Mast Cells Influence Sex-Specific Responses to Joint Injury in a Murine Post-Traumatic Osteoarthritis Model

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INTRODUCTION: Biological sex is one of many factors influencing osteoarthritis (OA) development. Clinically, knee OA is more prevalent in women over the age of 55, with women reporting greater pain severity compared to men.[1,2] In mouse models of post-traumatic osteoarthritis (PTOA), however, research has largely focused on males, as they develop more severe degeneration, limiting our understanding of sex differences. Sex differences in MC-associated diseases – including allergy, anaphylaxis, irritable bowel syndrome and rheumatoid arthritis – are well established, with females often experiencing increased prevalence and severity.[3-6] Our lab has previously identified a critical role for mast cells (MCs) in PTOA pathogenesis. Specifically, MC-deficient (KO, Kit$^{w\text{-}sh}$) mice develop less severe PTOA compared to wild-type (WT, C57BL/6) controls. In line with prior reports, we also observed that WT male mice exhibit greater joint damage than females following injury.[7] However, this sex difference is absent in MC-deficient mice, suggesting that MCs may be involved in the sex-bias seen in PTOA. To investigate the role of MCs in sex-differences, we combined histological assessment with bulk RNA sequencing of synovium in male and female mice to evaluate the contribution of MCs to sex-specific disease phenotypes in PTOA.

METHODS: All protocols were approved by the Institutional Animal Care and Use Committee. Fifty-four, 12-14 week-old, MC-deficient KO and WT mice were subjected to a non-invasive anterior cruciate ligament rupture (ACLR) of the right hindlimb to induce PTOA; the left limb served as a control. Mice were euthanized at 28 days post-injury and whole synovium (n=20; n=5/sex/genotype) was dissected from ACLR and contralateral knee joints and preserved in Trizol. RNA was extracted and bulk RNA sequencing was performed (100 bp paired-end, >40M reads/sample). Significant differentially expressed genes (DEGs) were determined using the limma package and gene set enrichment analysis (GSEA) using the fgsea package (R, version 4.4.1). In a separate group of mice (n=34; n=9 female/genotype, n=8 male/genotype) hind limbs were harvested, fixed, embedded in paraffin and stained with Saf-O/Fast Green. Joint tissue histopathology was scored using the OARSI scoring system. Statistical analysis was performed using a three-way ANOVA with repeated measures and Tukey's multiple comparisons where appropriate. Significance was set at $P<0.05$ (GraphPad Prism 9.4.1).

RESULTS: Histological assessment confirmed WT male mice developed significantly more severe PTOA following ACLR than WT females (Fig.1). In contrast, MC-deficient mice exhibited markedly attenuated joint damage across all parameters compared to WT (Fig. 1). Strikingly, no sex differences were observed in any histological outcome in KO mice (Fig. 1). RNA-seq analysis of synovium showed that KO males exhibited baseline upregulation of innate immune system–related pathways in contralateral limbs compared to KO females, consistent with WT results and previous literature (Fig. 2A).[7] Sex-specific differences were not observed in the pathway analysis of the injured limb of KO mice (Fig. 2B). Compared to WT mice, both male and female KOs exhibited downregulation of tissue remodeling-relevant pathways related to osteogenic and pro-fibrotic signaling, consistent with the marked disease protection we observe in KO mice (Fig. 3). Multiple cartilage and ECM associated genes (Col1a2, Col11a2, Fgfr2, Acan, Itga2) that were highly expressed in WT ACLR mice were significantly blunted in the injured limbs of KO mice compared to WT mice (Fig. 2C).

DISCUSSION: Taken together, these findings suggest that MCs are not only involved in inflammatory signaling but may also play a central role in driving structural changes in the joint. Both male and female KO mice showed reduced activation of pathways related to bone and tissue remodeling, accompanied by lower histopathology scores compared to WT mice. At baseline, both KO and WT male mice showed upregulation of immune related pathways in contralateral limbs compared to their female counterparts, indicating that male mice may be inherently primed toward heightened immune responsiveness. However, following injury this sex-specific bias was lost in KO mice, which also exhibited attenuated ECM remodeling and reduced cartilage degeneration compared to WT mice; these findings are supported by the blunted expression of collagen associated genes and pathways in KO mice. Additionally, although sex-specific differences were not seen at the pathway level, select immune-related genes (Pram1, Tnf, and Trem3) were upregulated in injured KO males compared to females, whereas this pattern was reversed in WT mice, suggesting a sex-dependent shift in inflammatory signaling following injury. As established drivers of inflammation, MCs may be required to sustain or amplify sex-specific immune responses within the injured joint environment. Without MC signaling or mediators, male immune priming may not escalate into aggressive tissue remodeling and joint degeneration observed in WT mice.

SIGNIFICANCE/CLINICAL RELEVANCE: MCs may significantly contribute to sex-specific differences in PTOA providing new insight into the biological basis of OA disparities and guiding the development of more tailored therapeutic approaches.

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IMAGES AND TABLES:

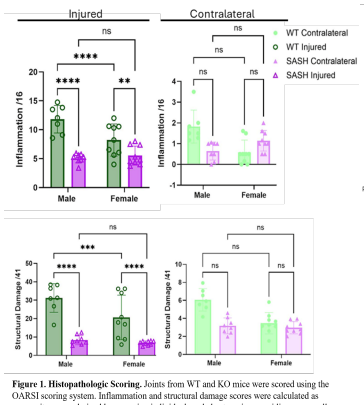

Figure 1. Histopathologic Scoring. Joints from WT and KO mice were scored using the OARSI scoring system. Inflammation and structural damage scores were calculated as composite scores derived by summing individual graded categories, providing an overall measure of joint pathology. Significant differences are indicated by * (P<0.05), **(P<0.01), *** (P<0.001), **** (P<0.0001)

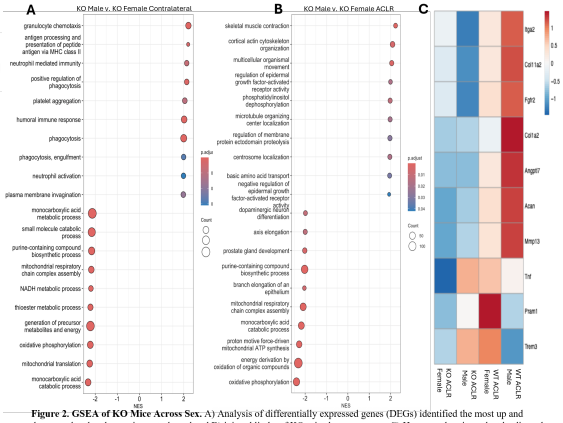

Figure 2. GSEA of KO Mice Across Sex. A) Analysis of differentially expressed genes (DEGs) identified the most up and downregulated pathways in contralateral and B) injured limbs of KO mice between sexes. C) Heatmap showing select leading edge genes from KO and WT male versus female comparisons.

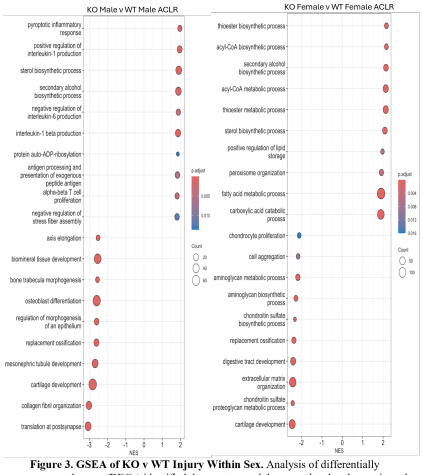

Figure 3. GSEA of KO v WT Injury Within Sex. Analysis of differentially expressed genes (DEGs) identified the most up and downregulated pathways in male and female injured limbs of KO and WT mice.