - cytosolic Poly(I:C) in combination with MPLA/ALUM can improve expression of activation marker levels above those observed with either adjuvant alone. This may prove particularly promising in improving the efficacy of existing ALUM- or MPLA-containing vaccines, through activation of T cell-mediated immunity.

Lactate alters metabolism in human macrophages and improves their ability to kill Mycobacterium tuberculosis

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To mount an immune response to infection, the macrophage alters its metabolism by increasing aerobic glycolysis and concomitantly decreasing oxidative phosphorylation; known as the Warburg effect. Consequently, lactate, the end-product of glycolysis, accumulates. The subsequent effect of lactate on surrounding macrophages is poorly understood. Mycobacterium tuberculosis (Mtb), the causative organism of Tuberculosis (TB), is phagocytosed by macrophages in the airways. Mtb infected macrophages upregulate aerobic glycolysis and effector functions to kill the bacteria. Although lactate has been considered a waste product of aerobic glycolysis, we hypothesised that lactate would impact subsequent immunometabolic responses and modulate macrophage function. Macrophages were cultured from blood of healthy individuals. Lactate treated human macrophages were stimulated with Mtb. Macrophages were then assessed for cytokine secretion and metabolic flux. Macrophages were then infected with Mtb in the presence or absence of lactate and their ability to kill Mtb was assessed by CFU. Lactate decreases glycolysis and increases oxidative phosphorylation in macrophages. When lactate-treated macrophages were stimulated with Mtb, glycolysis increased but oxidative phosphorylation remained stable. Lactate reduced secretion of TNF and IL-1β by macrophages in response to Mtb. Additionally, lactate improved Mtb bacillary killing by macrophages, through a mechanism that is partially, mediated by autophagy.

IL-17-producing CD4 tissue-resident memory T cells mediate protective immunity against Bordetella pertussis infection in the nasal mucosae

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Whooping cough is a severe respiratory disease cause by the Gram-negative bacterium Bordetella pertussis. Despite high vaccine coverage, a recent resurgence of disease has been observed, highlighting the need to better understand the mechanism of protective immunity. We found that IL-17A-producing CD4 tissue-resident memory T (TRM) cells are induced and persist in the nasal mucosae during B. pertussis infection. These cells expand rapidly during re-challenge and are associated with rapid clearance of a secondary infection with B. pertussis from the nasal cavity. Protection in the nasal cavity is lost in both IL-17-/- mice and mice depleted of CD4 T cells. Protective immunity is also associated with infiltration of a novel neutrophil population expressing Siglec-F, which have higher NETosis capacity when compared with conventional neutrophils. Depletion of neutrophils resulted in higher bacterial load in nasal cavity. Furthermore, accumulation of Siglec-F+ neutrophils was significantly reduced in IL-17-/- mice and mice depleted of CD4 cells. CXCL1, a chemokine required for neutrophil recruitment, is decreased in the nasal cavity of IL-17-/- mice, whereas intranasal IL-17A administration induced CXCL1 production. In addition to being a protective source of IL-17 during B. pertussis infection, we have also found that TRM cells can produce IL-17 in absence of TCR activation by stimulation with the cytokines or with unrelated PAMPS and pathogens. Our findings demonstrate that CD4 TRM cells are a major source of protective IL-17 in the nasal cavity and should be considered when informing future immunization strategies to control the transmission of B. pertussis.

Regulation of the JAK-STAT pathway by the non-structural proteins of the respiratory syncytial virus is dependent on cell line

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Respiratory Syncytial Virus (RSV) is the leading cause of bronchiolitis and viral pneumonia in infants, causing significant morbidity and mortality. RSV's impact on infants is mediated in part by its ability to limit signalling through the JAK-STAT pathway. Previous research has linked NS1 and NS2 to the subversion of IFN signalling, however, the majority of our knowledge comes from immortalised kidney cell lines. In order to establish a more physiologically relevant understanding of RSV's immune evasion strategies, we have studied its effects in both human alveolar and bronchial epithelial cell lines and primary human epithelial cells. We expressed NS proteins in alveolar epithelial cells (A549) and bronchial epithelial cells (BEAS-2b) and analysed their effect upon the IFN-a pathway. We discovered that the NS proteins have varying effects in each cell line, though both cell lines had decreased ISG expression with NS1, and variable SOCS expression with both NS1 and NS2. Our work has shown that the activity of NS1 and NS2 is cooperative and targets the activity of the JAK-STAT signalling at multiple points to limit the antiviral response, though the mechanism is dependent on the cell line used.

A novel role for IL-36 cytokines in enhancing endothelial cell barrier integrity

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Pathological increases in vascular permeability lead to oedema and swelling, causing a host of retinal and neurological disorders. Few barrier-enhancing factors have been discovered to specifically increase barrier integrity and make vessels resistant to fluid leakage. In this study, we explore the effects of IL-36 on angiogenic processes and vascular permeability in vivo, and in vitro in primary human microvascular endothelial cells of the central nervous system. IL-36β enhanced endothelial cell barrier function, reducing vascular permeability. IL-36 cytokines also induced primary endothelial cell proliferation, migration, and tube formation in vitro. Importantly, we demonstrate that the IL-36 signalling axis has potential to be useful clinically for reducing microvascular leakage, as the pro-angiogenic features of IL-36 in vitro are uncoupled from the potent ability of IL-36 to enhance vascular integrity in adult mice in vivo in an acute setting. Mechanistically IL-36 regulates endothelial cell tight/adherens junctions, in addition to inducing vessel remodelling and maturation providing a stabilised vascular network. Network analysis of RNA sequencing data support these functional assays. Our data present IL-36 cytokines as novel promoters of vascular integrity, with barrier enhancing properties that prevent pathological vascular permeability.

Aromatic ketoacids represent novel therapies for inflammatory diseases through activation of the Nrf2/HO-1 pathway and suppression of pro-inflammatory responses in primary human immune cells

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Trypanosoma brucei (T. brucei) are parasites best known for causing fatal human sleeping sickness. It has long been noted that Trypanosomiasis is accompanied by the excretion of high levels of trypanosome-derived ketoacids into the host's bloodstream. The aim of this study was to investigate the impact of trypanosome-derived ketoacids on immune-cell fate and function, using a wide range of methods including flow cytometry, western blotting, qPCR, ELISA, FLIM and Seahorse. We first demonstrate that T. brucei strongly upregulate the stress-response protein Heme Oxygenase-1 (HO-1) in primary murine glia and macrophages in vitro. This upregulation can be attributed to the specific aromatic ketoacids secreted by T. brucei. We report that T. brucei ketoacids are capable of inducing HO-1 in human dendritic cells (DC). Additionally, we present data to support Nrf2 activation as the mechanism of action by which these ketoacids upregulate HO-1 expression. We demonstrate that these ketoacids show immunomodulatory properties in DC by limiting maturation and suppressing production of pro-inflammatory markers, inhibiting the differentiation of pathogenic T helper cell subsets. We also show that ketoacids are capable of modulating DC cellular metabolism, favouring oxidative phosphorylation. Finally, we demonstrate that these ketoacids are capable of inhibiting proliferation and pro-inflammatory cytokine production in cells isolated from patients with Inflammatory Bowel Disease. Therefore, this study not only reports a novel immune-evasion mechanism potentially employed by T. brucei to suppress the host immune response, the ketoacids investigated also represent a new class of HO-1 inducer with therapeutic potential for the treatment of inflammatory conditions.

Cytokine synergy and glycolysis used to promote aggressive phenotype in synovial fibroblasts of children with Down's syndrome-associated arthritis

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Background: Down's syndrome-associated arthritis (DA) is a more common and clinically distinct disease compared to juvenile idiopathic arthritis (JIA). This study aims to identify the underlying mechanisms involved in driving synovial fibroblasts (FLS) activation and destructive capacity.
Methods: Primary DA-FLS were isolated from synovial biopsies from children with DA. DA-FLS were cultured with IL-17a, IFN-g and GM-CSF in the presence or absence of TNF-a. Culture supernatants were harvested and IL-6, IL-8, MCP-1 and RANTES levels quantified by ELISAs. Leukocyte adhesion was assessed by leukocyte-DA-FLS adhesion assays. Flow cytometric analysis was used to examine DA-FLS adhesion molecules (VCAM-1, ICAM-1) and chemokine receptors (CXCR3, CXCR4). The two major energy pathways glycolysis (ECAR) and oxidative phosphorylation (OCR) were quantified by the Seahorse XFe96 Analyser following the same stimulations as before.
Results: TNF-a, IL-17a and IFN-g induced IL-6, IL-8, RANTES and MCP-1. TNF-a, IL-17a, IFN-g and GM-CSF increased leukocyte adhesion to DA-FLS. TNF-a and IFN-g upregulated cell-surface expression of ICAM-1, VCAM-1, CXCR3 and CXCR4. IFN-g potentiated the effects of TNF-a on IL-6 and MCP-1 while decreasing IL-8. This synergy was also demonstrated for ICAM-1, VCAM-1, CXCR3, CXCR4 expression. Synergy between TNF-a and IL-17a switches DA-FLS to use ECAR.
Discussion: DA-FLS are transformed from quiescent to energetic. DA-FLS function is regulated by differential cytokine stimulation, with TNF-g and IFN-g demonstrating potent synergistic induction of adhesion, inflammatory and chemokine receptor expression. Meanwhile, cytokine synergy between TNF-a and IL-17a favours glycolysis suggesting complex cytokine signalling pathway mediate these effects resulting in a more aggressive phenotype.

Dysregulation of IL-17 and Calprotectin in the female reproductive tract affects pregnancy outcome following assisted reproductive therapy

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Embryo implantation is a critical stage of assisted reproductive technology (ART) and an appropriate endometrial immune environment is fundamental for a successful pregnancy. Here, we compared endometrial tissue from women who underwent successful (N=9) versus unsuccessful (N=11) ART cycles, to identify differences in their immune profile. Mid-luteal phase endometrial biopsies were collected from 20 nulliparous women with unexplained infertility, who were undergoing ART; RNA-sequencing analysis was performed and endometrial and serum levels of IL-17A were measured by ELISA. Immune cell profiling was performed using Cibersortx. IL-17A and calprotectin, the Antimicrobial peptide (AMP) dimer constituted by S100A8 and S100A9, were identified by immunohistochemistry in endometrial biopsies. AMP release upon IL-17A treatment in female reproductive tract (FRT) cells was measured by real-time qPCR. Data were analysed using GraphPad Prism. RNAseq revealed 204 differentially expressed genes (DEG) and particularly over-represented pathways analysis showed S100A9 and the 'IL-17 signalling pathway' to have decreased expression in the 'pregnant' group. IL-17A protein levels were significantly increased in serum and endometrial tissue from 'non-pregnant' women, despite immune cell profiling showed similar proportions in the two groups. Immunohistochemistry on endometrial biopsies, displayed IL-17A and calprotectin in the endometrial stromal compartment to correlate with CD45 expression. In vitro stimulation of FRT epithelial cells by IL-17A stimulated AMPs expression. Differential expression of IL-17A and related genes may reflect functional changes in endometrial innate immunity, modifying receptivity for embryo implantation. Abnormal levels of circulating IL-17A may provide a screening tool for patients likely to have negative reproductive outcomes in ART.

Regulation of IL-36 receptor driven skin in inflammation in psoriasis

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IL-36 family cytokines play and orchestrating role in driving psoriatic skin inflammation. However, the specific mechanisms through which these cytokines mediate such effects and how they are regulated, have yet to be determined. In this study, we generated a new transgenic mouse lacking Il36r expression specifically in keratinocytes (Il36rδK mice) and demonstrated that these mice are protected against psoriasiform inflammation to the same level as mice with global deficiency in Il36r. After IMQ induced psoriasiform inflammation Il36rδK mice displayed significantly reduced expression of IL-17a, IL-23, IL-22 and CXCL1, alongside reduced infiltration of neutrophils and IL-17A expressing γδ T cells in the inflamed skin. These data identify keratinocytes as the key responsive cell in mediating IL-36 driven skin inflammation. We have also identified Sigirr as an important negative regulator of Il36r signaling in keratinocytes, using Sigirr-/- mice. These mice were found to exhibit enhanced psoriasiform skin inflammation which was successfully reversed upon treatment with an anti-IL-36r blocking mAb. Significantly, an analysis publicly available gene expression databases demonstrated that while the levels of expression of Il36 family genes are elevated in psoriatic lesional skin, expression levels of SIGIRR are reduced, indicating an important regulatory role for this molecule in human psoriatic disease. In conclusion, these data identify keratinocytes as the major cell in orchestrating IL-36 driven psoriatic inflammation and demonstrate that Sigirr plays an important regulatory role in dampening these responses in both mice and humans.

Investigating the role of the complement system in the radioresistance of rectal cancer

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Background: Poor pathological response to neoadjuvant chemoradiation therapy (neo-CRT) is a clinical problem in rectal cancer. There is a need to determine molecular factors influencing response to neo-CRT and identify predictive biomarkers. Evidence supports a role for the complement system in tumourigenesis and therapeutic response.
Methods: Radiosensitivity of colorectal cancer (CRC) cell lines (HCT116, SW837, HRA-19, SW1463) was assessed by clonogenic assay. Gene expression was assessed by qPCR. Protein expression and anaphylatoxin production was assessed by ELISA. Transient C3 knockdown was achieved by siRNA reverse transfection. Regulatory protein and receptor expression was determined by flow cytometry. Serum C3a levels (n=39) were assessed by ELISA.
Results: HRA-19 cells are significantly more radioresistant compared to SW837 and HCT116 cells, whilst HCT116 cells are the most radiosensitive. Complement proteins and anaphylatoxins were produced by CRC cells. Protein production positively correlated with surviving fraction at 1.8Gy of radiation, a clinically-relevant dose. CRC cells expressed complement regulatory proteins and receptors. C3 knockdown in HRA-19 cells was associated with increased radiosensitivity following 2Gy of radiation. C3a levels were elevated in pre-treatment sera from rectal cancer patients with a subsequent poor pathological response to neo-CRT, when compared to good responders (p=0.039).
Conclusions: Increased complement expression in CRC cells is associated with radioresistance. Transient C3 knockdown increased radiosensitivity of CRC cells, suggesting a functional role for complement in the radioresponse. In patients, increased pre-treatment serum C3a was associated with subsequent poor responses to neo-CRT, highlighting complement as a potential predictive biomarker of response to neo-CRT in rectal cancer.

Immune-metabolic regulation in adipose tissue: Implications for dendritic cell maturation and metabolism

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Oesophageal Adenocarcinoma (OAC) is the most strongly associated cancer with obesity. This study aims to elucidate what influence adipose tissue metabolism and its secretome have in treatment resistance and whether obesity alters this response. Following patient consent, ex-vivo Visceral Adipose Tissue (VAT) explants were exposed to increasing doses of radiation. Agilent Seahorse Xfe24 was used to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in VAT explants and MitoStress test in dendritic cells (DCs) treated with Adipose Conditioned Media (ACM). Levels of DC maturation following irradiated ACM exposure were analyzed by flow cytometry. ACM was analyzed via MSD 54plex ELISA to assess secreted factors on angiogenic, chemokine, cytokine, inflammatory, TH17 or Vascular injury panels. VAT explant energy metabolism showed a significant increase for OCR and ECAR with obesity and increasing radiation exposure. DCs treated with iACM showed decreased expression of CD11c, CD80, CD86, HLA-DR, CD40 and PD-L1 with increasing radiation and decreased HLA-DR and CD54 following ACM exposure from obese versus non-obese patients. DCs showed altered metabolic profiles following exposure to ACM. Altered secretion of proinflammatory mediators was observed from the obese adipose secretome compared with non-obese and with increasing radiation doses. We have demonstrated that obesity and increasing radiation doses can significantly alter the immune-metabolic influences of adipose tissue. Alterations in the secretome of adipose tissue could potentiate the tumour microenvironment and deleteriously affect immune cell function therefore further interrogation is required to fully elucidate the influence adipose tissue may have in treatment response.

Targeting metabolism in cardiovascular disease: investigating metabolic reprogramming of macrophages in atherosclerosis

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Background: Atherosclerosis is a chronic inflammatory disease predominantly mediated by classically activated M1 macrophages. It has been demonstrated that pro-inflammatory macrophages in atherosclerotic plaques have a glycolytic profile which correlates with decreased plaque stability and increased incidence of rupture and thrombosis. However, the environmental stimuli which drive metabolic reprogramming of macrophages remains to be investigated. Cholesterol crystals, which are found to accumulate at both early and advanced stages of atherosclerosis; are known to drive inflammatory responses in macrophages. The impact of cholesterol crystals on macrophage metabolism and polarization has not yet been examined. Purpose: The aim of this study is to examine the impact of cholesterol crystals in metabolic reprogramming and polarization of macrophages. Methods: Primary human macrophages were treated with cholesterol crystals (500 µg/ml) over 24 hours in the presence/absence of the glycolytic inhibitor, 2-deoxyglucose (25 mM). mRNA expression was assessed by qPCR and cytokine production was assessed by ELISA. Macrophage metabolism was examined assessed using fluorescence lifetime imaging microscopy (FLIM) and Agilent Seahorse assays and pcr/westernblot. Mitochondrial morphology was assessed through confocal imaging. Results: Cholesterol crystals drive macrophage polarization towards an M1 pro-inflammatory phenotype. Seahorse and FLIM analysis revealed that cholesterol crystals drive metabolic reprogramming towards glycolysis with increased expression also observed of surrogate markers of glycolysis. Finally, cholesterol crystal induced inflammatory responses were attenuated upon inhibition of glycolysis.

Conclusion: This study demonstrates for the first time that cholesterol crystals alter macrophage metabolism and drive M1 polarization in primary human macrophages, highlighting metabolism as a therapeutic target to combat disease.

Understanding innate immune responses during early Mycobacterium tuberculosis infection: a role for your friendly neighbourhood neutrophil?

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Understanding innate immune responses during early tuberculosis (TB) infection could help pave the way for more efficacious treatment options. Alveolar macrophages are the first line of defence against TB infection. Other immune cells, including neutrophils, can also respond early and can kill the invading pathogen. Mycobacterium tuberculosis (Mtb), the bacteria that causes TB, can manipulate immune responses to allow it to thrive within macrophages and neutrophils. Finding ways to understand and target the macrophage-neutrophil axis during Mtb infection could have profound clinical benefit. PBMCs were isolated from healthy blood donors to obtain human monocyte-derived-macrophages (hMDMs). hMDMs were infected with Mtb at a multiplicity of infection of 1-10. Three hours post infection, unphagocytosed Mtb was washed off, the hMDMs incubated for 24 hours and macrophage-conditioned medium (MΦ-CM) collected. CD15+ human neutrophils were isolated from fresh human blood and treated with 20% MΦ-CM. Neutrophil metabolism and effector functions were assessed by Seahorse and flow cytometric analysis, respectively. We demonstrate that MΦ-CM induces a shift to glycolysis and oxidative phosphorylation in healthy neutrophils. Moreover, we show that MΦ-CM promotes neutrophil activation, as evidenced by decreased CD62L levels upon MΦ-CM treatment. Preliminary evidence also suggests that MΦ-CM can reduce the migratory capacity of these neutrophils and induce NETosis. Collectively, our data suggest that MΦ-CM can alter the bioenergetics and functionality of healthy neutrophils. We are currently examining the effect of MΦ-CM on neutrophil longevity and its ability to kill Mtb.

Intracellular survival of Staphylococcus aureus within phagocytes is facilitated by the modulation of multiple host cell pathways

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Multiple studies have shown that Staphylococcus aureus is an adept intracellular pathogen and can hijack phagocytes for host dissemination often referred to as the Trojan horse theory. However, the host pathways involved with facilitating intracellular survival remain unclear. To address this, an intracellular survival assay was established in murine bone marrow derived macrophages (BMDMs) infected with S. aureus strains, USA300, PS80 or Newman. Multiplex gene expression analysis of 733 myeloid-specific genes was performed using the Nanostring nCounter platform. Significant changes in gene expression was observed in BMDMs infected with all strains compared to uninfected cells with Intracellular S. aureus particularly associated with type 1 interferon signaling and the adenosinergic pathway. Each strain induced both pathways, however, differential gene expression was observed in a strain dependent manner with Newman showing overall enhanced ISG production and pro-apoptotic gene expression of trail & daxx. In contrast, USA300 showed enhanced expression of several genes related to the adenosinergic pathway including CD38 and HIF-1α compared to uninfected and Newman infected BMDMs and importantly the anti-inflammatory adenosine receptor Adora2a. Subsequent experiments demonstrated that blocking Adora2a in human neutrophils resulted in a significant reduction in intracellular survival whilst blocking apoptosis in Newman-infected BMDMs leads to enhanced intracellular survival. Taken together, our work suggests that IFN-I suppress intracellular survival of Newman within phagocytes by inducing apoptosis whilst activation of the adenosinergic pathway by USA300 potentially enhances intracellular survival. Altogether, we highlight two important pathways potentially modulated by S. aureus to facilitate intracellular survival.

Investigating the utility of CX3CR1 antagonism to promote NK cell migration towards tumour in obesity associated cancer

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Oesophageal adenocarcinoma (OAC) is an aggressive obesity-associated cancer, with a dismal 5-year survival rate of <20%. OAC patients face poor treatment response rates of <30% and urgently require new therapeutics. Our group have previously shown fractalkine drives natural killer (NK) cell migration to omentum in OAC patients, where their viability and function is altered. We propose targeting the CX3CR1: Fractalkine pathway holds therapeutic potential in OAC. NK cells were treated with OAC patient derived adipose conditioned media (ACM) +/- CX3CR1 antagonist. Their chemotaxis towards tumour conditioned media (TCM) was measured. PBMC were treated with fractalkine +/- dynamin inhibitor Dynasore or at 4°C and CX3CR1 expression was measured by flow cytometry. PBMC were treated with fractalkine for 2 or 24 hours and subsequently placed in a fractalkine free environment. Culture in ACM significantly decreased NK cell migration towards TCM (p=0.02). Pre-treatment with a CX3CR1 antagonist reversed the modulatory effects of ACM on NK cell migration towards TCM (p=0.01). Dynasore (p=0.0089) or at 4°C (p=0.0099) significantly attenuated fractalkine-mediated reduction of CX3CR1 surface expression on NK cells. Removal to a fractalkine free environment partially restored CX3CR1 expression (p=0.0124). Our data demonstrate that fractalkine-mediated reduction of CX3CR1 expression by NK cells occurs via endocytosis. Furthermore, OAC patient-derived ACM significantly decreases the capacity of NK cells to migrate towards TCM and this can be reversed by CX3CR1 antagonism. Our data provide novel insights into fractalkine-mediated regulation of CX3CR1 expression and the therapeutic utility of CX3CR1 antagonism to redirect NK cells towards tumour in obesity-associated cancer.

A Crohn's disease-associated pathobiont synergise with NSAID to promote inflammation and cell death in susceptible host via the caspase-8/NLRP3 axis

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Non-steroidal anti-inflammatory drugs (NSAIDs) are believed to exacerbate inflammation in patients with inflammatory bowel disease (IBD), but the mechanisms regulating NSAID-induced symptoms are unknown. Pathobionts such as adherent-invasive Escherichia coli (AIEC) are widely prevalent in the mucosa of Crohn's disease (CD) patients and considered relevant to CD pathogenesis. Caspase-8 is a protein regulating programmed cell death, intestinal homeostasis and inflammation. We hypothesise that the presence of AIEC might explain the NSAID-induced symptomatic worsening in IBD. Using IL-10-/- mice, we show an aggravation of colitis in AIEC-colonised mice fed on a NSAID supplemented diet. Activation of NLRP3 inflammasome, caspase-8 and cell death executors, like caspase-3, PARP and Gasdermin D, was observed in these mice. However, IL-10-/- mice colonised with AIEC alone or fed on a NSAID supplemented diet alone did not develop colitis, highlighting the synergistic effect of both AIEC and the NSAID. Using small-molecule inhibitors targeting NLRP3 and caspase-8, we show an amelioration in colitis due to a reduction in pro-inflammatory cytokines, M1 macrophages, cell death (apoptosis/pyroptosis) and improved barrier function. In conclusion, our findings provide evidence and mechanistic insights into how NSAID and an opportunistic CD-associated gut pathobiont can synergise to worsen IBD symptoms and inflammation. The data suggest that targeting caspase-8 and NLRP3 could be a potential therapeutic strategy for IBD patients with NSAID-worsened inflammation.