The role of oxytocin-primed human mesenchymal stem/stromal cells in reversing macrophage pro-inflammatory phenotype

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Disclosures: None

INTRODUCTION: The histological profile of the synovium in OA preclinical models[1] and often in OA patients[2] is mainly characterized by synovial lining hyperplasia and IFP fibrosis. The activation of pro-inflammatory signals from macrophages residing within synovium/IFP contribute to these histological changes and is closely linked to the progression of OA. Our recent work showed that human IFP-derived mesenchymal stem cells (IFP-MSCs) shift IFP macrophages from a pro-inflammatory M1 to the anti-inflammatory M2 phenotype in an acute preclinical model of inflammation[3]. Previous literature suggests that oxytocin (OT) has a stimulatory effect on chondrogenesis[4] and strongly influences MSC therapeutic potential[5]. On this basis, our hypothesis is that OT enhances IFP-MSC anabolic and immunomodulatory attributes, leading in an M2 macrophage polarization.
METHODS: Human IFP-MSC cultures were generated under regulatory-complaint conditions using human platelet lysate medium (PL Bioscience). IFP-MSC were primed using TIC inflammatory/fibrotic cocktail (15 ng/ml TNFα, 10 ng/ml IFNγ, 10 ng/ml CTGF) with and without OT for 72 h. Non-induced and OT-induced TIC cohorts were evaluated for their transcriptional profiles using multiplex methods (Qiagen). Human monocytes (THP-1, ATCC) were differentiated into macrophages using PMA/IO (Phorbol 12-myristate 13-acetate/Ionomycin) and polarized to M1 macrophages by M1-macrophage generation medium (PromoCell). PMA/IO-stimulated THP-1/IFP-MSC co-cultures were performed for 2 days and macrophages status was assessed using macrophage polarization qPCR multiplex array (ScienCell).
RESULTS: OT exposure of TIC-primed IFP-MSC resulted in increased expression for 23 genes related to immunomodulatory and anabolic actions. Notably, *PROM1* (stemness marker), *BMP7* (anti-inflammatory/differentiation marker), and *IL10* (anti-inflammatory marker) genes showed >2-fold expression increase in OT-induced compared to non-induced TIC cohorts. Upon IFP-MSC exposure, PMA/IO-stimulated macrophages molecular profiling indicated a strong gene expression shift towards an M2 macrophage polarization. Specifically, two characteristic M2-polarization macrophages markers, *IL12A* and *CD200R1*, showed increased expression levels when macrophages were exposed to IFP-MSC.
DISCUSSION: Our study identified distinct immunomodulatory and anabolic gene profiles for the OT-induced IFP-MSC under inflammatory conditions. Importantly, IFP-MSC could effectively polarize M1 pro-inflammatory macrophages to the anti-inflammatory M2 phenotype. Therefore, our preliminary data suggest that OT can effectively modulate IFP-MSC immunomodulatory properties.
SIGNIFICANCE/CLINICAL RELEVANCE: These findings lay the groundwork for enhancing MSC immunomodulatory potency in addressing the immune-mediated inflammatory joint conditions such as OA.
ACKNOWLEDGEMENTS: Study supported by NIH/NIAMS grant number 5R21AR080388-02

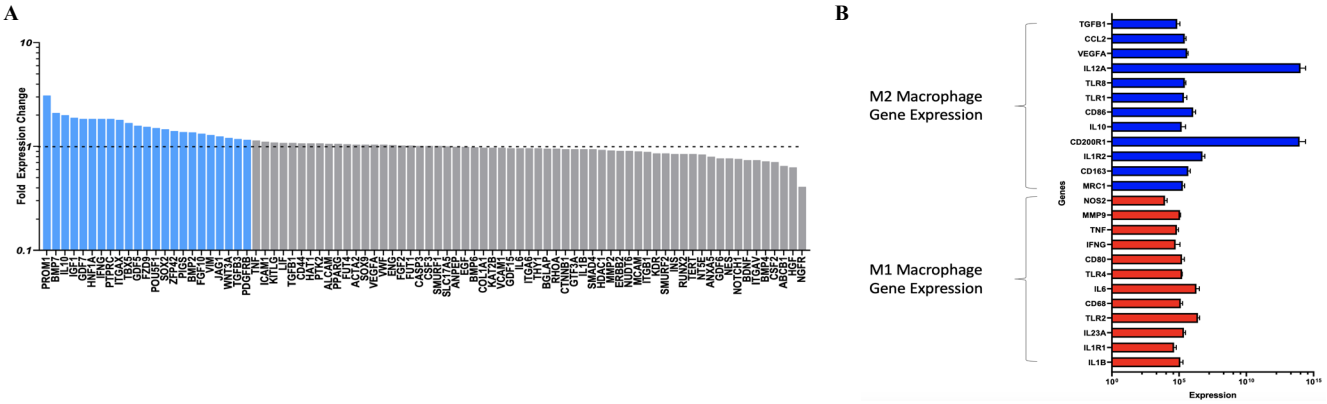

Figure 1: (A) Oxytocin exposure of TIC-primed IFP-MSC resulted in increased expression for 23 out of 85 genes related to immunomodulatory and anabolic actions (PROM1, BMP7, IL10, IGF1, GDF7, HNF1A, IFNG, PTPRC, ITGAX, TBX5, GDF5, FZD9, POU5F1, SOX2, ZFP42, PIGS, BMP2, FGF10, VIM, JAG1, WNT3A, TGFB3, PDGFRB). (B) Upon IFP-MSC exposure, PMA/IO-stimulated macrophages molecular profiling indicated a strong gene expression shift towards an M2 macrophage polarization.

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