APPLIED IMMUNOLOGY

TIME: 14.30 – 15.15

LOCATION: BERWICK ROOM

IMPACT OF ANAESTHESIA AND ANAESTHETIC TECHNIQUES ON IMMUNE FUNCTION AND CELLULAR POPULATIONS IN PATIENTS UNDERGOING SURGICAL RESECTION OF BREAST CANCER

David Ou, Northern Institute for Cancer Research, Newcastle University

Background: Despite advances in surgical treatments for breast cancer, it remains a major cause of morbidity and mortality. It is established that a robust immune system is associated with better outcomes, yet increasing evidence indicate the operative process can be immunosuppressive, especially with anaesthetics.

Aims: In breast cancer patients undergoing surgery: 1) Assess whether a change in the different immune population subsets of peripheral blood mononuclear cells (PBMCs) occur. 2) Identify changes in immune function. 3) Identify if specific anaesthetic drugs contribute to immunosuppression.

Methods: Blood samples were collected from breast cancer patients (n=20) pre-operatively, post-anaesthesia, and post-operatively. PBMCs were isolated and underwent flow cytometry to identify major immune populations and enzyme-linked immunosorbent spot (ELISpot) for IFN-γ to assess function. Single-drug spike-in ELISpots were used to assess drug effect on immune activity.

Results: All ELISpot stimulants showed highly significant decreases in immune response post-operatively. Natural killer (NK) activity decreased post-anaesthesia ($p<0.0001$) and post-operatively ($p<0.0001$). T-cell activity also decreased in response to viral (EBV, CMV) and breast cancer (MUC-1) antigen challenges post-operatively ($p<0.0001$, $p=0.0027$, $p=0.0002$). Functional decreases were also observed in single drug spiking experiments. No significant changes in PBMC populations were found in flow.

Conclusion: Immune activity is suppressed by both anaesthetic drugs and likely even more by the surgery itself. Specific anaesthetic drugs indicated severe immunosuppression ex vivo and support further investigations. Flow cytometry results suggest PBMC populations are not affected by drugs or surgery, observed decreases in IFN-γ producing cells are specific to drug and stress-induced functional suppression rather than cell loss.

THE ROLE OF SPHINOGSINE-1-PHOSPHATE IN VASCULAR PERMEABILIT

Georgie Wilkins, Institute of Cellular Medicine, Newcastle University

Kidney transplantation is the preferred treatment for end-stage kidney disease, however, in the UK there is a shortfall of kidneys available to transplant. To overcome this shortfall, "marginal" organs are now accepted from the elderly, donors with comorbidity or those that have had prolonged ischaemic times. However, these are particularly susceptible to ischaemia reperfusion injury (IRI), which can lead to loss of graft function, compounding the organ shortfall.

Ex vivo normothermic kidney perfusion (EVNKP) is a novel method that can be used to improve the quality of these "marginal" organs. The ex vivo nature of perfusion allows for the directed delivery of therapeutics. We have hypothesised that delivery of a barrier enhancing drug, a sphingosine-1-phosphate receptor (S1PR) agonist, could reduce the consequences of IRI by reducing vascular leak and leukocyte infiltration.

Through in vitro studies we have shown that S1PR expression is affected by mimics of ischaemia and reperfusion. Furthermore, we have shown that

treatment of endothelial cells with an S1PR1 agonist caused reductions in endothelial permeability, trans-endothelial neutrophil chemotaxis and cell-cell contact phosphorylation. Therefore, as we have found that current perfusion methods contain insufficient S1PR ligands to cause a barrier enhancing effect, supplementation of ex vivo perfusion circuits with an S1PR agonist may prove useful in reducing the consequences of IRI in kidney transplantation.

INVESTIGATING THE IMMUNOBIOLOGY OF CERAMIC BIOMATERIALS: AN EXAMINATION OF MACROPHAGE PHENOTYPIC CHANGES AND THE ROLE OF TLR4

Shannon Jamieson, Institute of Cellular Medicine, Newcastle University

Background: The use of ceramic implants for total joint replacement (TJR) is increasing year-on-year. This is because they are thought to be hard-wearing and bio-inert. The most commonly used ceramic implants are generated from an Alumina (Al_2O_3) and Zirconium (ZrO_2) composite.
Aim: To investigate immune responses to ceramics in a human macrophage model (THP-1 cell line).
Methods: THP-1 macrophages in culture were treated with different doses of Al_2O_3 or ZrO_2 for 24 hours. A TLR4-specific (CLI-095) small molecule inhibitor was used to assess whether TLR4 signalling was affected by ceramic treatment. ATP for 1 hour following ceramic treatment was also tested to assess inflammasome activation. Lipopolysaccharide (LPS) treatment was used as a positive control and untreated cells as negative control throughout. Enzyme-linked immunosorbent assay (ELISA) was used to assess protein secretion. Quantitative polymerase chain reaction (qPCR) was used to compare relative gene expression of inflammatory genes to untreated controls.
Results: THP-1 macrophages treated with Al_2O_3 or ZrO_2 experienced significant increases in inflammatory gene expression ($p<0.0001$) and protein secretion ($p<0.001$). Moreover, CLI-095 pre-treated cells experienced significant decrease in IL-8 gene expression ($p<0.0001$) and protein secretion ($p<0.0001$) compared to those without inhibitor treatment. IL-1β secretion and expression was significantly increased following ceramic and ATP treatment ($p<0.0001$).
Discussion: These findings show that ceramic oxides are capable of eliciting inflammatory responses in human macrophages and that TLR4 activation is key in ceramic-mediated inflammation.
Conclusion: Use of TLR4 antagonists may decrease adverse reactions to ceramics in patients.

THE DEVELOPMENT AND ACCEPTABILITY OF A NEW RNA-BASED POINT-OF-CARE TEST TO DIFFERENTIATE BACTERIAL AND VIRAL INFECTION IN HIGH RISK CHILDREN

Emily Rowlands, Institute of Cellular Medicine, Newcastle University

Background: Current clinical investigations cannot differentiate between bacterial and viral infections in >50%, resulting in unnecessary antibiotic treatment and missed serious bacterial illness. Improved diagnostic tests are required, particularly for children at high risk of infection (HR).
Aims: To investigate the acceptability to patients and feasibility of RNA Sequencing (RNASeq) in developing a new ribonucleic acid (RNA)-based point-of-care test (POCT) to determine fever aetiology in HR children.
Design: Prospective cohort and qualitative methodology.
Subjects: HR children with fever >38.0°C, presenting to the Emergency Department or other appropriate ward of 12 European hospitals from 12/12/16–02/05/19; Patients and families attending a Great North Children's Hospital (GNCH) Bone Marrow Transplant Unit event.
Outcomes: White cell counts (WCCs) producing the minimum RNA yield required for RNASeq (1µg); WCCs in subsets of HR children; Views of HR patients and families on a new POCT.
Results: 403 febrile episodes were included. 59.0% had a diagnosis of malignancy. 33.0% of infections remained undiagnosed after clinical investigations.

RNAseq would be feasible for 1.0ml samples with WCCs >0.9x10^9/L. The median WCC was 4.0x10^9/L (IQR 0.9-9.6), however many children with malignancy had WCCs <0.9x10^9/L. Patients value at-home treatment and antibiotic minimisation. They demonstrated trust in a POCT, particularly when offered in hospital. Safety and reassurance were important themes.

Conclusions: RNASeq would be feasible for 1.0ml samples from most HR children, but not in many with malignancy. Families would trust and accept a new POCT, with appropriate safety and reassurance.