Comparative analysis of macrophage activation in the synovium of healthy and osteoarthritic equine joints

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Abstract

Background: Synovitis is a major component of osteoarthritis and is driven primarily by macrophages. Synovial macrophages are crucial keepers of joint health by driving joint homeostasis, tissue repair and inflammation resolution (M2-like phenotype), but can also induce an inflammatory response (M1-like phenotype) when these regulatory functions are overwhelmed. Macrophage phenotypes in synovium from osteoarthritic and healthy joints are poorly characterized. Defining the patterns of synovial macrophage phenotypes in normal and osteoarthritic joints is paramount for developing targeted therapeutic approaches. The objective of this study was to compare patterns of macrophage activation phenotypes in healthy and osteoarthritic equine joints. We hypothesized that synovium from osteoarthritic joints would have increased M1-like:M2-like ratios compared to normal joints.

Methods: Gross evaluation of the articular surfaces, histology (H&E) and immunohistochemistry for M1-like (CD86), M2-like (CD206, IL-10), and pan macrophage (CD14) markers was performed on synovial biopsies from healthy (n=29) and osteoarthritic equine joints (n=26). Cytokines (MCP-1, IL-10, PGE2, IL-1β, IL-6, TNF-α, IL-1ra) and growth factors (GM-CSF, SDF-1α+β, IGF-1, and FGF-2) in synovial fluid were quantified.

Results: All macrophage markers were co-expressed in all joints with minimal differences between OA and normal joints. Intensity of expression varied with degree of synovial inflammation. CD14, CD86, CD206, and IL-10 were more highly expressed in grossly inflamed osteoarthritic synovium, with CD86 most highly expressed. Synovial fluid MCP-1 was higher in osteoarthritic joints while SDF-1 was lower. Overall, concentrations of synovial fluid IL-10 and PGE2 was not different between OA and normal joints. Increased CD14/CD86/CD206/Il-10 expression in the synovium was associated with synovial hyperplasia, consistent with macrophage recruitment and activation in response to higher demands for repair.

Conclusions: Macrophages are not as clearly defined in vivo as they are in vitro. The course of an effective response to injury in joints should start with inflammation and be followed inflammation resolution, both of which centrally driven by macrophages. Combined, our findings suggest that
homeostatic mechanisms from synovial macrophages are impaired in OA, resulting in unresolved, chronic joint inflammation. Therapeutic approaches aimed at recovering mechanisms of macrophage-driven synovial homeostasis may be more effective in treating osteoarthritis than simply inhibiting inflammation.

**Full-text**
Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

**Tables**
Due to technical limitations, tables are only available as a download in the supplemental files section.

**Figures**

![Figure 1](image_url)

**Figure 1**
Representative images demonstrating (arrowheads) the differences between normal and osteoarthritic (OA) joints for Intimal Hyperplasia and Subintimal Edema.
Figure 1

Representative images demonstrating (arrowheads) the differences between normal and osteoarthritic (OA) joints for Intimal Hyperplasia and Subintimal Edema.
Compared to osteoarthritic joints with no or minimal signs of inflammation (A), OA joints exhibiting gross signs of synovitis (D; black arrow), exhibited increased histological changes such as severe cell infiltration and hyperplasia of the synovial intima with shedding of its outermost layer (B; black arrowhead), markedly increased vascularization (C; white arrowheads), or a combination of both (E). Marked synovial and sub-synovial edema were also frequent findings (B & F; white arrows).
Figure 2

Compared to osteoarthritic joints with no or minimal signs of inflammation (A), OA joints exhibiting gross signs of synovitis (D; black arrow), exhibited increased histological changes such as severe cell infiltration and hyperplasia of the synovial intima with shedding of its outermost layer (B; black arrowhead), markedly increased vascularization (C; white arrowheads), or a combination of both (E). Marked synovial and sub-synovial edema were also frequent findings (B & F; white arrows).
Figure 3

Representative immunohistochemistry sections from normal and OA equine synovial membrane at low (top 2 rows; scale bar=100 µm) and high magnification (bottom 2 rows; scale bar = 50 µm) from the same histological section and demonstrating the median staining scores for macrophage markers (CD14, CD206 [M2], CD86 [M1], and IL-10 [M2]).
Figure 3

Representative immunohistochemistry sections from normal and OA equine synovial membrane at low (top 2 rows; scale bar=100 µm) and high magnification (bottom 2 rows; scale bar = 50 µm) from the same histological section and demonstrating the median staining scores for macrophage markers (CD14, CD206 [M2], CD86 [M1], and IL-10 [M2]).
Figure 4

Sets of representative immunohistochemistry sections from normal and grossly inflamed OA equine synovial membrane from the same horse (2 different horses; scale bar = 150 µm) demonstrating increased staining intensity and distribution for all selected markers in OA joints, denoting more consistently marked increases for CD86 staining.
Sets of representative immunohistochemistry sections from normal and grossly inflamed OA equine synovial membrane from the same horse (2 different horses; scale bar = 150 µm) demonstrating increased staining intensity and distribution for all selected markers in OA joints, denoting more consistently marked increases for CD86 staining.

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