BSI Bristol Immunology Group:
The immunology of long-haul disease
Monday 19 June, 2023
Bristol Beacon, Bristol

MONDAY 19 JUNE

08:45	Registration
09:20	**Welcome** Lindsay Nicholson, University of Bristol, UK
	SESSION 1 – Infection and Immunopathology Chair(s): Laura Rivino & Simon Eastham, University of Bristol, UK
09:30	**Comorbidities in African adolescents with perinatally-acquired HIV infection – a tale of two viruses?** Sarah Rowland-Jones, University of Oxford, UK – *invited speaker*
09:55	**Neutrophils as protagonists in severe malaria** Borko Amulic, University of Bristol, UK – *invited speaker*
10:20	**CD14 marks tissue CD8+T-cells instructed by myeloid cells and modulated by LPS** Laura Pallett, University College London, UK – *selected short talk*
10:40	**The senescent secretome promotes expression of the fetal receptor PLVAP in human hepatic endothelium to promote monocyte transmigration.** Shishir Shetty, University of Birmingham, UK - *selected short talk*
11:00	Refreshment break, posters and meet the exhibitors
	SESSION 2 – Autoimmunity, Inflammation and Immune Privilege Chair(s): Lindsay Nicholson & Claire Naveh, University of Bristol, UK
11:30	**Fine-tuning costimulation blockade in autoimmunity** Lucy Walker, University College London, UK – *invited speaker*
11:55	**Antigen-specific immune intervention in long-haul autoimmune diseases** David Wraith, University of Birmingham, UK – *invited speaker*
12:20	**Canagliflozin impairs T-cell effector function via metabolic suppression in autoimmunity** Benjamin Jenkins, Swansea University, UK - *selected short talk*

12:40	**IL-23 drives uveitis by acting on a novel population of resident ocular T cells** David Copland, University of Bristol, UK - *selected short talk*
13:00	Lunch, posters and exhibition
	SESSION 3 – Cancer Immunology Chair(s): Gareth Jones & Michaela Gregorova, University of Bristol, UK
14:15	**Breaking Down Multiple Barriers to Tumour Immunity: how to trigger the perfect storm?** Awen Gallimore, Cardiff University, UK – *invited speaker*
14:40	**Hedgehog signaling in immune cells: novel biology and treatment opportunities** Maike De La Roche, University of Cambridge, UK – *invited speaker*
15:05	**Investigating the impact of radiation on the immunopeptidome of colorectal cancer cell lines** Jessica Oliver, Cardiff University, UK - *selected short talk*
15:25	**TIM3 is a context-dependent co-regulator of cytotoxic T cell function** Hanin Alamir, University of Bristol, UK - *selected short talk*
15:45	Refreshment break, posters and exhibition
16:00	**What we've learnt about immune inflammation and defence from studies of COVID** Peter Openshaw, Imperial College London, UK – *key note speaker*
16:45	Prizes and concluding remarks
17:00	Networking reception
18:00	Meeting close

Poster presentations

P.01 CAR-TREG cell therapies and their future potential in treating ocular autoimmune conditions.

Alan Abraham, Ophthalmology Research Group, Academic Unit of Ophthalmology, Translational Health Sciences, University of Bristol, UK

P.02 The longitudinal loss of islet autoantibody responses from diagnosis of type 1 diabetes occurs progressively over follow-up and is determined by low autoantibody titres, early-onset, and genetic variants.

C.L. Williams, Diabetes and Metabolism, Bristol Medical School, University of Bristol, UK

P.03 Feeding and autoimmunity in children with Down Syndrome evaluation study (FADES): Evidence of early autoimmunity to insulin.

Georgina Mortimer, Diabetes, Bristol Medical School, Translational Health Sciences, University of Bristol, UK

P.04 Impaired immune response to dengue viral infection in children and young adults with obesity

Michaela Gregorova, University of Bristol, UK

P.05 Modulation of pro-inflammatory and pro-fibrotic monocyte phenotype by novel mitochondria-targeted hydrogen sulfide delivery.

E. Leonova, University of Exeter, UK

P.06 Suppression of the function of human anti-tumour T cells with a defined T cell receptor by human tumour cell spheroids

Amal Alsubaiti, University of Bristol, UK

P.07 Immature Neutrophils: Showing their age

Claire Naveh, University of Bristol, UK

P.08 Mesenchymal stem cells as a therapeutic agent for SARS-CoV-2 infection

Erly Savitri, School of Cellular and Molecular Medicine, University of Bristol, UK

P.09 Circulating white blood cell traits and colorectal cancer risk: A Mendelian randomization study

Andrei-Emil Constantinescu, MRC Integrative Epidemiology Unit at the University of Bristol, UK, Bristol Medical School, Population Health Sciences, University of Bristol, UK, School of Translational Health Sciences, Bristol Medical School, University of Bristol, UK

P.10 Assessing changes in the ocular micro-environment during inflammatory arthritis to understand co-existing arthritis and uveitis

S. Eastham, University of Bristol, UK

P.11 Can the cross-reactivity of therapeutic TCRs be determined in silico?

Annabelle Hartt, School of Biochemistry, University of Bristol, UK

P.12 Hemozoin, a by-product of malaria infection, suppresses the neutrophil oxidative burst.

Chinelo Etiaba, University of Bristol, UK

P.13 Analysis of NK-cell function during dengue viral infection in children and young adults

Marianna Santopaolo, Divya Diamond, School of Cellular and Molecular Medicine, Faculty of Life Sciences, University of Bristol, UK

P.14 Impact of cryopreservation on immune cell metabolism as measured by SCENITH

Curtis Luscombe, University of Bristol, UK

P.15 Autoimmune Disease and Cancer Crosstalk: A Shared Neo-Antigen inhibits the anti-tumour immune response

Maryam Alismail, School of Cellular and Molecular Medicine, University of Bristol, UK

P.16 The impact of L-arginine deprivation on CMV and SARS-CoV-2-specific T-cell proliferation

AV Ramesh, University of Bristol, UK

The British Society for Immunology like to thank the following sponsors for their support:

Selected talks abstracts

Canagliflozin impairs T-cell effector function via metabolic suppression in autoimmunity

Benjamin J. Jenkins[1], Julianna Blagih[2,3], Fernando M. Ponce-Garcia[1], Mary Canavan[4], Nancy Gudgeon[5], Simon Eastham[6], David Hill[6], Megan M. Hanlon[4], Eric H. Ma[7,8], Emma L. Bishop[5], April Rees[1], James G. Cronin[1], Elizabeth C. Jury[9], Sarah K. Dimeloe[5], Douglas J. Veale[10], Catherine A. Thornton[1], Karen H Vousden[2], David K. Finlay[11], Ursula Fearon[4], Gareth W. Jones[6], Linda V. Sinclair[12], Emma E. Vincent[13,14], Nicholas Jones[1*]

[1]Institute of Life Science, Swansea University Medical School, Swansea University, UK, [2]The Francis Crick Institute, UK, [3]University of Montreal, Canada, [4]Molecular Rheumatology, School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland, [5]Institute of Immunology and Immunotherapy, Institute of Metabolism and Systems Research, College of Medical and Dental Sciences, University of Birmingham, UK, [6]Cellular and Molecular Medicine, University of Bristol, UK, [7]Department of Metabolism and Nutritional Programming, Van Andel Institute, USA, [8]Rheos Medicines, Cambridge, USA, [9]Centre for Rheumatology Research, Division of Medicine, University College London, UK, [10]EULAR Centre of Excellence, Centre for Arthritis and Rheumatic Diseases, St Vincent's University Hospital, Ireland, [11]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland, [12]Division of Cell Signalling and Immunology, School of Life Sciences, University of Dundee, UK, [13]School of Translational Health Sciences, University of Bristol, Bristol, UK, [14]Integrative Epidemiology Unit, School of Population Health Science, University of Bristol, UK

Augmented T-cell function leading to host damage in autoimmunity is supported by metabolic dysregulation. Targeting immunometabolism for the treatment of autoimmune disease by repurposing clinically approved metabolic modulators, such as those used to treat people with type 2 diabetes (T2D), is therefore an attractive avenue. Canagliflozin – a member of the newest class of T2D drug, sodium glucose co-transporter 2 (SGLT2) inhibitors – has known off-target effects including inhibition of mitochondrial glutamate dehydrogenase (GDH) and complex I. To date, the known effects of SGLT2 inhibitors on human T-cell function are extremely limited. Here, we show that canagliflozin-treated human CD4+ T-cells are compromised in their ability to activate, proliferate and initiate effector functions such as cytokine production. We demonstrate that canagliflozin inhibits the T-cell receptor signalling cascade, impacting on downstream ERK and mTORC1 activity, concomitantly associated with reduced c-Myc. Compromised c-Myc levels were encapsulated by a failure to engage translational machinery resulting in impaired metabolic protein and solute carrier production amongst others. Importantly, canagliflozin treatment of T-cells derived from patients with autoimmune disorders (systemic lupus erythematosus and rheumatoid arthritis) significantly impaired their effector function. Taken together, our work highlights a potential therapeutic benefit for repurposing canagliflozin as an intervention for T-cell mediated autoimmunity.

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CD14 marks tissue CD8+T-cells instructed by myeloid cells and modulated by LPS

Laura J. Pallett[1], Leo Swadling[1], Mariana Diniz[1], Alexander A. Maini[2], Marius Schwabenland[3], Adrià Dalmau Gasull[3], Jessica Davies[1], Stephanie Kucykowicz[1], Jessica K. Skelton[4], Niclas Thomas[1], Nathalie M. Schmidt[1], Oliver E. Amin[1], Upkar S. Gil[l5], Kerstin A. Stegmann[1], Alice R. Burton[1], Emily Stephenson[6], Gary Reynolds[6], Matt Whelan[1], Jenifer Sanchez[7], Roel de Maeyer[2], Clare Thakker[1], Kornelija Suveizdyte[1], Imran Uddin[1], Ana M. Ortega-Prieto[4], Charlotte Grant[8], Farid Froghi[8], Giuseppe Fusai[8], Sabela Lens[9], Sofia Pérez-del-Pulgar[9], Walid Al-Akkad[10], Giuseppe Mazza[10], Mahdad Noursadeghi[1], Arne Akbar[2], Patrick T. F. Kennedy[5], Brian R. Davidson[8,10], Marco Prinz[3], Benjamin M. Chain[1], Muzlifah Haniffa[6], Derek W. Gilroy[2], Marcus Dorner[4], Bertram Bengsch[3], Anna Schurich[7] & Mala K. Maini[1]

[1] Division of Infection & Immunity, Institute of Immunity & Transplantation, University College London, UK, [2] Division of Medicine, University College London, UK, [3] Institute of Neuropathology, University of Freiburg, Germany, [4] Department of Medicine, Imperial College London, UK, [5] Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK, [6] Biosciences Institute, Faculty of Medical Sciences, Newcastle University, UK,[7] School of Immunology and Microbial Sciences, Kings College London, UK, [8] Division of Surgery, University College London, UK, [9] Liver Unit, Hospital Clinic, IDIBAPS and CIBEREHD, University of Barcelona, Spain, [10] Institute for Liver & Digestive Health, University College London, UK.

Background and Aims: The liver is bathed in bacterial products, including lipopolysaccharide transported from the intestinal portal vasculature, but is able to maintain a state of tolerance that is then exploited by persistent pathogens and tumours. The cellular basis mediating this tolerance, yet allowing a switch to immunity or immunopathology, needs to be better understood for successful immunotherapy of liver diseases.

Method: We analysed the phenotype and function of CD14-expressing CD8 T cells directly ex vivo from resected/explanted human liver and explored their derivation, functionality, expansion and LPS-responsiveness in multiple in vitro and in vivo models.

Results: Here we show that a variable proportion of CD8+ T cells compartmentalized in the human liver co-stain for CD14 and other prototypic myeloid membrane proteins and sit in close proximity to CD14high myeloid cells in the liver. CD14+CD8+ T cells exhibit increased turnover, activation and constitutive immunomodulatory features with high homeostatic IL-10 and IL-2 production ex vivo, and enhanced antiviral/anti-tumour effector function after TCR engagement. Whereas stimulation via CD14 by bacterial lipopolysaccharide not only increases the frequency of CD14+CD8+ T cells in vitro and in vivo but skews their function towards the production of chemotactic and regenerative cytokines. This CD14+CD8+ T cell profile seen ex vivo in tissues, can be recapitulated by the acquisition of membrane proteins—including the lipopolysaccharide receptor complex—from mononuclear phagocytes, resulting in augmented tumour killing by TCR-redirected T cells in vitro.

Conclusion: A proportion of CD8 T cells compartmentalised in the liver express CD14/TLR4/MD2, recapitulated in vitro by membrane acquisition from mononuclear phagocytes. Thus, bacterial products in the gut-liver axis and tissue stromal factors can fine tune liver immunity by driving myeloid instruction of CD8+ T cells with immunomodulatory ability.

The senescent secretome promotes expression of the fetal receptor PLVAP in human hepatic endothelium to promote monocyte transmigration.

Alex L Wilkinson[1], Samuel Hulme[1], Paul Horn[1], Marco Y. W. Zaki[2], Matthew Hoare[3], Daniel A Patten[1], Shishir Shetty[1]

[1]University of Birmingham, UK, [2]University of Minia, Egypt, [3]University of Cambridge, UK

Chronic inflammatory liver disease (CLD) is a global challenge leading to organ failure or cancer development. Liver sinusoidal endothelial cells (LSEC) which line the sinusoidal channels of the liver, regulate immune cell recruitment. LSEC undergo significant phenotypic changes in CLD and yet the factors that drive this process and the impact on their function as a vascular barrier and gatekeeper for immune cell recruitment are poorly understood. Recent single cell studies have identified the fetal receptor, Plasmalemma vesicle-associated protein (PLVAP), as a marker of disease-associated LSEC. This led us to study the expression of PLVAP in CLD and its functional role in primary human LSEC.

We found increased PLVAP in scar associated LSEC, correlating with markers of tissue senescence in CLD. Furthermore, exposure of human LSEC to the senescence associated secretory phenotype (SASP) in vitro led to a significant upregulation of PLVAP. Using flow based adhesion assays we studied the contribution of PLVAP in SASP-driven leukocyte recruitment across LSEC. We found that leukocyte subset recruitment in this setting was characterised by paracellular transmigration of monocytes whilst the majority of lymphocytes migrated transcellularly through LSEC. Knockdown studies and antibody blockade confirmed that PLVAP selectively regulated monocyte transmigration. Bulk RNA sequencing and confocal imaging demonstrated that PLVAP regulated LSEC junctional integrity by regulating Phosho-VE-cadherin expression and endothelial gap formation.

Our studies suggest that tissue senescence promotes re-emergence of the fetal receptor PLVAP in LSEC to promote monocyte recruitment. PLVAP may therefore be a potential endothelial target to treat CLD and its complications.

IL-23 drives uveitis by acting on a novel population of resident ocular T cells

David A Copland[1,3,5], Robert Hedley[2,5], Amy Ward[1,5], Colin J Chu[1,3,5], Jonathan Sherlock[2,4,5], Andrew D Dick[1,3,5]

[1]Translational Health Sciences, Ophthalmology, University of Bristol, UK, [2]Kennedy Institute of Rheumatology, University of Oxford, UK, [3]Institute of Ophthalmology, University College London, UK, [4]Janssen, USA, [5]ORBIT Consortium ORBIT — The Kennedy Institute of Rheumatology

Purpose: Acute anterior uveitis is a frequent ocular co-morbidity strongly associated with the Spondyloarthropathies (SpA), a group of chronic inflammatory diseases affecting the joints, skin, and gut of patients. Pathology at these different anatomical sites is driven by local resident populations of interleukin (IL)-23 responsive lymphoid cells. We therefore sought evidence for a resident population of IL-23 responsive cells within the ocular entheseal tissues.

Methods: B6(Cg)-Tyrc-2J/J (albino) and C57BL/6J IL-23R-eGFP reporter mice were used to determine tissue localization and phenotype of resident CD3+ populations in normal mouse anterior uvea. Perfused eyes were optically cleared for whole tissue Ce3D Lightsheet imaging. Flow cytometry (FACS) and ImageStream®X MK II analysis was performed to immunophenotype cells. To evaluate IL-23 responsiveness, C57BL/6J or Rag2-/- mice received intravitreal (IVT) injection of ShH10 adeno-associated virus (AAV) encoding an IL-23 hyperkine. Clinical assessment (Fundus & OCT) was performed to determine onset of clinical inflammation. Dissected anterior and posterior uvea were processed for FACS enumeration of CD45+ infiltrate.

Results: Ex vivo tissue imaging of naïve eyes reveals CD3+IL-23R+ cells are located across multiple tissues of the anterior uvea, including the sclera, iris, and ciliary body. FACS identifies 50-100 cells/eye, and defines the naive phenotype as CD3+CD4-CD8-TCRγδ+RORγt+IL-23R+. In vivo, IL-23 over-expression elicits clinical inflammation in anterior and posterior segments of the eye by day 12. FACS confirms a significant increase in CD45+CD3+ cell number in anterior and posterior compared to eyes receiving control ShH10 vector. Ex vivo stimulation of anterior uvea shows increased IL-17A production by CD3+γδTCR+ from cells following IL-23 overexpression. ShH10-mediated IL-23 expression does not elicit clinical inflammation or infiltration of CD45+ cells in Rag2 deficient mice.

Conclusions: A novel population of ocular T cells defined by CD3+CD4-CD8-TCRγδ+IL-23R+ expression resides within the anterior uvea of the mouse eye. Localised ocular cytokine expression demonstrates that resident IL-23R+ IL-17A producing cells are both necessary and sufficient to drive uveitis in response to IL-23. Further characterization of this population is warranted.

TIM3 is a context-dependent co-regulator of cytotoxic T cell function

Hanin Alamir[1], Carissa C.W. Wong[1], Amal Alsubaiti[1], Grace L. Edmunds[1], James Boyd[1], Tressan Grant [1,2], David J. Morgan[1], Christoph Wülfing[1].

[1]School of Cellular and Molecular Medicine, University of Bristol, UK, [2]Department of Life Sciences, University of Bath, UK

Cytotoxic T lymphocytes (CTLs) are essential effectors in the anti-viral and anti-tumour immune response and attractive targets in cancer immunotherapy. Although CTLs can directly recognise and kill tumour cells, tumour cells suppress CTLs by different mechanisms. This project investigated the upregulation of the inhibitory receptor T-cell immunoglobulin and mucin domain 3 (TIM3). TIM3 is expressed on T cells after chronic antigen exposure and marks the most exhausted tumour infiltrating CTLs in multiple solid tumours. However, it is unclear whether TIM3 directly regulates CTL function. Also, despite a predominantly inhibitory role in vivo, TIM3 promotes cellular activation in T cells, and roles of putative ligands in TIM3 function are controversial. Therefore, we aimed to determine the effect of TIM3 on direct CTL anti-tumour functions and how TIM3 ligand Galectin9 regulates its function. We employed three-dimensional (3D) tumour spheroids that effectively induce CTL suppression similar to the in vivo tumour microenvironment in comparison to conventional two-dimensional (2D) tumour cell culture. In the 3D model, TIM3 significantly inhibited CTL cytotoxicity and cytoskeletal polarisation as a key mechanism of effective cytolysis in murine and human CTLs. Acute blockade of TIM3 by monoclonal antibodies reversed this inhibition. In contrast, in the 2D model, TIM3 stimulated CTL effector functions. Expression of Galectin9 on tumour cells further suppressed CTL killing ability in the 3D spheroid model. In the 2D, however, Galectin9 enhanced TIM3 costimulatory functions. In summary, our data suggest

that TIM3 functions as a context-dependent coregulatory receptor as supported by the expression of its ligand Galectin9.

Investigating the impact of radiation on the immunopeptidome of colorectal cancer cell lines

Jessica Oliver[1], Kirti Pandey[2], Stephanie Burnell[1], Anthony Purcell[2], Awen Gallimore[1], Andrew Godkin[1]

[1]Cardiff University, UK, [2]Monash University, Australia

There is renewed focus in the potential of radiotherapy to stimulate T cell recognition of tumour cells, through mechanisms that include the upregulation of HLA-I on the tumour cells. The complex array of peptides displayed by HLA molecules are termed the immunopeptidome and its studying is a powerful tool for identifying actionable cancer antigens and their T cell epitopes. It is crucial to understand how radiotherapy modifies the immunopeptidome to develop targeted approaches to cancer. Thus, the aim of this research was to profile the immunopeptidome pre- and post-radiotherapy of colorectal tumours.

Irradiation of a malignant colorectal cell line, SW480, provided a proof-of-principal that radiation increases HLA-I expression. Subsequently, we investigated whether the immunopeptidome of these cells was altered by irradiation by investigating eluted peptides from purified HLA-I molecules from irradiated and non-irradiated cells and identifying them by tandem mass spectrometry.

Irradiated cells demonstrated a significant increase in the total number of unique peptides presented at the tumour cell surface, with a unique radiation-induced peptide repertoire emerging, exhibiting peptides with C and/or N terminal extensions. Additionally, the peptides isolated from the irradiated cells were sourced from more diverse antigens with more distinct peptides representing each source protein. This was also evident for tumour-associated antigens (TAAs) with enhanced presentation of peptides from proteins associated with poor prognosis in colorectal cancer.

This research establishes that irradiation impacts the immunopeptidome and alters what is available for scrutiny by T cells. Increased presentation of TAAs may heighten tumour immunogenicity and facilitate tumour eradication.

Poster abstracts

P.01 CAR-TREG cell therapies and their future potential in treating ocular autoimmune conditions.

Alan Abraham[1], Panayiotis Maghsoudlou[1,2], David Copland[1], Lindsay Nicholson[1], Andrew Dick[1,3]

[1]Ophthalmology Research Group, Academic Unit of Ophthalmology, Translational Health Sciences, University of Bristol, UK, [2]University of Bath, UK, [3]UCL Institute of Ophthalmology, UK

Ophthalmic autoimmune and autoinflammatory conditions cause significant visual morbidity and require complex medical treatment complicated by significant side effects and lack of specificity. Regulatory T cells (Tregs) have key roles in immune homeostasis and in the resolution of immune responses. Polyclonal Treg therapy has shown efficacy in treating autoimmune disease. Genetic engineering approaches to produce antigen-specific Treg therapy has the potential for enhanced treatment responses and fewer systemic side effects. Cell therapy using chimeric antigen receptor modified T cell (CAR-T) therapy, has had significant success in treating haematological malignancies. By modifying Tregs specifically, a CAR-Treg approach has been efficacious in preclinical models of autoimmune conditions leading to current phase 1-2 clinical trials. This educational poster summarises CAR structure and design, Treg cellular biology, developments in CAR-Treg therapies, and discusses future strategies to apply CAR-Treg therapy in the treatment of ophthalmic conditions.

P.02 The longitudinal loss of islet autoantibody responses from diagnosis of type 1 diabetes occurs progressively over follow-up and is determined by low autoantibody titres, early-onset, and genetic variants.

C.L. Williams, R. Fareed, G.L.M. Mortimer, R.J. Aitken, I.V. Wilson, G. George, K.M. Gillespie, A.J.K. Williams, The BOX Study Group, A. E. Long.

Diabetes and Metabolism, Bristol Medical School, University of Bristol, UK

The clinical usefulness of post-diagnosis islet autoantibody levels is unclear and factors that drive autoantibody persistence are poorly defined in type 1 diabetes (T1D). Our aim was to characterise longitudinal loss of islet autoantibody responses after diagnosis in a large, prospectively sampled UK cohort.

Participants with T1D [n=577] providing a diagnosis sample [range -1.0-2.0 years] and at least one post-diagnosis sample (<32.0 years) were tested for autoantibodies to glutamate decarboxylase 65 (GADA), islet antigen-2 (IA-2A), and zinc transporter 8 (ZnT8A). Select HLA and non-HLA SNPs were considered. Non-genetic and genetic factors were assessed by multivariable logistic regression models for autoantibody positivity at initial sampling and autoantibody loss at final sampling.

For GADA, IA-2A, and ZnT8A, 70.8%, 76.8% and 40.1%, respectively, remained positive at final sampling. Non-genetic predictors of autoantibody loss were low baseline autoantibody titres ($p<0.0001$), longer diabetes duration ($p<0.0001$), and age-at-onset under 8 years ($p<0.01$-0.05). Adjusting for non-genetic covariates, GADA loss was associated with low-risk HLA class II genotypes ($p=0.005$), and SNPs associated with autoimmunity RELA/11q13 ($p=0.017$), LPP/3q28 ($p=0.004$), and negatively with IFIH1/2q24 ($p=0.018$). IA-2A loss was not associated with genetic factors independent of other covariates, while ZnT8A loss was associated with the presence of HLA A*24 ($p=0.019$) and weakly negatively with RELA/11q13 ($p=0.049$).

The largest longitudinal study of islet autoantibody responses from diagnosis of T1D shows that autoantibody loss is heterogeneous and influenced by low titres at onset, longer duration, earlier age-at-onset, and genetic variants. These data may inform clinical trials where post-diagnosis participants are recruited.

P.03 Feeding and autoimmunity in children with Down Syndrome evaluation study (FADES): Evidence of early autoimmunity to insulin.

Georgina Mortimer[1], Georgina Williams[2], Sam Leary[2], Stu Toms[2], Julian Hamilton-Shield[2], Kathleen Gillespie[1]

[1]Diabetes, Bristol Medical School, Translational Health Sciences, University of Bristol, UK, [2]Biomedical Research Centre, Translational Health Sciences, Bristol Medical School, University of Bristol, UK

Children with Down syndrome (DS) have an increased risk of autoimmune conditions including diabetes, coeliac, and thyroid disease. FADES was developed to study the association between early feeding, infections, antibiotic use, gut microbiome, and autoimmunity in children with DS.

In-depth feeding and medical questionnaires, stool, urine, and capillary blood samples were collected at birth, 6 months, and annually until the study end and/or 7 years of age. DNA was tested for T1D HLA class II susceptibility haplotypes. Serum was tested for autoantibodies associated with islet, celiac, and thyroid autoimmunity using radioimmunoassay and luciferase immunoprecipitation systems.

Since 2014, 116 infants (Male n=56) were recruited at mean age 16.8 weeks (S.D. 9.8 weeks). Initial questionnaires were completed by 89% of the participants with samples obtained from 72%. Thus far, autoantibodies associated with type 1 diabetes, celiac disease, and thyroid disorders were identified in 10/71 (14.0%) in the last available sample including 6/71 (8.5%) positive for insulin autoantibodies (IAA), the youngest seroconverting at 0.5 years and the eldest at 5 years. Genetic samples were available from 5/7 with IAA, none had T1D HLA class II susceptibility haplotypes; two individuals were heterozygous for the protective DRB01*15-DQB01*0602.

FADES has created a bank of samples and information to be analysed to explore the effects of early life influences on autoimmunity. Initial results support an increased frequency of islet autoimmunity in children with DS. This cohort should prove a valuable resource for future research into autoimmunity in DS.

P.04 Impaired immune response to dengue viral infection in children and young adults with obesity

Michaela Gregorova[1], Nguyet Minh Nguyen[2], Ho Quang Chanh[2], Nguyen Thi Xuan Chau[2], Dong Thi Hoai Tam[2], Tran Thuy Vi[2], Duyen Huynh Thi Le[2], Cao Thi Tam[3], Hoa Vo Thi My[2], Marianna Santopaolo[1], Sophie Yacoub[2,4,] Laura Rivino[1]

[1]University of Bristol, UK, [2]Oxford University Clinical Research Unit, Vietnam, [3]Hospital for Tropical Diseases, Vietnam, [4]Centre for Tropical Medicine and Global Health, Oxford University, UK

Background: Obesity, an increasing global health problem, has been associated with development of severe. But the exact role of immune cells and mechanism underlying obesity as a risk factor is still not fully understood. Our study investigates the phenotype/functionality of T-cells and their association with body mass index and dengue outcomes.

Methods: Multiparameter flow cytometry was used to evaluate the phenotypic/functional characteristics of T-cells in a Vietnamese cohort of acute non-severe and severe dengue patients (day 3 and 8 illness onset). Magnitude, phenotype and multifunctionality of DENV-specific T-cells were assessed by intracellular cytokine staining.

Results: Obesity associated with more dysfunctional and impaired phenotype of CD8+ T, mainly their cytotoxic ability, suggesting altered immune responses with potentially impaired capacity to clear dengue virus. Moreover, these altered cellular phenotypes were more marked in severe disease and strongly correlated with increasing BMI and serum leptin levels. In both patient groups (obesity and normal weight), DENV-specific CD8+ T-cells expressed high levels of MIP-1β and CD107a, with variable levels of IFN-γ, TNF-α and IL-2 cytokines. DENV-specific CD8+ T-cell responses were higher at day 3 in severe dengue patients and patients with obesity compared to non-severe and normal weight patients but were significantly lower at day 8.

Conclusions: We identified alterations in immune responses to dengue virus in patients with obesity compared to patients with normal weight, which were more marked in severe disease and secondary infection. Moreover, our data suggest potential impairment in the anti-viral capacity of T-cells in patients with obesity, particularly during severe and secondary dengue infection.

P.05 Modulation of pro-inflammatory and pro-fibrotic monocyte phenotype by novel mitochondria-targeted hydrogen sulfide delivery.

E. Leonova [1], S.L. Barratt[2], M.A. Lindsay[3], M. Whiteman1, C.J. Scotton[1]

[1]University of Exeter, UK, [2]North Bristol NHS Trust, UK, [3]University of Bath, UK

Background: Idiopathic pulmonary fibrosis (IPF) is a progressive and incurable disease requiring novel therapeutics. High baseline monocyte count associates with increased risk of progression and mortality in IPF. Perturbations in monocyte phenotype and mitochondrial function, IL6 production, ACOD1 gene expression and metabolism have been observed in IPF patients. IPF patients also have reduced biosynthetic capacity for the immunomodulatory gas hydrogen sulfide (H2S) - an endogenous regulator of mitochondrial function and inflammation. However, the impact of H2S modulation in monocytes is unknown.

Aims: Investigate the anti-fibrotic and anti-inflammatory effects of mitochondrial-targeted H2S donor compounds (mtH2SD) in human monocytes.

Methods: Fresh monocytes were isolated from healthy donors (n=11), stimulated with IFNγ/TNFα/IL-4 (20ng/ml) +/- mtH2SD (100nM) for 24 hours prior to multispectral flow cytometry, Seahorse metabolic assays, ELISA, qPCR analysis.

Results: In vitro mtH2SD treatment of stimulated monocytes resulted in reduced pro-fibrotic marker levels (CD206 and CD163; $p<0.05$), more energetic and glycolytic metabolism, augmentation of anti-fibrotic ACOD1 expression, decreased IL-6 production and gene expression ($p<0.01$).

Conclusions: Modulation of phenotype and metabolism, pro-fibrotic and pro-inflammatory gene expression by mtH2SD may have therapeutic potential for IPF. Future work will recapitulate these data in IPF-derived monocytes.

P.06 Suppression of the function of human anti-tumour T cells with a defined T cell receptor by human tumour cell spheroids

Amal Alsubaiti[1], Hanin Alamir[1], Christopher Holland[2], Christoph Wuelfing[1]

[1]University of Bristol, UK, [2]immunocore, UK

Cytotoxic T cells commonly recognise tumour antigens and have the ability to kill tumour target cells. However, such killing is often suppressed within the tumour microenvironment. T cells eventually lose their ability to proliferate and release effector cytokines and cytotoxic granules that are essential for effective elimination of tumour cells. As one mechanism of such suppression, T cells increase expression of inhibitory receptors, including T-cell immunoglobulin mucin-3 (TIM-3). Overcoming T cell suppression is a fundamental strategy in cancer immunotherapy. Robust in vitro cell-based assays are required to optimise therapeutic approaches to do so. Here we utilize 3D tumour cell spheroids and the lentiviral expression of the IG4 T cell receptor recognising a peptide derived from the NYESO melanoma tumour antigen in primary human T cells for the development an assay focussing on CTL function. As key features of our assay, killing of tumour target cells was peptide-dependent, IG4-expressing T cells acquired a suppressed phenotype through incubation with the spheroids and such suppression was further enhanced by TIM3 expression.

P.07 Immature Neutrophils: Showing their age

Claire Naveh, Borko Amulic, Ashley Toye, Christopher Rice, Kieran Roberts

University of Bristol, UK

Neutrophils are the most abundant white blood cells in the circulation, being the first responders to a site of infection. Their antimicrobial arsenal includes phagocytosis, degranulation of antimicrobial peptides, production of reactive oxygen species, neutrophil extracellular trap (NET) formation and cytokine release. Whilst this antimicrobial arsenal makes neutrophils essential for clearing pathogens, inappropriate or over activation may also cause damage to the host. Numerous inflammatory diseases including malaria and severe COVID-19 are associated with the appearance of immature neutrophils in circulation. These immature neutrophils are an indicator of poor clinical prognosis; however, little is known in terms of their function and mechanism in severe inflammatory disease. We hypothesise that immature neutrophils have altered functional properties that distinguish them from mature neutrophils. To address this we used two experimental systems: GCSF-treated healthy donors with circulating pools of primary immature neutrophils, and in vitro differentiation of human CD34+ stem cell derived cultured neutrophils. For both systems, we tested antimicrobial functions including NETosis, degranulation, cytokine production and mitochondrial content. It was clear that in both systems immature neutrophils differed from mature healthy neutrophils, but further investigation is needed to elucidate how this may be relevant for pathogenesis of inflammatory disease.

P.08 Mesenchymal stem cells as a therapeutic agent for SARS-CoV-2 infection

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Background and Purpose:

The highly contagious SARS-CoV-2 gives a variety of symptoms, ranging from asymptomatic cases to severe and long-term debilitating symptoms. Dysregulated immune response is strongly associated with disease severity, lung tissue damage, and long-term functional disability. Human mesenchymal stem cells (MSCs) are multipotent, self-renewing cells that have an immunomodulatory, anti-inflammatory, and regenerative properties. We hypothesized that MSCs can dampen lung inflammation and repair lung tissue damage caused by the virus. The aim of study was to investigate the immunomodulatory and regenerative properties of MSCs on SARS-CoV-2 infection of lung cells.

Materials and Methods:

The human adenocarcinoma lung epithelial cell line, Calu-3, was used to model lung tissue air-liquid interface (ALI) in vitro. The model was infected with SARS-CoV-2 and co-cultured with MSCs (n=4) for 1 and 4 days. The impact of MSCs on Calu-3 was evaluated by measuring gene expression of inflammatory cytokines and immunostaining of epithelial integrity markers.

Results:

MSCs significantly reduced the inflammatory markers IL4, IL6, IL8, IL13, and TNFα and increased the expression of epithelial integrity marker, CDH1, 1 and 4 days after infection. The disruption of lung epithelial tight junction was repaired by the MSCs at both time points, while human dermal fibroblast (HDF), as MSC control cells, did not impact the infected cells.

Conclusion:

MSCs can reverse the damage to lung epithelial cells caused by SARS-CoV-2 infection by inhibiting inflammatory cytokines and improving the integrity of epithelial cells. These findings support the potential for using MSCs or their by-products in cell therapy modalities for long COVID patients.

P.09 Circulating white blood cell traits and colorectal cancer risk: A Mendelian randomization study

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Colorectal cancer (CRC) is one of the most common cancers in the UK and accounts for around 10% of cancer deaths worldwide. Previous studies have suggested a role for immune cell subtypes in colorectal cancer, with eosinophils having a protective effect and neutrophils having a detrimental effect. Here, we aimed to investigate the effect of circulating immune cell counts (ICCs) on CRC risk using Mendelian randomization (MR). Genome-wide association study (GWAS) summary statistics for ICCs were accessed from a comprehensive meta-analysis (N=562,132 Europeans), and for CRC overall and by site (colon, proximal colon, distal colon and rectal) through a large meta-analysis (58,221 cases and 67,694 controls in the Genetics and Epidemiology of Colorectal Cancer Consortium, Colorectal Cancer Transdisciplinary Study, and Colon Cancer Family Registry). We performed univariable (UV) and multivariable (MV) MR analyses to assess the effect of ICCs on CRC risk. The inverse-variance weighted UVMR analysis showed evidence of an effect on CRC risk for basophils (overall CRC - OR: 0.88, CI(95%): 0.78-0.99, P=0.04), eosinophils (overall CRC - OR: 0.93, CI(95%): 0.88-0.98, P=0.01), and overall ICCs (colon - OR: 0.91, CI(95%): 0.85-0.99, P=0.02). These results were corroborated by sensitivity UVMR analyses (MR-PRESSO, Cochran's Q test, MR-Egger). The MVMR method provided evidence of an effect for eosinophils (Overall

CRC - OR: 0.88, CI(95%): 0.80-0.97, P=0.01) and lymphocytes (Overall CRC - OR: 0.84, CI(95%): 0.76-0.93, P=0.0007) on overall CRC risk. Our study provides evidence that circulating immune cells play a role in CRC aetiology, laying the path for targeted mechanistic studies.

P.10 Assessing changes in the ocular micro-environment during inflammatory arthritis to understand co-existing arthritis and uveitis

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An association exists between sight-threatening uveitis and inflammatory arthritis. Approximately 11-30% of children with juvenile idiopathic arthritis and up to 50% of patients with spondyloarthropathies develop uveitis. However these coexisting conditions are often studied separately, here we determined how the ocular microenvironment is impacted in arthritis using experimental arthritis models and tracking ocular immune changes.

Antigen-induced arthritis (AIA) and experimental autoimmune uveitis (EAU) were established in wild type and IL-27R-deficient (Il27ra-/-) mice. Clinical disease was assessed by histopathology, flow cytometry and optical coherence tomography (OCT) imaging.

Following induction of arthritis, ocular OCT analysis suggested sub-clinical changes in leukocyte infiltration. Consistent with this, at time points reflecting the peak of joint swelling and synovial CD4+ T-cell infiltration in AIA, a concurrent increase in ocular-infiltrating CD4+ and CD8+ T-cells, CD11b+ myeloid cells and Ly6G+ neutrophils was observed. As flares of arthritis and uveitis often coincide in patients, we tested whether recurrent flares of AIA led to clinical uveitis. Here, the ocular infiltrate mirrored the pattern of flare-remission seen in the synovium, but multiple flares did not exacerbate the ocular immune response. AIA in Il27ra-/- mice resulted in exacerbated arthritis and an increase in ocular infiltrating leukocytes, particularly CD11b+ and Ly6G+ populations. Parallel investigations of EAU in Il27ra-/- mice revealed an earlier disease onset and an increase in ocular infiltrating leukocytes.

In summary induction of arthritis promotes an ocular leukocyte infiltrate reminiscent of sub-clinical changes preceding uveitis. IL-27 limits ocular leukocyte infiltration during arthritis and uveitis highlighting its regulatory action and therapeutic potential.

P.11 Can the cross-reactivity of therapeutic TCRs be determined in silico?

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During cell-mediated adaptive immunity, human leukocyte antigen (HLA) proteins present short peptides made from degraded intracellular proteins on the cell surface. T-cell receptors (TCRs) can bind to non-self peptide-HLA (pHLA) molecules, leading to T-cell signalling events that induce the death of the antigen presenting cell. TCR affinity and specificity need optimising for therapeutic applications, as natural TCRs have low affinity and high cross-reactivity (i.e. bind to peptides that are not the intended target).

A consequence of engineering high-affinity TCRs is often a reduction in specificity, which can cause off-target toxicity in patients. Previous studies have demonstrated that TCR cross-reactivity can be due to factors such as energetic hotspots, conformational flexibility, and rigid binding mechanisms. Here we show how biomolecular simulations can be used in combination with experimental work to investigate these factors in a series of affinity-enhanced TCRs to understand and predict cross-reactivity. We

determine that the cross-reactive and specific TCRs show differences in energetic interactions with the HLA as well as in CDR loop flexibility. Simulation of the TCR-pHLAs with known off-target peptides indicates that cross-reactivity may be predicted during high-affinity TCR design. Overall, this research offers valuable insights into the applications of biomolecular simulation in therapeutic TCR development and improves understanding of TCR specificity.

P.12 Hemozoin, a by-product of malaria infection, suppresses the neutrophil oxidative burst.

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Malaria is an inflammatory disease with a global incidence of around 220 million cases per year. Plasmodium falciparum, the causative organism of the most severe type of malaria, is a eukaryotic parasite endemic in sub-Saharan Africa. It invades and replicates in erythrocytes, often leading to life-threatening symptoms. Additionally, malaria patients have increased susceptibility to invasive bacterial infections such as Salmonella and Staphylococcus aureus, although the reason is not fully understood. Neutrophils suppress bacterial pathogens through oxidative burst, where NADPH oxidase (NOX2) assembles at phagosomal membranes and produces reactive oxygen species (ROS). Interestingly, neutrophils from malaria patients are often observed to have phagocytosed hemozoin (HZ), an inert crystal by-product from hemoglobin digestion by Plasmodium. We assessed the impact of synthetic HZ (sHz) on the oxidative burst of human neutrophils in response to bacteria. We found that neutrophils treated with sHz had reduced ROS production in response to Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli. This was confirmed by microarray data, which demonstrated that HZ downregulates p47phox, one of the NOX2 components. In conclusion, HZ has a suppressive effect on neutrophil antimicrobial function and might explain the increased rate of bacteraemia seen in malaria patients.

P.13 Analysis of NK-cell function during dengue viral infection in children and young adults

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Background/Aim: Dengue virus (DENV) is a flavivirus causing the most prevalent mosquito-borne viral disease afflicting humans. During uncomplicated dengue, Natural Killer (NK)-cells are robustly activated and proliferating (Zimmer at al 2019, Nature communication). However, in severe dengue NK-cells display decreased expression of markers of activation and cytotoxicity (Vuong et al 2022 J Infect Dis). Here, we evaluate NK-cell expression of activating and inhibitory receptors and their anti-viral function in association with disease severity.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from patients with confirmed dengue and <72 hours of fever at the Hospital of Tropical Diseases, Vietnam. Ex vivo isolated PBMCs were thawed and stained with fluorescently-labelled antibodies and analyzed by flow cytometry to assess NK-cell phenotype. NK-cell function was analyzed after overnight resting or stimulation of PBMCs with IL-12/IL-18 and subsequent co-culture with K562 target-cells. Cytokine/chemokine production (IFN-g, TNF-a and MIP-1β), degranulation (CD107a) were measured by flow-cytometry.

Results/Conclusion: Our preliminary data from the analysis of N=19 samples show decreased expression of activation markers including NKG2D in NK-cells from severe compared to non-severe patients. However, decreased ex vivo NK-cell activation was not accompanied by impaired degranulation potential and decreased production of effector cytokines of NK-cells. Indeed severe patients have an increased percentages of functional IFN-g and CD107a producing NK-cells compared to non-severe dengue. This data suggest that NK-cells may not be intrinsically impaired in severe dengue.

P.14 Impact of cryopreservation on immune cell metabolism as measured by SCENITH

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Immunometabolism is a rapidly expanding field, offering novel insights into the mechanisms through which cellular metabolic processes tightly regulate immune cell function and activity. Clinical samples are commonly cryopreserved to facilitate biobanking and high-throughput batch analysis, but this process is thought to influence cellular bioenergetics (e.g. as measured through Seahorse XFe96 bulk analysis). Recently described by Argüello et al and gaining significant attention in the field, SCENITH is a novel single-cell method for ex vivo functional metabolic profiling of immune cells. Our study aimed to investigate the impact of cryopreservation on immune cell metabolism as measured by SCENITH.

We present a number of central findings. Firstly, T cells undergoing activation with a CD3/CD28 stimulus are less readily metabolically/translationally reprogrammed following cryopreservation. Secondly, we find that the process of cryopreservation introduces a metabolic artefact that favours glycolysis and impairs oxidative phosphorylation, potentially indicating a degree of mitochondrial dysfunction. Despite this artefact, we demonstrate that SCENITH is still clearly able to differentiate between metabolically distinct cell populations following cryopreservation (e.g. by comparing the metabolic profiles of CD69 positive and negative T cells, or classical and non-classical monocytes).

In summary, this poster will present several novel findings that will prove to be of interest to those studying immunometabolism in relation to a broad range of disease categories (including infection, auto-immunity and cancer), highlighting considerations and troubleshooting tips for those wishing to employ SCENITH in their research.

P.15 Autoimmune Disease and Cancer Crosstalk: A Shared Neo-Antigen inhibits the anti-tumour immune response

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The autoimmune disease is associated with an increased susceptibility to cancer. A continuous immune response against self-antigens, coupled with potential mechanisms of immune suppression, could hinder the immune system's ability to detect and combat early-stage tumors. Our objective was to elucidate mechanisms of crosstalk between autoimmune disease and cancer using a mouse model featuring a shared neo-antigen, the influenza haemagglutinin, between pancreatic tissue and a specific tumour cell line. A crucial aspect of persistent immune responses is the upregulation of inhibitory receptors. Further linking autoimmune and anti-cancer immune responses, blocking inhibitory receptors in cancer immunotherapy commonly results in significant adverse autoimmune effects. Therefore, in our investigation of the relationship between autoimmune disease and cancer, we also blocked the inhibitory receptor PD-1. In our mouse model, the presence of the shared antigen enhanced tumour growth and suppressed T-cell infiltration in tumors. Furthermore, it impaired the upregulation of co-

stimulatory receptors on CD4 cells. Within the short 16-day timeframe of tumor growth, we did not observe any discernible effect of anti-PD1 treatment.

P.16 The impact of L-arginine deprivation on CMV and SARS-CoV-2-specific T-cell proliferation

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Background

Critical illness is associated with striking abnormalities of innate and adaptive immunity. The increased incidence of secondary infections due to weakly virulent organisms, e.g. herpesvirus reactivation, strongly suggests T-cell suppression. Existing literature suggests that the amino acid L-arginine is essential for T-cell proliferation, and critical illness is a low arginine state, but these experiments have not examined antigen-specific responses. Here we examine whether altering arginine availability affects in vitro human CMV and SARS-CoV-2-specific memory T-cell responses.

Methods

Cellular proliferation was measured in a dye-dilution proliferation assay using CellTrace Violet (CTV) (Thermofisher). CTV-loaded peripheral blood mononuclear cells (PBMCs) from healthy donors with previous CMV or SARS-CoV-2 infection were cultured in media containing different L-arginine levels (0mM, 0.115mM, 1.15mM). Antigen-specific stimulation (HLA-A*02-restricted NLVPMVATV epitope (pp65 495-503) for CMV; Spike protein peptide pools for SARS-CoV-2) was compared to non-specific stimulation (phytohaemagglutinin (PHA) and anti-CD3/CD28 beads).

Results

CD4 and CD8 T-cells cultured in the absence of arginine are smaller and do not proliferate when activated non-specifically (PHA or anti-CD3/CD28 beads). When T-cells are stimulated via the T-cell receptor using NLV peptide or Spike peptides pools, proliferation was reduced in the absence of arginine, but not abolished.

Discussion

In contrast to non-specific stimulation, T-cells stimulated via the T-cell receptor can proliferate in the absence of arginine, albeit at reduced capacity, suggesting they can withstand low arginine states. Further work is needed to determine whether this is a memory T-cell phenomenon or due to differing mechanisms of T-cell activation, and whether effector function is affected.