# IL-17A Induces Distinct Functional Differences Between Two Novel Mesenchymal Stem Cell Populations Identified At The Human Enthesis

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## Background

Ankylosing spondylitis (AS) is associated with entheseal inflammation and new bone formation. Resident immune cell populations including IL-23 produce myeloid and lymphocyte producing IL-17A and IL-22 cells are known to be present at the enthesis (1, 2). The IL-23/17 axis is known to influence tissue repair responses including osteogenesis in addition to driving inflammation. Surprisingly, enthesis resident mesenchymal stem cells (MSCs) have not been phenotypically or functionally characterised in relationship for this milieu.

## Objectives

To determine the presence of MSCs in the human entheseal tissue and to investigate the effect of spondyloarthritis associated pro-inflammatory cytokines on MSCs osteogenesis and adipogenesis.

## Methods

Samples from healthy spinous process and interspinous ligament (10 male, 10 female, median age = 49) were divided into entheseal soft tissue (EST) and peri-entheseal bone (PEB) and enzymatically digested (1). MSCs content was assessed using a CFU-F assay. Flow cytometry was used to examine expression of MSCs specific markers (3) in passage 1 plastic adherent cultures. Following osteogenic, chondrogenic and adipogenic inductions, osteogenesis was assessed by alkaline phosphatase and alizarin red staining and by measurement of calcium accumulation. Chondrogenesis and adipogenesis were assessed using glycosaminoglycan assay and Oil Red O staining respectively. Complete osteogenic and adipogenic media were supplemented with IL-17A (50ng/ml), IL-22 (10ng/ml) or TNF-α (1ng/ml) to determine the effect of these cytokines on adipogenesis and osteogenesis.

## Results

The CFU-F number in the EST digests (median 0.67±3.05%) was 6.7-fold higher compared to donor-matched PEB (median 0.1±1.53%) (n=16 pairs, $p<0.001$). Cultured cells were positive for expression of MSC markers CD73, CD90, CD105 (median 98.49±0.74%) and negative for CD14, CD19, CD34, CD45 and HLA-DR (median 0.58±1.76%). Although both populations had potential of tri-lineage differentiation, PEB MSCs produced more calcium (average 175% higher, n=11 pairs, $p<0.05$) and less lipid (average 75% lower, $p<0.05$). Calcium accumulation was significantly reduced in PEB MSCs exposed to any cytokine ($p<0.05$). In contrast addition of IL-17A to EST MSCs significantly increased calcium accumulation (by 50%, n=10 pairs, $p<0.05$) and reduced lipid accumulation (by 50%, n=5 pairs, $p<0.01$). The addition of TNF-α also reduced lipid accumulation (by 18%, n= 5 pairs, $p<0.010$).

## Conclusions

Both the EST and PEB contain cells that meet the ISCT criteria for MSCs. However, MSCs from these sources are functionally distinct in terms of their differentiation potential and response to inflammatory cytokines. IL-17A is capable of enhancing osteogenesis and impairing lipid accumulation in entheseal soft tissue MSCs. These findings are potentially important in explaining the altered bone and fat phenotype observed in AS as the aberrant new bone formation arises in this tissue (4).

## References

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