Imaging mass cytometry reveals distinct cellular phenotypes in CD14-deficient mouse synovium.

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INTRODUCTION: Growing evidence has revealed that inflammation is a major driver of osteoarthritis (OA). However, previous consideration of OA as a noninflammatory disease placed early focus on mechanical and structural characterization. As a consequence, there is a knowledge gap with respect to the full description of the inflammatory state across tissues within the knee joint (synovium, meniscus, cruciate ligaments, etc.) during OA progression. Of these tissues, the synovium has been identified as a reservoir of not only inflammatory mediators but also innate (monocyte/macrophages) and adaptive (T- and B-cells) immune cells. Both the diverse cell populations and unique structure of the synovium, including the lining and sublining layers, undergo unique inflammatory-mediated degenerative changes. CD14, a co-receptor to inflammatory toll-like receptor (TLR) signaling and subsequent macrophage activation, has also been identified as being upregulated in OA synovium and, in our prior work, we showed that global genetic CD14 deficiency in mice is protective against PTOA related bone-remodeling and mobility dysfunction.1 Imaging mass cytometry (IMC) is an emerging technology that allows for the spatial localization of molecular species across tissue samples, facilitating investigation of cellular subtypes throughout diverse tissue structures, such as the synovium, as they change with disease. Utilizing this technology, we hypothesized that CD14 deficiency would modulate the innate immune cell profiles within the synovium during OA progression.

METHODS: CD14 knockout (CD14-KO) mice: Global CD14 deficient mice of C57BL/6 background were obtained from Jackson Laboratories (003726).4 OA model (n=5): Distalization of the medial meniscus (DDM) surgery was performed to induce OA in skeletally mature (10-12 wk old) CD14-KO or C57BL/6 (WT) mice (n=5). Tissues were pooled for each biological replicate, collected at 0 (preop), 4, 8, or 16-wks post-surgery, and cells were isolated enzymatically. Cell suspensions were split in half and stained with antibodies for monocyte (CD45, Ly6C), and macrophage (CD45, CD64) cell markers or T cell (CD45, CD3) and T-helper cell (CD45, CD3, CD4) markers. Multicolor flow cytometry was performed (BD LSR II), and data was analyzed with FlowJo software (Version 10). Multicolor/macrophage populations were expressed as percent of the CD45+ population, T cell populations were expressed as percent of the CD45+ or CD3+ populations. IMC (n=5, 4wks-post DMM): Whole knee joints were fixed, decalcified, paraffin embedded, and sectioned. Sagittal sections underwent heat-mediated antigen retrieval, and overnight incubation with a 22-marker multiplex panel of metal-conjugated antibodies, followed by incubation with Intercalator-Ir nuclear stain, and imaging using a Hyperion Imaging System (Bruker). Cellular phenotype analysis using imaging mass cytometry (t-SNE) revealed 12 unique cell populations across combined experimental synovial regions, with clustering by differential expression of vascular density (CD31), nerve (PGP9.5), monocyte/macrophage (Ly6C, F4/80, CD68, MHC-II, CX3CR1), T-cell (CD3), fibroblast, and other immune cell markers (Fig. 3A,B). The identified clusters could be localized throughout synovial lining and sublining layers (Fig. 3C, Fig. 4), and evaluation of cells within unique phenotype clusters revealed significant decreases in Cluster 2 (p=0.021) and Cluster 8 (p=0.033), and an increase in cluster 5 (p=0.026) in CD14-KO synovium compared to WT at 4wks post DMM (Fig. 3D).

DISCUSSION: Flow cytometry analysis revealed the significant within the synovium following injury, providing mechanistic support for how CD14 deficiency may be protective against PTOA-associated pathology and mobility dysfunction. SIGNIFICANCE: These results reveal that CD14 deficiency produces distinct immune cell clusters with distinguishable spatial organization within the synovium following injury, providing mechanistic support for how CD14 deficiency may be protective against PTOA-associated pathology and mobility dysfunction.


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