Macrophage depletion attenuates pain-like behaviors and alters DRG neuron molecular signaling in osteoarthritic mice of both sexes

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INTRODUCTION: Osteoarthritis (OA) is one of the leading causes of chronic pain and disability. Yet, management of OA pain remains poor, and often relies on analgesics with limited efficacy. Recent literature points to the emerging role of innate immunity in mediating OA pain. The knee joint is innervated by sensory neurons whose cell bodies reside in the lumbar level dorsal root ganglia (DRGs). We previously found increased levels of F4/80+ macrophages in the knee-innervating DRG, 8 weeks after OA surgery was surgically induced in the mouse knee, coinciding with onset of behaviors indicative of persistent pain1. In addition, we identified gene clusters via single cell RNA-sequencing (scRNAseq) that suggested the presence of a variety of immune cell types, including macrophages, in the DRGs of naïve mice. Therefore, the objectives of this study were to determine the effect of macrophage depletion on pain-like behaviors, joint damage, and DRG molecular changes in both male and female mice with OA.

METHODS: All animal experiments were approved by our Institutional Animal Care and Use Committee. We performed destabilization of the medial meniscus (DMM) on male or partial meniscectomy (PMX) surgery on female mice, age 12 weeks old at time of surgery, in Macrophage Fas-Induced Apoptosis (MaFIA) mice2. We evaluated hind paw mechanical allodynia (using von Frey fibers) and knee hyperalgesia using pressure application measurement (PAM) in both males and females, and weight bearing in female mice. We depleted macrophages at 8-weeks or 16-weeks post DMM surgery in male mice and 12-weeks post PMX surgery in female mice using AP20187 (Tocris), which binds to the transgenic CSF1R-eGFP receptor in macrophages and induces apoptosis. For flow cytometry, the ipsilateral L3-L5 DRGs were collected at the time points above (pooled two mice per sample, n=6-13 mice per group; n=3-6 for flow cytometry). DRGs were digested using collagenase IV and DNase I to make a single cell suspension, subsequently cells were counted and stained before running through the LSR Fortessa flow cytometer using antibody panels to detect PE-CD45, AF700-CD3, BV711-CD11b, PE/Cy7-MHCII, PerCp/Cy5.5-Ly6G, APC-F4/80, BV421-CD163, BV605-CCR2 (BioLegend), and Aqua-Live/Dead stain (ThermoFisher), and CSF1R-eGFP was detected endogenously. Analysis was completed using FlowJo software. In a separate cohort, we examined DRG molecular changes post macrophage depletion at 8-weeks post DMM surgery and naïve age matched controls treated with Vehicle or AP20187 (n=5/group, 4 groups: DMM Vehicle, DMM AP20187, Naïve Vehicle, & Naïve AP20187) from male MaFIA mice by bulk RNAsequencing. We utilized PantherDB for pathway analysis based on input of genes p<0.05 and BioVenn to look at pathways that overlapped per group. Statistical analysis was achieved by two-tailed t-tests done at each time point.

RESULTS: To determine the role of macrophages in mediating OA pain, we performed macrophage depletion using the MaFIA mouse model. Systemic macrophage depletion 8-weeks after DMM resulted in some alleviation of mechanical allodynia and knee hyperalgesia (Fig. 1A+B) with some mice returning to normal thresholds. Depletion at 16-weeks post DMM in males also resulted in attenuation of mechanical hypersensitivity in OA mice of both sexes. DRG macrophages were previously demonstrated to contribute to joint damage, and DRG molecular changes in the DRGs of naïve mice. Therefore, the objectives of this study were to determine the effect of macrophage depletion on pain-like behaviors, joint damage, and DRG molecular changes in both male and female mice with OA.

DISCUSSION: Here, we showed that macrophage depletion causes a variety of molecular changes within the DRG and leads to attenuation of pain-like behaviors in OA mice of both sexes. DRG macrophages were previously demonstrated to contribute to joint damage and DRG molecular changes in the DRGs of naïve mice. Therefore, the objectives of this study were to determine the effect of macrophage depletion on pain-like behaviors, joint damage, and DRG molecular changes in both male and female mice with OA.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates that DRG macrophages play a role in mechanical sensitization in mice with OA. These studies have significant clinical relevance for the development of targeted analgesics for OA pain.


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