Promoting immunology research in Yorkshire
13 May 2025
Abstracts

Flash talks

P.01 **Ontogeny-independent expression of LPCAT2 in granuloma macrophages during experimental visceral leishmaniasis**

Dr Shoumit Dey, University of York, UK

Granulomas are organized inflammatory lesions that form in response to persistent stimuli such as infections. Murine infection with Leishmania donovani results in the formation ofgranulomas around infected Kupffer cells in the liver and serves as a well-defined model of immune granuloma formation. The formation and resolution of granulomatous inflammation requires dynamic shifts in immune cell activation states, imposing significant metabolic demands. As mediators of energy homeostasis and cell signaling, lipids and lipid metabolism play a key role in regulating immune cell function during inflammation and the response to infection. However, the extent to which alterations in lipids are spatially linked to altered immune cell transcription has yet to be resolved. In this study, we performed a multimodal imaging analysis combining MALDI mass spectrometry, spatial and single cell transcriptomics, proteomics of flow-sorted macrophages and histopathology of L. donovaniinduced hepatic granulomas. Using this spatially-integrated approach, we identified LPCAT2-mediated membrane re-modelling of myeloid cells as a novel feature of these granulomas. Our study provides new insights into local immunometabolic changes associated with granuloma formation and macrophage activation.

P.02 **Discovering new therapeutic targets by systematic genome scale human receptor interaction screening: Identifying Human CD200 Receptor as a target for immunoregulation by pathogens**

Dr Cristina Viola, University of York, UK

Autoimmune diseases encompass a diverse group of disorders in which the immune system erroneously targets self-antigens, affecting 8–10% of the global population. Conventional therapies broadly suppress immune activity, which, while effective in controlling disease symptoms, increases long-term susceptibility to infections and malignancies. There is a critical need for targeted immunomodulatory strategies that restore immune tolerance without inducing generalized immunosuppression. However, identifying optimal immune receptors for therapeutic intervention remains challenging due to the complexity of immune signaling pathways. Parasitic organisms, which have co-evolved with the human immune system over millennia, naturally modulate immune responses to establish chronic infections. Understanding how parasites engage immune receptors may provide insights into novel

therapeutic targets for autoimmune disease. Plasmodium vivax, the second leading cause of malaria, can cause relapses months to years after initial infection. A large superfamily of highly variable Plasmodium interspersed repeat proteins (PIRs or VIRs in P. vivax) are displayed on the surface of Plasmodium-infected cells. PIR family diversity and presence on the infected cell surface make them likely candidates for agents of immune evasion. VIRs screened against a library of extracellular human leukocyte proteins using an in-house bespoke interaction assay (AVEXIS) identified a specific interaction with the human immunoregulatory protein CD200R. Characterisation of eight CD200R-binding VIRs reveal diversity in binding mode, affinity and activity. Our results show that in vitro, recombinant VIRs are more potent inhibitors than known CD200R agonists.

P.03 **Unveiling the differences in adaptive immune response to staphylococcal protein A that are associated with specific clinical conditions**

Alex Bear, University of Sheffield, UK

Staphylococcus aureus is a commensal gram-positive bacterium that, while a part of our commensal flora, can cause a wide array of infection syndromes. Despite regular exposure to the bacteria a wide array of virulence factors prevent the formation of an effective immune response or the development of a vaccine. A key virulence factor is staphylococcus protein A (SpA) a superantigen that plays a wide array of roles in limiting the immune response including the prevention of IgG response, the binding of B cells and their subsequent depletion. I aim to show that there is a significant difference in immune response between patients with bacteraemic vs non-bacteraemic infections.

Patients with S. aureus infections were recruited from Sheffield teaching hospitals with plasma and PBMC samples taken at 2 times points; the first 7-14 days post onset of infection and the second 3-6 months post infection. A multiplexed bead assay was developed to assess relative IgG levels in the plasma, with 5 antigens selected for inclusion. Additionally, a 20 colour spectral flow cytometry assay was developed to assess the B cell memory repertoire and the methodology of B cell death in the PBMCs of participants.

The multiplexed bead assay showed significant differences in the circulating antibody titres against SpA between individuals with bacteraemic infections vs. non-bacteraemic infections, these differences were also significant when compared to other infections or to healthy individuals. There was also a significant difference observed between the antibody titres at the visit 1 timepoint vs the visit 2 timepoint for SpA and CP5.

Preliminary flow cytometry results show key differences in the phenotypic profile of the memory B cell compartment in those infected with S. aureus.

During S. aureus infections it is possible that there is a generation of an antibody response against SpA due to the noted significant differences observed in IgG titres however, these antibodies may not be able to confer protection.

P.04 **Modulating macrophage phenotypes in a zebrafish infection model**

Zoë Speirs, University of Sheffield, UK

Tuberculosis (TB), caused by Mycobacterium tuberculosis, is a global health emergency. A rise in antimicrobial resistance means there is an urgent need to develop new therapeutics, such as host-directed therapies (HDTs) which improve host responses to infection. Macrophages are key innate immune cells that can kill Mtb, however, Mtb dampens the macrophage pro-inflammatory response, shifting them towards an anti-inflammatory phenotype to promote survival. Macrophages polarise on a spectrum from pro- to anti-inflammatory phenotypes, with inducible nitric oxide synthase and arginase often used as markers for each state respectively.

Here, Mycobacterium marinum (Mm) infected zebrafish larvae are used to model TB to address how macrophage behaviours are dysregulated in vivo. As zebrafish are a natural host for Mm, they closely recapitulate human TB pathogenesis, including the formation of organised granuloma structures. By using under 5-day old larvae, we replace the use of protected animal species often used in TB research. We aim to characterise whether a new arginase transgenic line TgBAC(arg2:GFP) can be used as an in vivo reporter for anti-inflammatory macrophages and use it to determine when and how macrophages switch behaviours during infection.

The highest proportion of arg2:GFP+ macrophages was seen at 3 days post-infection (dpi), with only 12.5% of these cells containing bacteria. Live Mm was required to induce macrophage arg2:GFP and it was dependent on the RD1 virulence locus, suggesting that arg2:GFP expression is induced by Mm ESX-1 secreted virulence factors. Time-lapse imaging at 3dpi identified that arg2:GFP+ macrophages were significantly smaller and rounder than arg2:GFP- macrophages. Analysis revealed a higher mean velocity for arg2:GFP+ macrophages (1.28mm/s vs. 1.52mm/s) and these faster cells correlated with a rounder morphology. These differences corroborate previous in vitro studies in mouse and human models which show that anti-inflammatory macrophages are rounder and faster, providing evidence for the use of the arg2:GFP line as anti-inflammatory macrophage reporter. Identifying HDTs that modulate macrophage phenotype to increase bacterial clearance whilst retaining tissue integrity is essential. Genetic stabilisation of hypoxia inducible factor (HIF)-2a resulted in leukocyte arg2:GFP upregulation, supporting previous in vitro work showing arginase to be HIF-2a target. Importantly, we have shown that inhibition of the early anti-inflammatory signal by downregulating HIF-2a is host protective in Mm infection.

This work highlights the potential for arg2:GFP to be used as a reporter for the identification of HDT targets, such as HIFs, which could modulate macrophage function to clear mycobacterial infection.

P.06 **Malat1 controls Th2 differentiation in a sex specific manner**

Mags Gwynne, University of York, UK

Beyond sex hormone and chromosome-driven mechanisms, little is known about cell intrinsic mediators of immune sexual dimorphism. Here, we show that long intergenic non-coding RNA Malat1 is required for appropriate cytokine expression in female, but not male, Th2 cells. Malat1 deficiency impairs in vitro Th2 differentiation of naïve CD4+ T cells from female mice, characterised by transcriptome-wide suppression of differentiation and cytokine expression, particularly affecting IL10. Upon IL10R blockade, a pronounced effect is also seen on IL4 and

IL13. Mechanistically, naïve CD4+ T cells from Malat1-/- female mice demonstrate altered early activation kinetics and impaired early differentiation in their gene expression programme, including up-regulation of an interferon stimulated gene (ISG) module. This is followed by suppression of IL2Ra and IL2Rg expression and IL2-mediated differentiation. Mimicking the effect of Malat1 loss by maintaining early ISG expression in WT cells with IFNb treatment phenocopies the effects of Malat1 deficiency predominantly with regards to IL2Rg and IL10 expression in female-derived CD4+ T cells. Male CD4+T cell activation and Th2 differentiation are not sensitive to Malat1 loss due to stronger early activation, higher interferon responsive gene expression during early differentiation, maintenance of IL2Ra expression independently of Malat1, and lower sensitivity to exogenous IL2 during late differentiation. In vivo, female, but not male, Malat1-/- mice demonstrate altered Th2 differentiation characterised by a reduction in IL10+ Th2 cells in both lung and spleen following priming and challenge with Schistosoma mansoni eggs, a model of lung type 2 inflammation. Overall, these findings reveal Malat1 as a novel determinant of immune sexual dimorphism.

P.07 **Plasma cell parkour: Harnessing myeloma oncogenes to drive cell cycle re-entry**

Ben Kemp, University of Leeds, UK

Multiple myeloma is an incurable plasma cell neoplasm with complex genetic heterogeneity. Multiple myeloma progresses through a spectrum of disease from the benign monoclonal gammopathy of undetermined significance (MGUS) to aggressive plasma cell leukaemia. The establishment of MGUS is driven largely by chromosomal translocations in germinal centre B-cells that juxtapose oncogenes with the immunoglobulin heavy chain (IgH) locus on chromosome 14. Notable examples of such translocations include CCND1 (t11;14) and c-MAF (t14;16) genes. In contrast, the transition from the pre-malignant MGUS to malignant multiple myeloma is driven by acquisition of activatory point mutations in oncogenes and repression of tumour suppressor gene expression in terminally differentiated plasma cells.

While sequencing of myeloma patient genomes has provided insight into the genetic events which result in the development of the neoplasm, the individual and combinatorial impact of oncogenes on plasma cells to overcome quiescence, and re-enter cell cycle, remains elusive.

Using an in vitro plasma cell differentiation model, we have demonstrated that the over-expression of c-MYC, CCND1 and c-MAF as single oncogenic events alters the cellular phenotype and permits an enhanced proliferative burst in a subset of terminally differentiated plasma cells upon re-activation with immuno-stimulatory cytokines. However, the introduction of these oncogenes is insufficient to drive full transformation. To interrogate how acquisition of additional oncogenic events may facilitate transformation, the culture system has been adapted to allow sequential oncogene expression, thus enabling the creation of genetically customised in vitro models of human myeloma.

Poster presentations

P.08 **Intrinsic Folding Properties of the HLA-B27 Heavy Chain Revealed by Single Chain Trimer Versions of Peptide-Loaded Class I Major Histocompatibility Complex Molecules**

Izabela Lenart[123], Linh-Huyen Truong[45], Dinh Dung Nguyen[46], Olga Rasiukienė[4], Edward Tsao[1], Jonathan Armstrong[7], Pankaj Kumar[7], Kirsty McHugh[89], Branca I Pereira[1210], Balraj S Maan[411], Malgorzata A Garstka[12], Paul Bowness[8], Neil Blake[13], Simon J Powis[7], Keith Gould[14], Darren Nesbeth[15], Antony N Antoniou[415]

[1] Division of Infection and Immunity/Centre of Rheumatology, University College London, London, UK
[2] Centre of Rheumatology, University College London, London, UK
[3] Clinical Trials and Regulatory Affairs, Science Pharma, Warsaw, Poland
[4] Department of Applied Sciences, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK
[5] University of Oxford, Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Oxford, UK
[6] Medical Genetics Department, Medical Genetics Centre, Vinmec Research Institute of Stem Cell and Gene Technology, Hanoi, Vietnam
[7] School of Medicine and Biological Sciences Research Complex, University of St. Andrews, Scotland, UK
[8] The Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Science, Oxford University, Oxford, UK
[9] Jenner Institute, University of Oxford, Oxford, UK
[10] Research and Development Department, Chelsea and Westminster Hospital NHS Foundation Trust, London, UK
[11] School of Medical Education, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK
[12] Core Research Laboratory, Department of Endocrinology, National & Local Joint Engineering Research Center of Biodiagnosis and Biotherapy, Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, China
[13] Institute of Infection and Global Health, University of Liverpool, Liverpool, UK
[14] Wright-Fleming Institute, Imperial College London, London, UK
[15] The Advanced Centre for Biochemical Engineering, University College London, London, UK

Peptide-loaded Major Histocompatibility Complex (pMHC) class I molecules can be expressed in a single chain trimeric (SCT) format, composed of a specific peptide fused to the light chain beta-2 microglobulin (β2m) and MHC class I heavy chain (HC) by flexible linker peptides. pMHC SCTs have been used as effective molecular tools to investigate cellular immunity and represent a promising vaccine platform technology, due to their intracellular folding and assembly which is apparently independent of host cell folding pathways and chaperones. However, certain MHC class I HC molecules, such as the Human Leukocyte Antigen B27 (HLA-B27) allele, present a challenge due to their tendency to form HC aggregates. We constructed a series of single chain trimeric molecules to determine the behaviour of the HLA-B27 HC in a scenario that usually allows for efficient MHC class I molecule folding. When stably expressed, a pMHC SCT incorporating HLA-B27 HC formed chaperone-bound homodimers within the endoplasmic reticulum (ER). A series of HLA-B27 SCT substitution mutations revealed that the F

pocket and antigen binding groove regions of the HLA-B27 HC defined the folding and dimerisation of the single chain complex, independently of the peptide sequence. Furthermore, pMHC SCTs can demonstrate variability in their association with the intracellular antigen processing machinery.

P.09 **Ibrutinib enhances the bias of T cell responses towards staphylococcal superantigens sustaining inflammation in chronic lymphocytic leukaemia**

Fisal Tantoush[1], David Allsup[12], Leigh Naylor-Adamson[1], Frank Voncken[1], and Stefano Caserta[1]

[1] Biomedical Institute for Multimorbidity, Centre for Biomedicine, Hull York Medical School, Faculty of Health Sciences, University of Hull, Hull, UK
[2] Department of Haematology, Castle Hill Hospital, Hull University Teaching Hospital NHS Trust, Cottingham, UK

Chronic lymphocytic leukaemia (CLL) is an uncurable haematological malignancy, associated with significant infection morbidity. Bruton's tyrosine-kinase inhibitors (e.g., ibrutinib) have improved disease outcomes, but severe infections and poor immunization responses afflict patients. Recently, carriage of the endemic Staphylococcus aureus (SA) was associated with lymphocytosis and decreased survival in CLL patients. We then hypothesized that exposure to staphylococcal superantigens (SAgs), known to promote hyper-inflammatory responses, impairs immunity and increases severe infection risk in CLL patients. Herein, we evaluate the reactivity of T cells and CLL cells to SA SAgs, in cultures derived from ibrutinib-treated and untreated CLL patients. We found that ibrutinib-treated patients had less naive CD8+ T cells ($p=0.0348$), more checkpoint receptor (TIM-3) expression in memory T cells ($p<0.0001$), and lower IFNγ/cytokine responses in patient T cells ($p\leq0.0298$). Exposure to SA SAg further increased the accumulation of memory T cells with an exhaustion-phenotype, preferentially in cultures derived from ibrutinib-treated patients ($p\leq0.0350$). Nevertheless, staphylococcal SAgs could not induce regulatory T cells from CLL patients inasmuch as healthy donors ($p\leq0.0461$) and this was associated with accumulation of inflammatory T cells. Significantly, SAg-exposure enhanced inflammatory activation of CLL tumour cells, which acquired CD38, CD40, CD86, while downregulating CD27 ($p\leq0.005$), even in cultures from ibrutinib-treated CLL patients. Thus, we suggest that environmental SAg-exposure promotes the accumulation of pseudo-exhausted T cells, which induce/sustain tumour cell activation, not counteracted by ibrutinib. Our study critically helps understand the chronic inflammatory milieu in CLL patients, with implications for infection morbidity, disease aetiology and future interventions.

P.11 **Schistosoma mansoni granulin: novel ligand of neutrophil CD177 with immunomodulatory potential**

Martin Majer[1], Kelly Lee[1], Nicole Muller-Sienerth[2], and Cecile Crosnier[1]

[1] University of York, UK; [2] Wellcome Sanger Institute, Cambridge, UK

Schistosoma mansoni, a parasitic helminth that infects millions worldwide, evades the human immune system by secreting specific proteins that modulate immune cell function. This allows the parasite to survive for years in blood vessels. The current analysis follows up on a protein interaction study searching for novel binding partners for extracellular parasite proteins amongst a collection of over 700 human receptors.

We revealed a novel interaction between S. mansoni granulin (SmGRN) and CD177, a GPI-anchored glycoprotein predominantly expressed on neutrophils. CD177 plays a crucial role in inflammatory response activation, serving as a receptor for proteinase 3 (PR3). Our findings suggest that SmGRN has the potential to inhibit the binding of PR3 to CD177 and change PR3 activity. However, a direct effect of SmGRN on neutrophil activation was not observed within the scope of this study.

The interaction between SmGRN and CD177 suggests disruption of neutrophil-mediated inflammatory responses by interfering with PR3 signaling. These findings provide new insights into the mechanisms of S. mansoni immunomodulation, particularly its manipulation of neutrophil function, and highlight the complex strategies employed by helminths to establish and maintain chronic infection.

P.12 **Intracortical Microstimulation Induces Rapid Microglia Process Convergence**

Nathaniel P. Williams[12], Anna M. Kelly[1], X. Sally Zheng[1], Alberto L. Vazquez[234], X. Tracy Cui[124]

[1] Department of Bioengineering, University of Pittsburgh, United States
[2] Center for Neural Basis of Cognition, University of Pittsburgh and Carnegie Mellon University, United States
[3] Department of Radiology, University of Pittsburgh, United States
[4] McGowan Institute for Regenerative Medicine, University of Pittsburgh, United States

Intracortical microstimulation (ICMS) can generate useful percepts to restore vision and hearing when applied to the relevant regions of cortex in animals and humans and has great clinical translational potential for sensory restoration. The neuronal response to ICMS has been extensively studied at the cellular level through electrophysiology and 2-photon (2P) imaging studies. The response of non-neuronal cells and in particular of microglia, as well as the effects of ICMS on blood brain barrier (BBB) integrity, have yet to be understood. Here, we applied ICMS under 2P imaging in dual reporter mice expressing green fluorescent protein in microglia and red fluorescent Ca+ indicator in neurons. We also imaged vascular dye leakage to monitor BBB integrity throughout the experiment. We used clinically relevant waveform parameters and tested a range of current amplitudes. We found that, surprisingly, microglia respond rapidly within 15 minutes following stimulation via microglia process convergence (MPC) on areas of particularly high neural activity. The prevalence of MPC increased as current amplitude increased, but interestingly, it did not bear a relationship to the strength of the local electric field. Vascular dye penetration into brain tissue was higher for stimulated than unstimulated control animals and increased as ICMS current amplitude increased. Taken together, these results demonstrate a more rapid and active microglia and BBB response to ICMS than previously thought and highlight the need for future studies to understand the full biological response to ICMS and establish safety standards.

P.13 **Resurrecting the Ancient Glycome to explore the natural anti-Neu5Gc antibody response**

Esme Hutton[1], Yumiko Uno[12], Craig N. Robson[3], Martin A. Fascione[1], Nathalie Signoret[2]

[1] Department of Chemistry, University of York, UK
[2] Hull York Medical School, University of York, UK
[3] Centre for Cancer, Translational and Clinical Research Institute, Newcastle University

N-glycolylneuraminic acid (Neu5Gc) is a non-human sialic acid which is taken up from dietary sources and presented on the glycocalyx of human cells. This makes Neu5Gc a unique example of a ‘xeno-autoantigen’- a non-human, immunogenic molecule found on the surface of human

cells. Neu5Gc-reactive antibodies have implications for many aspects of human health, as a mediator of xenograft rejection, a driver of chronic inflammation, and as a potential serological cancer biomarker. However, current methods to detect and study anti-Neu5Gc antibodies rely on complex synthesis of glycan structures, animal handling expertise, or access to expensive reagents and equipment.

Here, we outline a simple workflow to enrich and detect natural anti-Neu5Gc antibodies from small volume human samples, compatible with samples collected during routine serological testing. This strategy uses human cells engineered to produce Neu5Gc by 'resurrecting' the enzyme required to synthesise it- CMAH. CMAH-transfected cells can then be exploited as a source of diverse Neu5Gc-containing human glycans, with parental cells used as a matched Neu5Gc-negative control.

This strategy successfully enriched Neu5Gc-specific antibodies from individual plasma specimens from healthy donors. Anti-Neu5Gc antibodies were detected in all donors regardless of age or sex, and were predominantly of the IgG isotype, despite some reports that anti-glycan antibodies are often low-affinity IgM antibodies. While reproducible individual differences in Neu5Gc-reactivity were observed between the donors, in all cases anti-Neu5Gc antibodies were capable of binding to Neu5Gc-containing glycans on whole human cells, supporting the hypothesis that they may play a physiologically relevant role in human health.

P.14 **Spatial Transcriptomic Mapping of the Wound Microenvironment: Unravelling Molecular and Gene Expression Correlates of Senescence in Chronic Wound Repair**

Lauren B. Colborn[12], Nina Rocha[2], Alexander Johns[2], Bezaleel Mambwe[3], Michael Peake[3], Abigail Langton[3], Matthew J. Hardman[2], Holly N. Wilkinson[2]

[1] Hull York Medical School, UK; [2] University of Hull, UK; [3] University of Manchester, UK

Chronic skin wounds affect 2.2 million elderly and diabetic patients in the UK, yet current treatments remain inadequate due to limited understanding of the molecular and cellular correlates of poor healing. Indeed, our group were the first to demonstrate that cellular senescence contributes to delayed wound repair in vivo. Thus, clearance of senescent cells could present a targeted therapeutic strategy to promote skin repair. Single-cell spatial transcriptomic technology provides an unprecedented opportunity to dissect the highly heterogenous wound microenvironment, revealing how the accumulation of senescent cells disrupts the otherwise tightly coordinated tissue repair response. Here, we construct a spatially resolved single cell atlas, comparing cell populations between low vs high senescent human chronic wounds (Xenium in situ, 10X Genomics). Our novel findings demonstrate elevation of pro-inflammatory and immune-related clusters in highly senescent wounds, whereas wounds exhibiting low levels of senescence were associated with active remodelling of the extracellular matrix. Differential gene expression analysis further identified exacerbation of SASP-associated gene pathways and loss of immune inhibitory mechanisms within the highly senescent wound microenvironment. Spatial proteomic imaging (MACSima platform, Miltenyi Biotec) validated these findings by characterising immunosenescence in patient chronic wounds. In summary, these data reveal a previously unappreciated role for immunosenescence in delayed wound repair, presenting an exciting avenue for therapeutic targeting.

P.15 **Discovering new immunotherapeutic targets using malaria parasites**

Joe P. Whitehead[12], Cécile Crosnier[13], Gavin J. Wright[123]

[1] Department of Biology, UK; [2] Hull York Medical School; [3] York Biomedical Research Institute, University of York, York, UK

Dysregulation of the immune response is mechanistically relevant to many diseases affecting large proportions of the population. In cancer, immune suppression can prevent tumour clearance, contributing to morbidity and mortality. Alternatively, certain illnesses, including auto-immune conditions, are the result of undesirable immune activity. As such, it is crucial to identify major immunoregulatory checkpoint receptors for therapeutic targeting. Plasmodium vivax is an etiological agent of malaria that causes chronic and often subclinical infections. Sequencing of the P. vivax genome identified an expansive and polymorphic gene family referred to as the VIRs (Vivax Interspersed Repeats), that constitute over 15% of the protein-encoding genes in the P. vivax genome. VIRs share some features with the RIFIN protein family, another highly polymorphic family found in P. falciparum, which directly interacts with human immunomodulatory receptors to promote parasite survival. To investigate the immunomodulatory role of VIRs, recombinant soluble VIR ectodomains were screened for interactions with human immune receptors using a high throughput approach, revealing a wide array of receptors targeted by VIRs, expressed on many immune cell subtypes. This experimental work interrogates the function of the VIR gene family, with the aim of identifying novel cell surface immunoregulatory mechanisms for therapeutic targeting.

P.16 **Probiotic Modulation of Macrophage and T Cell Responses in Infection-Induced Inflammation**

Jevan Terry[1], Stefano Caserta[12], Georgios Efthimiou[12]

[1] University of Hull, UK; [2] Hull York Medical School, UK

Pathogen-induced inflammation is essential in immunological responses to infection, but excessive activation can cause significant tissue damage and chronic inflammation. This dysregulation involves increased production of pro-inflammatory cytokines such as TNF-a, IL-6 and IL-1b alongside immune cell infiltration, exacerbating tissue damage rather than resolving it. Probiotics, which have been recognized for their immunomodulatory potential, offer a strategy for managing hyperactive immune responses. This study investigates how probiotic-derived signals influence macrophage polarisation and T cell-mediated inflammation to restore immune balance.

Thus far, standard growth curves have been created for pathogens Staphylococcus aureus, Acinetobacter baumannii, and Candida albicans, as well as probiotics: Saccharomyces boulardii, and Streptococcus thermophilus. These will guide dose-dependent exposure studies using ex vivo mouse peritoneal macrophages, where cell viability will be assessed via trypan blue exclusion assays. Future work will incorporate additional probiotics (Lactobacillus acidophilus, Lactobacillus bulgaricus, and Bifidobacterium lactis) and utilise human peripheral blood mononuclear cells (PBMCs) to characterise probiotic-driven macrophage activation, cytokine secretion (IL-10, IL-4, and IL-23) and T cell responses via flow cytometry.

By exploring the immunoregulatory effects of probiotics, this research aims to provide insight into their potential role in mitigating excessive inflammation caused by infectious diseases, contributing to a broader understanding of immune regulation in host-pathogen interactions and cytokine-mediated inflammation.

P.17 **Novel interactions between highly variable Plasmodium falciparum RIFINs and human immunomodulatory receptors**

Vincent Geoghegan, Cecile Crosnier, Adam Roberts, Jade Ketteringham, Gavin J. Wright

Department of Biology, Hull York Medical School, York Biomedical Research Institute, University of York, UK

Plasmodium falciparum parasites cause the most lethal form of malaria by replicating in host erythrocytes. During this intra-erythrocytic replication cycle, the parasites express members of a highly variable, multi-gene family known as the RIFINs, which are exported and displayed upon the surface of the infected erythrocyte. RIFINs are thus available to directly interact with and down-modulate the human immune system to promote survival of erythrocytes infected with parasites. To explore the extent of RIFIN interactions with human immune receptors, we have constructed a library of >3,000 unique RIFIN hypervariable domains from Plasmodium falciparum lab strains and field isolates. We successfully expressed 289 RIFIN V2 domains in HEK 293 cells or insect sf21 cells and screened for interactions against a panel of >600 human receptors. We discovered that RIFINs from multiple field strains target receptors in the human KIR2 family, which are important modulators of NK cell cytotoxic activity. NK cells are innate immune cells that can recognise and kill infected host cells. Our interaction data suggest that the parasite may use RIFINs to mimic binding of self-peptide-MHC to KIR2 receptors, thereby delivering signals to down modulate NK cell activity and escape NK cell mediated lysis.

P.18 **Investigation of agrin as a novel caspase-4 substrate**

Ella Barrett, Dr. Dave Boucher, Dr. Kim Robinson, Dr. Nathan Leborgne

University of York, UK

The inflammasomes, multiprotein complexes central to the innate immune system, mediate inflammatory responses to infections and cellular damage. The non-canonical inflammasome (NCI), characterised by caspase-4 activation, responds to intracellular lipopolysaccharide (LPS), a critical component of Gram-negative bacteria. Caspase-4 activation leads to the secretion of pro–inflammatory cytokines IL-1b and IL-18, as well as promoting pyroptotic cell death via Gasdermin D (GSDMD) cleavage).

Agrin, a heparan sulphate proteoglycan traditionally known for its role in neuromuscular junction formation, has been recently implicated in wound healing and immune responses.

Proteomic analysis conducted by our lab identified agrin as a novel substrate cleaved by caspase-4 during LPS-induced activation of the human NCI. Our study aims to characterise agrin's cleavage by caspase-4, validate its role during NCI activation, and elucidate the functional consequences of this process.

Using cellular immunology approaches and biochemistry assays, we demonstrate that agrin is cleaved by caspase-4 in response to intracellular LPS in epithelial cells and human macrophages. Our findings suggest that agrins plays a role in inflammasome-mediated inflammatory responses, potentially linking its extracellular matrix remodelling functions to immune signalling pathways. These results provide new insights into the molecular mechanisms underlying agrin's involvement in inflammation and tissue repair, offering potential therapeutic implications for targeting inflammasome pathways in disease contexts.

P.19 **Investigating Innate Lymphoid Cells in the context of Haematological Malignancies**

Jadie Acklam, University of York, UK

Innate lymphoid cells (ILCs) were discovered just over a decade ago as essential initiators of appropriate immune responses against bacteria, viruses, and parasites, but also as inappropriate drivers of autoimmune and allergic disease. My PhD research centres on the development and optimisation of a comprehensive flow cytometry panel that identifies and characterises various human ILC populations. Given the increasing evidence linking inflammation to multiple blood cancers, this project will leverage 45,000+ bone marrow and peripheral blood patient samples collected by the Haematological Malignancy Diagnostic Service at St James' Hospital in Leeds. By investigating ILCs in the context of blood cancer and within the bone marrow, this research ventures into a previously underexplored area. Moreover, our findings will be further contextualised by integrating epidemiological data from the Haematological Malignancy Research Network, based in York, to enhance the relevance and impact of our conclusions. This innovative and collaborative approach aims to deepen our understanding of ILC roles in haematological malignancies and to assess their potential as biomarkers for diagnosis and treatment strategies.

P.20 **Macrophage-derived developmental endothelial locus 1 (DEL-1) expression promotes an immunoprotective phenotype in experimental visceral leishmaniasis**

Jakub Lukaszonek[1], Najmeeyah Brown[1], Jessie Zenga[1], Elmarie Myburgh[12], James P. Hewitson[23], George Hajishengalis[4], Triantafyllos Chavakis[5], Paul M. Kaye[12], Ioannis Kourtzelis[12]

[1] Hull York Medical School, University of York, UK
[2] York Biomedical Research Institute, University of York, UK
[3] Department of Biology, University of York, UK
[4] Department of Basic and Translational Sciences, Penn Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA
[5] Institute for Clinical Chemistry and Laboratory Medicine, University Hospital and Faculty of Medicine, Technische Universität Dresden, Dresden, Germany

Tissue-derived homeostatic mediators are increasingly recognised as key modulators of immune cell behaviour across diverse microenvironments. Macrophages display remarkable functional plasticity shaped by local signals during infection - many of which remain incompletely defined. Leishmania spp. are intracellular parasites that cause leishmaniasis, a neglected tropical disease affecting millions, with manifestations ranging from cutaneous lesions to potentially fatal visceral infections. These parasites subvert immune control by establishing a replicative niche within phagosomes of host macrophages. Developmental Endothelial Locus-1 (DEL-1) is such homeostatic factor, with anti-inflammatory and pro-resolving functions during sterile inflammation. However, its potential role to intracellular pathogens remains uncharacterised. Here, we establish the novel immunomodulatory role of DEL-1 to intra-phagosomal infection through a murine model of visceral leishmaniasis induced by L. donovani.

In vivo infection was induced in wild-type C57BL/6, Del1-KO, and DEL-1-overexpressing transgenic mice via intravenous injection of L. donovani amastigotes. Parasite burden was quantified through in vivo imaging and histological analysis. Hepatic immune cell populations were assessed by flow cytometry.

Del1-KO mice exhibited significantly increased hepatic parasite loads and granuloma enlargement compared to WT at peak of infection. This heightened inflammatory pathology

included elevated frequencies of IFNγ-producing $CD4^+$ T cells and inflammatory Ly6Chi monocytes/macrophages expressing iNOS, MHC-II, and TNF. In contrast, mice with macrophage-specific overexpression of DEL-1 display reduced parasitic load and attenuated granuloma formation. Of note, endothelial-specific overexpression had no such effect.

Conclusions: Our findings establish DEL-1 as a novel regulator of macrophage-driven immunity during intracellular infection which may be applicable to other types of intra-phagosomal pathogens.

P.21 **Innate urothelial response to BK Polyomavirus drives interferon-mediated APOBEC3A mutational damage.**

George H. Hatton[1], Sally R. James[1], Jennifer Southgate[1], Simon C. Baker[1]

[1] Department of Biology, University of York, UK

Historically, urothelial carcinoma (UCa) was thought to arise from mutational damage primarily derived from tobacco smoke and exposure to industrial chemicals. However, recent identification of mutational signatures from antiviral 'APOBEC3' enzymes in UCa are suggestive of a viral aetiopathology. Whilst epidemiological evidence from immunosuppressed kidney transplant recipients supports BK Polyomavirus (BKPyV) as a risk factor for UCa, BKPyV is absent from the vast majority of UCa. Previous in vitro work by our group identified the capacity of BKPyV to induce mutagenic APOBEC3A/B enzymes in urothelial cells, however the mutational consequence and mechanism of this remains unknown.

Using RT-qPCR and immunoblotting, BKPyV was found to significantly induce transcription of IFNK and IFNL1, STAT2 phosphorylation, and induction of the ISG: IFIT1, consistent with an innate tissue-mediated antiviral response. Treatment with exogenous IFNα1 and IFNλ1 induced significant expression of APOBEC3A within differentiated urothelial cultures, while JAK inhibition (which blocks all interferon signalling) led to a complete loss of APOBEC3A protein induction during BKPyV infection. Immunofluorescence microscopy demonstrated induction of APOBEC3A expression in both infected and uninfected 'bystander' cells, providing a mechanism for generation of APOBEC3-mutated virally-negative tumours. Critically, nanorate sequencing of BKPyV infected urothelial cultures revealed the creation of APOBEC3 associated mutational signatures, as frequently observed in UCa.

This work provides mechanistic evidence that BKPyV infection induces an innate antiviral response in differentiated urothelial cultures through type I and III IFN signalling. Importantly, this response induces APOBEC3A in infected and uninfected cells, providing a novel mechanism for the generation of APOBEC3-mutated tumours lacking BKPyV.

P.22 **Macrophage phagocytosis as a modulator of trained innate immunity - dependent tumour suppression**

Alexandros Chatzis[12], Dave Boucher[23], Ioannis Kourtzelis[12]

[1] Hull York Medical School, University of York, UK; [2] York Biomedical Research Institute, UK; [3] Department of Biology, University of York, UK

There is an increasing need to better understand cancer pathogenesis to improve the efficacy of current immunotherapies. Myeloid cells in solid tumours display hallmarks of cancer. One of their major effector functions, named phagocytosis, regulates anti-tumour immunity. Trained innate immunity (TII) is induced via modulation of mature myeloid cells or their bone marrow progenitors and mediates sustained increased responsiveness to secondary challenges. Although TII has been linked to tumour suppression, its impact on phagocytosis in the tumour

setting remains elusive. Here, we investigated whether macrophage-dependent phagocytosis of tumour cells can be modulated through TII induction.

To assess phagocytosis, mice were pre-treated with β-glucan and bone marrow was isolated for macrophage differentiation. Macrophages were then co-cultured with tumour cells that were either apoptotic or opsonised with an antibody recognising a tumour antigen, to mimic efferocytosis and antibody-dependent cellular phagocytosis (ADCP), respectively.

TII did not have any impact in the modulation of ADCP. Interestingly, efferocytosis was decreased in macrophages from mice pre-treated with β-glucan, which was associated with reduced levels of active caspase-1 and IL-1β cytokine. Similarly, the mRNA levels of molecules promoting efferocytosis were down-regulated in macrophages from the β-glucan group. Combined in vitro treatment of macrophages from mice pre-treated with β-glucan and a commercially available MerTK tyrosine kinase inhibitor further decreased efferocytosis.

Our findings reveal a hitherto unknown role of TII in the regulation of anti-tumour immunity. Given that inhibition of efferocytosis has been linked to tumour suppression, this study may aid in improving existing cancer immunotherapies.

P.23 **Exploring the role of microRNA in Acne Vulgaris**

Prof. Dimitris Lagos, Prof. Alison Layton, Dr Kourtzelis, Ali Deghany

University of York, UK

Acne vulgaris is a prevalent inflammatory skin condition, especially affecting adolescents and young adults. While the causes of acne are multifactorial, including hormonal changes, bacterial colonization, and immune responses, recent research highlights the role of microRNAs (miRNAs) in its pathogenesis. miRNAs are small, non-coding RNA molecules that regulate gene expression post-transcriptionally, impacting various cellular processes such as inflammation, cell proliferation, and differentiation, all of which are central to acne development. Therefore, it is critical to explore the emerging role of miRNAs in acne vulgaris, focusing on their influence on inflammatory pathways and sebaceous gland activity. Acne-associated miRNAs (miR-21, miR-150, and miR-223) have been found to be significantly elevated in both plasma and tissue biopsies of individuals with different severity and scarring; making miRNAs a potential biomarker for prognosis. By elucidating these roles, miRNA-based therapeutics could offer targeted acne treatments, reducing reliance on antibiotics and hormonal therapies, which often have side effects. Further research into miRNA profiles in acne patients could provide valuable biomarkers for disease severity and treatment response, paving the way for precision medicine in dermatology.

P.24 **It takes two to tango: Ferroptosis and pyroptosis control response to intracellular LPS**

Ruth Gibbons, David Oppenshaw, David Kneafsey, Dave Boucher

Department of Biology, University of York, UK

Intracellular Lipopolysaccharide (LPS) detection leads to the activation of the non-canonical

inflammasome, a caspase-4/5 activating platform in humans. Upon activation, caspase-4 and -5 cleave Gasdermin D (GSDMD) to trigger pyroptosis. Our lab is identifying novel signalling pathways that regulate responses to LPS upon caspase-4 activation. A central hypothesis leading our research is that human caspase-4 can cleave unique substrates that control responses to infection. Using quantitative proteomic approaches, we identified novel caspase-4 substrates that promote the activation of ferroptosis. These novel substrates are cleaved by

caspase-4 directly, in primary human epithelial cells, macrophages, and neutrophils in response to LPS transfection and infection with the enteric pathogen Salmonella enterica. Cleavage of these substrates occurs in parallel with GSDMD, driving both death modalities during infections. Pharmacological targeting of ferroptosis impacts cell death in epithelial cells and neutrophils differently and impacts bacterial replication. Our results suggest that gram-negative bacterial infections trigger both pyroptosis and ferroptosis in human cells, highlighting a novel uncharacterised connection between these cell death modalities in human diseases.

P.25 **Novel Cell-autonomous mechanisms controlling intracellular bacterial infections**

Catherine Harper, Nathan Leborgne, Dave Boucher

University of York, UK

Cell-autonomous immunity is a critical first line of defence and refers to the intrinsic ability of individual host cells to detect and eliminate intracellular pathogens without relying only on external immune factors. Amongst these, the non-canonical inflammasome is vital in responding to gram-negative intracellular bacterial infections due to its ability to detect lipopolysaccharide (LPS), a major component of the outer membrane of the gram-negative bacteria. Cytosolic LPS is directly detected by caspase-4 and -5 in humans, triggering an inflammatory signalling cascade. A large proteomics screen from our lab was performed in human interstinal epithelial cells treated with LPS to activate caspase--4,-5 identified APOL2 as. putative . Here, I characterise APOL2 as a novel substrate of caspase-4 and identify its impact on inflammasome signaling. Although preliminary, our work identify a novel caspase-4 effector mechanisms controlling gram-negative infections.

P.26 **Cell death modalities and infections**

Adam Sung, Callum Robson, Dave Boucher

Department of Biology, University of York, UK

Salmonella enterica serovar typhimurium (S.Typhimurium) infections induce ranges of gastrointestinal and systemic disease in human hosts. Virulence and symptom severity are associated with two major regions lipopolysaccharide (LPS) and salmonella pathogenicity islands 1 and 2, type 3 secretion systems (SPI-1/SPI-2 T3SS). These ingrained systems produce effector proteins from bacteria vacuole into host target cells and promote bacterial survivals. Mutations to T3SS effector proteins or LPS region potentiates S.Typhimurium to further host recognition and stronger innate immune responses within primary human macrophage and epithelial cells and cell lines, particularly in inflammasome responses. Inflammasome activity triggering activations of cell host effector cysteine proteases, canonical caspase 1 or non-canonical caspase 4/5, leading to subsequent pyroptosis of pathogen. Our project aims to further define the activations of these inflammatory pathways in response to mutated T3SS or LPS S.Typhimurium infections within human macrophages, epithelial or THP-1 cells. Furthermore, SPI-2 T3SS proteases GtgE and SseL were purified and exogenous expression found may potentially trigger GBP-1 activation, a precursor to the non-canonical pathway. We also show S.Typhimurium mutants elicit variable effects of cell deaths and inflammatory pathways within our target host cells, further improving nature of understanding in human host pathogen interactions.

P.27 **Innate recognition of PAMPs**

Barwin Viswanathan, Davey Kneafsey, Ioannis Kourtzelis, Dave Boucher

University of York, UK

The innate immune system sense a range of molecules associated with infectious agents. Recognition of defined ligands called Pathogens-associated Molecular Patterns (PAMPs) activate specific signalling pathways to control infections and maintain homeostasis. One such pathway is the inflammasome, which activates signalling proteases called caspases to generate inflammation by processing cytokines and killing cells. My project aims to identify novel receptors sensing PAMPs in the cytosol of human Keratinocytes. Here, I will discuss my initial findings which uncovered new PAMPs activating inflammasomes and the characterisation of the downstream immune response observed. Overall, we hope that our work will lead to the identification of novel inflammasome-activating receptors that control infections in the human skin.