Sexual dimorphism in production of specialized pro-resolving mediators after anterior cruciate ligament transection

Chilan B. G. Leite[1], Luciana P. Tavares[2], Julie Mekhail[1], Hannah Fricke[1], Gergo Merkely[1], Jessica Lehoczky[1], Janey Whalen[1], Julia F. Charles[1,2], Christian Lattermann[1]

[1]Department of Orthopedic Surgery, [2]Department of Medicine, Brigham and Women's Hospital - Harvard Medical School, Boston, MA

Presenting Author: cbleite@bwh.harvard.edu

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INTRODUCTION: It is generally recognized that male animals develop more severe disease in posttraumatic osteoarthritis (PTOA) mouse models. Previous investigations have demonstrated a protective effect of female sex against PTOA development after joint injury. Yet, the biologic mechanisms behind this are unknown. Anterior cruciate ligament (ACL) tear is a prevalent knee injury that results in immediate activation of inflammatory responses. Unresolved inflammation after ACL tear can cause degenerative changes to cartilage and bone that culminates in PTOA. Resolution of inflammation is an active process modulated by the specialized pro-resolving mediators (SPMs), polyunsaturated fatty acid derivatives with anti-inflammatory and regenerative properties. While previous investigation from our group has demonstrated SPM production post-ACL injury in mice, the existence of sexual dimorphism in SPM production remains unexplored. This study aims to investigate the sexual differences in the inflammatory and proresolutive response over time following an ACL transection (ACLT). We hypothesize that female mice have less severe PTOA, and this is associated with higher production of maresin 1 (MaR1) and resolvin D1 (RvD1), two SPMs derived from omega-3 fatty acids.

METHODS: Eight-weeks old male and female C57BL6/J mice underwent ACLT, and at days 1, 3, 7, 14-, 21-, 28- and 56-days post-injury (n=6 per time point), euthanasia was performed for collection of synovial fluid and tibiofemoral joint. Both knees were collected, and the contralateral (unoperated) knee served as control. Synovial fluid was used for total cell counting and measurement of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and SPMs (MaR1 and RvD1). Histological OARSI score and microCT score were performed at 56 days post-surgery to determine the presence and severity of PTOA. To investigate whether SPM signaling plays distinct roles between sexes, ACLT was performed in male and female 8-week-old LGR6 knockout (LGR6 is the receptor for MaR1) and wild-type (WT) mice. After 56 days post-ACLT, synovial fluid cell count, histology and microCT analyses for PTOA were performed. Two-way ANOVA and Tukey's post hoc tests were used for statistical analysis. Statistical significance was set at $p < 0.05$. This study was approved by our institutional animal care and use committee.

RESULTS: Consistent with findings in other PTOA models, female mice were relatively protected from developing OA changes after ACLT (Fig.1). ACLT led to an overall similar synovial fluid leukocyte recruitment in male and female mice, although females had a transiently lower leukocyte count on day 3 post ACLT ($p<0.01$). Additionally, both sexes demonstrated similar increases in synovial fluid pro-inflammatory cytokines that peaked in the first day (TNF-α and IL-1β) or at day 3 (IL-6) post-injury and declined over time (Fig. 2A). Conversely, the magnitude and temporal pattern of SPMs (MaR1 and RvD1) were different between sexes, with female mice having a later but higher peak concentration of SPMs ($p<0.0001$) (Fig. 2B). In mice lacking the MaR1 receptor LGR6, both male ($p>0.05$) and female LGR6 KO ($p<0.0001$) mice had increased synovial fluid leukocytes in comparison to WT mice. PTOA severity in LGR6 KO mice was increased in both male and females compared to WT mice, and loss of LGR6 reduced sex differences in PTOA outcome (Fig. 3).

DISCUSSION: This study shows that ACL transection causes a similar proinflammatory response in both male and female mice. However, the production of proresolutive molecules represented by the SPM production is late and higher in females. Interestingly, female mice had less severe signs of PTOA in comparison to males. When evaluating the effect of LGR6 deficiency in PTOA development, both male and female mice knockout for this receptor presented worst signs of PTOA. Therefore, sexual dimorphism in SPM production was observed, in which higher production of SPM as observed in females may be associated with less severe PTOA development.

SIGNIFICANCE/CLINICAL RELEVANCE: This project explores the temporal and sex specific patterns of inflammation and its resolution within the joint after injury, and may address knowledge gaps related to role, magnitude, and potential therapeutic role of SPMs in early PTOA. The existing clinical treatment approaches have yet to effectively tackle persistent inflammation in PTOA. This study offers valuable perspectives into the chronic inflammatory process, with the overarching objective of facilitating robust translational investigation.

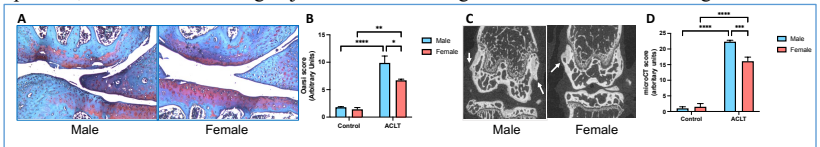

Figure 1. Females are relatively protected from PTOA after ACL transection. ACLT was performed on 8-week-old C57BL/6J male and female mice, and knee histology and microCT assessed 8 weeks post-operatively. **(A)** Saf-O/Fast Green staining of male (left) and female (right) ACLT knee showing articular cartilage loss, fissures, and erosions consistent with PTOA development **(B)** with higher OARSI score in males. **(C)** MicroCT images of male (left) and female (right) ACLT knee showing the presence of osteophytes (arrows), **(D)** with worst score in males. N=3/sex Mean score ± SEM., *p<0.05, **p<0.01, ****p<0.0001.

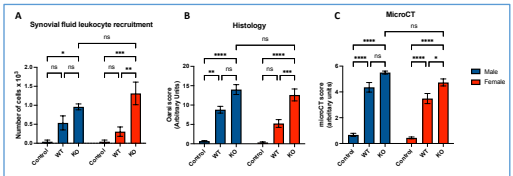

Figure 3. ACLT in LGR6 knockout (KO) and wild type (WT) mice. (A) ACLT KO animals presented higher cell recruitment in comparison to WT mice, particularly in females. LGR6 KO mice had worst signs of PTOA, with higher **(B)** OARSI score and **(C)** microCT score in both male and female mice. N=5-8/group; mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

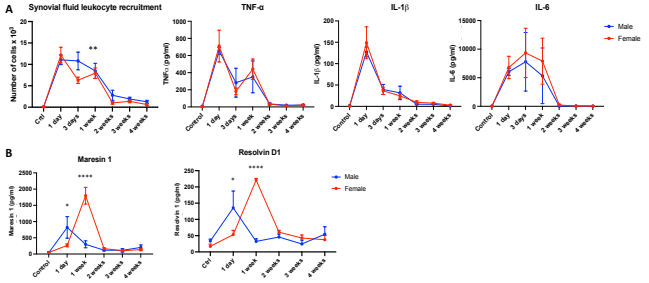

Figure 2. Inflammatory response to ACLT according to each sex. (A) Pro-inflammatory response: synovial fluid leukocyte recruitment and levels of TNF-α, IL-1 and IL-6 are elevated 1 day after ACL surgery and gradually decrease over time. No differences between the sexes are observed, except for higher cell recruitment in males at day 3 post-surgery. **(B)** Proresolutive response: synovial fluid levels of Maresin 1 and Resolvin D1 per time point after ACLT (by ELISA). Female mice have a later and higher concentration of Maresin 1 and Resolvin D1. N=3/sex/time; mean ± SEM; *p<0.05, ****p<0.0001.