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# **Innate Lymphoid Cells are Present at Normal Human Entesis Providing a Potential Mechanism for Spondyloarthritis Pathogenesis**

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## **Background and objectives**

The pathogenesis of murine spondyloarthritis (SpA) has been intimately linked to the presence of IL-23 responsive, innate like lymphocytes at peripheral and spinal entesis. Human SpAs are associated with SNPs in genes related to the IL-23 pathway and drugs that block IL-12/23 have shown efficacy. We hypothesised that the normal human entesis has a population of resident innate lymphoid cells (ILCs) that could be key in governing enthesal immune homeostasis partly via interaction with resident mesenchymal stromal cells (MSCs).

## **Materials and methods**

Normal spinal entesis were harvested from patients undergoing spinal decompression surgery and enzymatically digested prior to sorting or flow cytometry. Immunophenotyping and cell sorting was performed on entesis samples harvested from 6 patients and unmatched peripheral blood. The expression of ROR $\gamma$ t and key immunomodulatory transcripts was tested in sorted populations by RTqPCR. Anterior cruciate ligament and Achilles entesis were obtained from patients with knee OA and Achilles tendon rupture and analysed by immunohistochemistry (IHC). Adherent cells from enthesal digest were cultured under standard MSC culture conditions and expression of known MSC markers was assessed by flow cytometry.

## **Results**

All sorted samples contained ILC3s, median proportion 0.09% (range 0.015-0.63). Transcript analysis confirmed the expression of ROR $\gamma$ t transcript in sorted ILC3 populations. ILC3s expressed 51-fold greater relative expression of ROR $\gamma$ t in comparison to unsorted mononuclear cells. 5 of 6 sorted samples contained ILC2s, median proportion 0.20% (range 0-0.49). ROR $\gamma$ t expression was detected in knee OA and there was widespread expression of ROR $\gamma$ t in inflammatory infiltrates in injured entesis as shown by IHC. Culture expanded adherent cells grew in characteristic fibroblastoid colonies and expressed phenotypic markers consistent with bone marrow derived MSCs.

## **Conclusions**

Our findings show that both ILCs and MSCs are present in the normal human spinal enthesis. ILCs may also be greatly increased in frequency following injury. The co-localisation of ILC and MSC populations at the enthesis suggests a potential link between cellular dysregulation of the IL-23/17 axis and human SpA pathology.