

Mast Cells Mediate Acute Inflammatory Responses following Glenoid Labral Tears

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INTRODUCTION: Glenoid labrum injuries frequently occur because of repetitive movements or traumatic dislocation events. Despite current success with arthroscopic techniques, multiple revision surgeries of glenoid repair are often required. Failure of labrum healing can lead to degenerative deterioration of the labral tissue and joint instability. It is not clear how labral tears elicit inflammatory responses and lead to labral degeneration. Mast cells (MCs) have been established as crucial players in inducing acute inflammatory responses following soft tissue injury by inducing histaminic responses via degranulation responses. In a recent study, we unexpectedly discovered degranulating MCs in the synovial tissue following labrum tear. Based on this initial observation, we hypothesized that MC activation is responsible for synovial inflammation, leading to labral degeneration following labral tear. Here, the role of MC activation in labral degeneration and shoulder function was investigated *in vivo* using a glenoid labrum tear rat model in which MC stabilizer cromolyn sodium was used to significantly reduce mast cell degranulation [1-3].

METHODS: This study was approved by the IACUC. Labral tears were generated according to published protocols [4, 5] and modified to include surgical repair using suture anchors. Sprague-Dawley rats (200-300g weight, n=24) were randomly divided into 3 groups, with 2 end points (1 and 3 weeks), and of 4 rats each: 1) Healthy, 2) Injured, 3) Injured + Cromolyn. **Animal model.** A 2-3 cm incision was made longitudinally along the posterior aspect of the right shoulder. The shoulder was dislocated to expose the glenoid cavity. An ophthalmic blade was used to create a small laceration in the anteroinferior labrum (4:00 – 5:00) at the level of the glenoid rim to simulate a Bankart lesion. A suture anchor site was produced using a 0.3 mm diameter tungsten carbide drill bit. 5-0 non-absorbable suture was fed through the drill hole, joint capsule, and tightened with the knot resting on the capsular edge of the labrum (to avoid obstructing the joint articulation). The shoulder was then relocated, the subcutaneous tissue repaired with 4-0 non-absorbable sutures, and the skin closed using wound clips. MC activation was attenuated using pharmaceutical grade cromolyn sodium. Animals were given daily intraperitoneal injections of cromolyn (dissolved in sterile saline) for 7 days, starting on the day of surgery at 80 mg/kg body weight. **Functional assessment.** Animals were confined to a walkway underlined with white paper with an inkpad to mark paws and a dark shelter at each end [6]. Step width (distance between the hands perpendicular to the direction of travel), step length (distance from hand strike to subsequent hand strike of the opposite hand), and stride length (distance from hand strike to subsequent hand strike of the same hand) were measured to evaluate shoulder function before and every 48 hours after injury. **Histological analysis.** At 0, 1, and 3-weeks post-surgery, analyses of cell/tissue responses were performed to assess the structure and composition of the injured tissues (H&E), inflammatory responses (CD11b, MMP-13, and IL-1 β), and extracellular matrix (ECM) degeneration (Toluidine Blue). Images were quantified using ImageJ and statistical analysis was performed using GraphPad Prism software, with differences considered significant when p-value was <0.05.

RESULTS: Histological analysis. As expected, there were abundant activated MCs present in the synovium of labral tears nearby vasculature and muscle surrounding the glenohumeral joint (**Fig. 1A**). The number of activated MCs increased significantly in week 1 and maintained a similar level in week 3 (**Fig. 1B**). In support of our hypothesis, cromolyn significantly reduced the percentage of activated MCs in synovia by ~40% and ~30% at 1- and 3-weeks, respectively (**Fig. 1B**). Cromolyn treatment was also found to reduce both synovial and labral inflammatory responses. Specifically, histological analysis revealed that there were significantly larger number of CD11b+ inflammatory cells in synovia at 1- and 3-week following labral tear. Subsequently, cromolyn reduced the number of CD11b+ cells in synovial tissues reduced by ~3X and ~4X at 1- and 3- weeks, respectively (**Fig. 1C**). Synovia of injured labra also exhibited a significant increase in the release of inflammatory products—IL-1 β (~8-9X) and MMP-13 (~7-8X) than healthy controls at week 1 and 3. Torn labral tissues exhibited ~5X and ~7-8X higher IL-1 β and MMP-13 concentration than healthy, respectively. With cromolyn, labral IL-1 β intensity reduced by ~2X at each time point and labral MMP-13 intensity slightly reduced at week 1 and by ~3X at week 3. The extent of labral degeneration was evaluated by staining for GAG (**Fig. 1D**). As expected, we found that there was major GAG loss observed (>50%) in injured labra alone as early as 1 week compared to healthy. In contrast, cromolyn treated labral exhibited reduced ECM loss compared to injured controls at by ~40% at both time points (**Fig. 1E**). **Functional assessment.** By 3 weeks, injured rats with suture-anchor alone had significantly more reduction (~20%) from their initial step length and stride length, while rats with cromolyn treatment had full shoulder functional recovery with no significant difference from their baseline step and stride lengths.

DISCUSSION: Here, we provide evidence that MC activation and synovial inflammation play a critical role in the pathogenesis of labral degeneration. With labral tears, we found that significant inflammatory responses present in the synovial tissue were elicited by MC activation. Such MC-mediated inflammatory responses propagated from synovial tissue towards inside the labral tissue. These findings support that the inflammatory players found in the labral matrix likely originated from the inflamed synovium. Cromolyn treatment can reduce synovial inflammatory responses, minimize labral degeneration, and facilitate functional recovery. The overall results support that MC stabilizers may be a promising treatment option for acute inflammation following traumatic shoulder events such as labral tears. Further studies would be needed to determine the optimal dosage and duration for full MC stabilization or whether other pathways are involved in MC activation and subsequent labral degeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: Arthroscopic repair of glenoid labrum with suture anchors is one of the commonly used surgical procedures. However, long-term tissue and joint degeneration remain a clinical challenge, leading to the persistence of pain, instability, and functional limitations. These findings support the idea that MCs stabilizer may be used as a complementary therapeutic option in the treatment and repair of labral tears by reducing injury-associated inflammatory responses and tissue damage.

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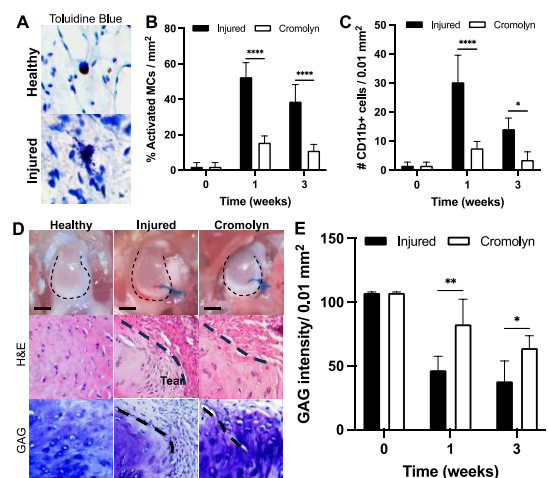


Figure 1. (A) Naïve & degranulated MCs can be found in the synovial tissue near healthy and 1-week injured labrum. (B) Synovial MC activation and (C) CD11b+ cell density following labral tear. (D) Gross images of rat labrum 3-weeks after surgery. Scale bar = 1 mm. (E) Representative H&E and GAG images of injured labra at 3 weeks. (F) Labral GAG intensity.