Abstract Title- *In vitro* Human enthesitis model with induced IL17A and TNFα from CD4+ and CD8+ lymphocytes and effect of pharmacological antagonism with Janus Kinase and retinoic acid receptor-related orphan receptor γ inhibition

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Background: Animal models of experimental spondyloarthritis (SpA), human genetics and therapies support a central role for adaptive immunity in disease pathogenesis. However, there is very limited data on whether the human enthesis harbours conventional CD4+ and CD8+ T-cells.

Objectives: To investigate whether spinal enthesis including peri-entheseal bone and peripheral blood harboured resident CD4+ and CD8+ conventional T-cells and to evaluate the effects of therapy in blocking the production of pivotal SpA-related cytokines TNF and IL-17A. Additionally, to investigate T cell plasticity via gene expression of Th17 and Treg markers following in vitro induction of entheseal inflammation.

Methods: Healthy interspinous ligament and spinous process with matched blood were harvested from patients undergoing elective surgery for the correction of mechanical spinal defects (n=13). Entheseal soft tissue (EST) and peri-entheseal bone (PEB) were separated and digested. Following the isolation of CD4+ and CD8+ T-cells lymphocytes from PEB and peripheral blood, the conventional T lymphocytes were investigated using ELISA's and RNA extraction for qRT-PCR to assess T effector cell markers. Cells were stimulated using an anti-CD3/CD2/CD28 beads with and without the presence experimental RORγt inhibitor (RORγti), Tofacitinib, methotrexate (MTX), and phosphodiesterase type 4 inhibitor (PDE4i).

Results: Following stimulation, CD4+ T-cells produced more TNF and IL-17A than CD8+ T-cells ($p<0.05$), IL-17A was robustly detected in CD4+ but not CD8+ T-cells. TNF and IL-17A production from CD4+ T-cells was effectively inhibited by Tofacitinib ($p<0.05$), while RORγti only reduced IL-17 secretion highlighting it's specificity in the IL-17A signalling pathway. MTX and PDE4i treated cells had no significant impact on reducing IL-17A production in either cell population. MTX also had no impact on reducing TNF production in either cell population, however PDE4i treated cells did reduce TNF production in both cell populations in blood.

CD4+ and CD8+ T-cells showed increased expression of the Treg lineage specific gene FOXP3 compared to DMSO control ($p=0.002$) where PDE4i and methotrexate treatment caused the highest relative expression fold change in CD4+ PEB and CD8+ blood respectively (4.33±0.97, 347.89±347.35). The pleiotropic cytokine TGFβ showed varying results in PEB and blood, with a complete downregulation in CD4+ PEB and an upregulation in CD8+ Blood, methotrexate treatment caused the highest relative expression fold change (19.66±17.78), which may suggest a Th17 phenotypic shift in PEB and an immunomodulatory shift in CD8 Blood, this is also supported by RORC and IL-6 upregulation in CD4 PEB in PDE4i treated cells ($p=0.045$ and 0.001 respectively).

Conclusions: This is a novel finding of conventional CD4+ and CD8+ enthesis resident T-cells that exhibit regulatory transcript expression in health. Where TGFβ expression may highlight T cell plasticity, promoting a Th17 phenotype in PEB and an immunomodulatory phenotype in blood. Induced IL-17A was robustly inhibited by RORγt inhibition.