The Role of Mast Cells in Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is characterized by progressive joint degeneration and chronic inflammation. The mast cell (MC) is one of the major immune cells found in OA synovium and is often present near blood vessels and nerve endings in the synovium. MCs are known to orchestrate inflammation by releasing numerous stored granules with enzymes and inflammatory mediators, such as tryptase, chymase, TNF, leukotrienes, as well as synthesizing inflammatory cytokines as such IL1 and IL6 (Fig. 1A). Recently, mice deficient in MCs were found to have lowered DMM-induced OA, suggesting a requirement of MCs for OA progression [1]. However, how MCs affect OA is still unclear. In this study, we demonstrated that MC plays a key role in OA progression by promoting catabolic gene expression and contributing to OA pain.

METHODS: OA patient synovium was obtained from Tufts Medical Center after knee replacement surgery under IRB exempt status. All animal care and experimental procedures are approved by IACUC at Tufts University and Mass General Hospital. 10-week male C57BL/6 mice (The Jackson Laboratory) were subjected to ACLT or sham surgery on the right knees, with the left knees intact. Mice were euthanized at 12 days or 42 days after surgery for the assessment of MC number and OA damage. OA damage was assessed using the modified MANKIN system [2]. MC degranulating stimulator C48/80 (Sigma-Aldrich) or PBS control were intra-articular (IA) injected at day 28 after ACLT. 7 days after injection, nociceptive pain was assessed by von Frey test at 7 days post-injection and results were shown as the ratio of the right knee over the left knee to normalize the individual differences of mice. We used the following antibodies and compounds for flow cytometry (FACS) and immunostaining: CD117 (APC conjugate, Invitrogen) and FcεRI (FITC conjugate, Invitrogen), IL-6, tryptase, and chymase (Abcam), PGP9.5 (Proteintech) and avidin (Alexa Fluor 488 conjugate, Invitrogen). Human LAD2 MCs were activated by IgE (1μg/mL, Sigma-Aldrich) on day 1 followed by anti-IgE (1μg/mL, Invitrogen) on day 2, following standard protocols. MC-conditioned medium was collected on day 3 of culturing and used for treating human primary chondrocytes (NDRI) and synoviocytes (Cell Applications) at a 1:1 mixture with chondrocytes or synoviocytes culturing medium. Statistical analysis was done using GraphPad Prism 9, $p < 0.05$ will be considered significant.

RESULTS: 1. MCs are present in human and mouse OA synovium and have distinct markers. Toluidine blue was used to identify MCs. MCs are widely spread in human OA synovium (Fig. 1A) and can be isolated by the surface markers CD117 and FcεRI via FACS (Fig. 1B). In ACLT OA mouse, MCs were found in the synovium expressing MC-specific protease such as tryptase and chymase, as well as inflammatory cytokines such as IL-6. However, while all cells express tryptase, only some cells express chymase or IL6, suggesting heterogeneity of MCs (Fig. 1C. Red arrow: tryptase+/chymase+/IL-6+ MCs, blue arrow: tryptase+/chymase+/IL-6- MCs, purple arrow: tryptase+/chymase-/IL-6+ MCs). **2. MCs numbers correlate with OA severity and MC conditioned medium induces MMP expression.** We found that MC numbers at 12 days or 42 days after ACLT surgery and the level of cartilage destruction were positively correlated (Fig. 2A). To test whether MCs have effects on joint cells, human LAD2 MC conditioned medium was used to treat human primary articular chondrocytes and synoviocytes. The level of MMP expression was increased, suggesting MCs are capable to promoting catabolic activities (Fig. 2B). Our findings suggest that the MCs are poised to contribute to catabolic gene expression and chronic OA inflammation. **3. MCs are located near nerve endings and MC activation increases nociceptive pain in OA.** Because MCs were found near nerve endings in OA synovium (Fig. 3A), we intraarticularly injected C48/80, a compound well established to induce MC degranulation, into OA joints at 28 days after ACLT. Von Frey assay showed a significantly increased nociceptive pain, which can be induced by joint injury and trauma, in C48/80 injected joints 1 week after C48/80 treatment (Fig. 3B). In addition, C48/80 itself did not lead to increased MMP production in chondrocytes or synoviocytes or nociceptive pain (data not shown). These data suggest the increased nociceptive pain in C48/80 injected ACLT joints was directly caused by the activation of MCs, not indirectly resulting from the responses of chondrocytes and synoviocytes.

DISCUSSION: Our results showed that MCs contribute to OA development by inducing catabolic gene expression and increasing nociceptive pain. However, what inflammatory mediators from MCs are responsible for these activities and what regulates MC activities remain to be investigated. The crosstalk between MCs and nerve endings may exist as a two-way process. It is known that MCs are readily activated by substance P, which is well known to be released by nerve endings [3]. Thus, it is possible that pain may also exacerbate MC activation and cartilage damage.

SIGNIFICANCE: This study provides much-needed insights into MC in OA disease progression and pain. The understanding of MC suggests the potential target to prevent OA disease progression or relieve OA symptoms to improve the quality of life for OA patients.

REFERENCES: [1] Wang Q. eLife. 2019. [2] Hui Mingalone CK. Osteoarthr. Cartil. 2022. [3] Tsilioni. J Pharmacol Exp Ther. 2016

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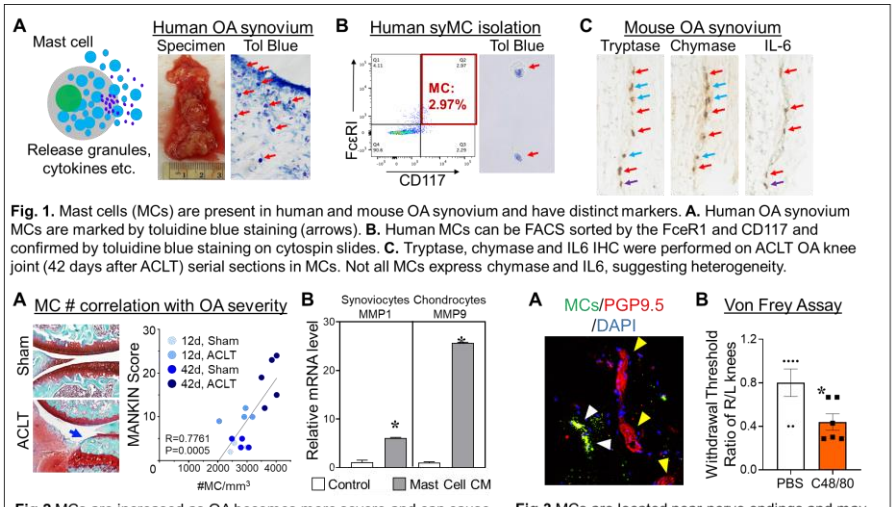

Fig. 1. Mast cells (MCs) are present in human and mouse OA synovium and have distinct markers. **A.** Human OA synovium MCs are marked by toluidine blue staining (arrows). **B.** Human MCs can be FACS sorted by the FceR1 and CD117 and confirmed by toluidine blue staining on cytospin slides. **C.** Tryptase, chymase and IL6 IHC were performed on ACLT OA knee joint (42 days after ACLT) serial sections in MCs. Not all MCs express chymase and IL6, suggesting heterogeneity.

Fig.2 MCs are increased as OA becomes more severe and can cause catabolic gene expression. **A**. MC numbers and OA severity are positively correlated in mouse ACLT joints. **B**. Activated human LAD2 MC conditioned medium can lead to increased MMP expression in synoviocytes and chondrocytes after 2 days of culturing. *:p<0.05.

Fig.3 MCs are located near nerve endings and may contribute to OA pain. **A**.Immunofluorescence staining showing MCs (avidin, green) and never endings (PGP9.5, red) in the synovium. DAPI: blue. **B**. C48/80 injection into the ACLT mice results in increased nociceptive pain in von Frey assay. *:p<0.05.