Early anterior cruciate ligament reconstruction in a murine model is associated with reduced local lymph node responses

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INTRODUCTION: The optimal timing of anterior cruciate ligament reconstruction (ACLR) remains a subject of debate among experts in the field. While previous and ongoing studies have identified the impact of early versus delayed reconstruction on arthrofibrosis and adjacent soft tissue injury, the immune system has not been well studied in this context of injury. Innate and adaptive immune cells constantly patrol the body, and following injury, these cells coordinate with tissue-resident cells for repair processes. Tissue-specific lymphatics then bring these cells to the draining lymph node (LN), where they interact with the LN stromal compartment that is known to support and regulate immune responses. Here, to better understand how immune cells respond to injury and surgical intervention, we sought to characterize the effect of ACLR timing on local and systemic immune responses.

METHODS: Forty-eight 12-week-old male B6 mice were randomized to one of three conditions: closed ACL rupture followed by immediate ACLR, closed ACL rupture followed by delayed ACLR 7 days post-injury (dpi), or closed ACL rupture only. The mice were then sacrificed 28 days post-operatively alongside a cohort of age- and sex-matched uninjured mice (Figure 1). To identify the draining LN of the knee joint, 2 µL of 2% Evans Blue Dye (EBD) was injected intraarticularly, and mice were sacrificed 5 minutes later. Ipsilateral iliac LNs (iLNs) and spleens were harvested to assess for local and systemic immune responses, respectively. These tissues were mechanically and enzymatically digested and passed through 70 µm filters to remove undigested debris. Total cell counts were determined using a Z Series Coulter Counter. Cells were stained, passed through a FACS Symphony A3, and analyzed on FlowJo. All statistics were calculated with GraphPad Prism.

RESULTS: EBD assay confirmed that the primary draining LN of the knee joint was the iLN (Figure 2). iLN cellularity and multiple cell populations, including monocytes, neutrophils, resident dendritic cells (rDCs), migratory dendritic cells (mDCs), natural killer (NK) cells, CD4+ T cells, T regulatory cells (Tregs), CD8+ T cells, B cells, and IgG+ plasma cells (PCs), were elevated in all surgical groups compared to non-surgical groups (Figure 3). iLN stromal cells, including blood endothelial cells (BECs), lymphatic endothelial cells (LECs), and fibroblastic reticular cells (FRCs) were also elevated in the surgical groups, consistent with expansion of the stromal compartment to support and regulate immune cells. Within the surgical groups, iLN cellularity and LEC counts were elevated in the delayed surgery group compared to the immediate surgery group (Figure 3). Myeloid, T, and B cell populations were unchanged between immediate and delayed surgery, though there was a trend toward greater cell counts in the delayed cohort. Mice that were injured, but did not receive surgery, saw an abrogated response. There was a decrease in spleen total cellularity after injury and surgery with a less pronounced difference in specific cell populations (Figure 4).

DISCUSSION AND CONCLUSION:

There are signs of greater immune activity, as evidenced by larger LNs, with delayed surgery compared to early surgery. This correlates with ongoing studies demonstrating reduced joint function and increased osteoarthritis development in the delayed surgery group. Whether greater LN activity reflects or contributes to ongoing joint disease will need further investigation, but our results suggest that greater immune activity is a characteristic of delayed, rather than immediate, surgery.