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P232 -The development of multi-parameter flow cytometry panels to identify immune cells in renal transplant recipients and healthy controls

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Introduction:
Cardiovascular disease is a major cause of morbidity and mortality for renal transplant recipients (Collins, Foley, Gilbertson, & Chen, 2015), with infection and malignancy also limiting graft and patient survival (Bamoulid et al., 2016). Immunosuppressive medications alter innate and adaptive immunity and can result in immune dysfunction. Over-suppression of the immune system can result in open windows of opportunity for patients to contract an infection (Walsh et al., 2011), and under-suppression can result in graft rejection, limiting survival rate. Therefore, it is vital to monitor immune status in this population in clinical settings and in research. Our aim was to design multi-colour flow cytometry panels to assess lymphoid and myeloid cell populations from human peripheral blood mononuclear cells from renal transplant recipients. These panels cover cell types such as T cells, B cells, natural killer (NK cells), dendritic cells (DCs) and monocytes as well as phenotypic and chemokine migratory receptors of these populations.
Methods: A 10-colour lymphocyte panel and two 6-colour myeloid panels (monocytes and dendritic cells) have been designed to assess changes in the frequency and phenotype of cells within the peripheral blood of renal transplant recipients undergoing defined exercise programmes. Peripheral blood mononuclear cells were isolated from whole blood of renal transplant recipients (n=5, age=50±6 years, eGFR=46±18 mL·min·1.73m2, BMI=27±1) and healthy controls (n=3, age=38±17, eGFR=>90 mL·min·1.73m2, BMI=29±1). These cells were stained with lymphoid lineage markers: CD3, CD4, CD8a, CD19, CD56, and function associated markers: CD45RA, CD45RO, CD127, CD25, and CD197. Monocyte subsets were identified using HLA-DR, CD14, CD16, and their migratory potential determined using CCR2, CCR5 and CX3CR1. Dendritic cell populations were identified using HLA-DR, CD1c, CD11c, CD141, CD123 and CD14.
Results/ Discussion: The panels allow reproducible detection of cell populations in peripheral blood cells from renal transplant recipients and healthy volunteers. These panels are now being used to assess immune cell populations in renal transplant recipients undergoing three distinct exercise protocols. Determining how cell populations change in response to exercise will prove information regarding the impact of exercise on immune status in this population.