

Postoperative Physical Activity in the Setting of Delayed ACL Reconstruction is Associated with Increased Local and Systemic Immune Responses in Mice

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Introduction: The local and systemic immune responses to anterior cruciate ligament (ACL) injury and ACL reconstruction likely have substantial effects on graft-to-bone healing and patient recovery; however, the immune cell profile following ACL injury and reconstruction has not been well-characterized. The primary aim of the study was to evaluate the local and systemic immune response to ACL injury and reconstruction using a murine ACL reconstruction model. The secondary aim of the study was to evaluate the impact of postoperative physical activity on the immune cell profile.

Methods: Following IACUC approval (IACUC approval number: 2019-0034), fifty-five 11-week-old male C57BL/6 mice were randomized to one of 3 groups: (1) closed ACL rupture only ("closed" group, n = 15), (2) closed ACL rupture followed by immediate ACL reconstruction ("immediate" group, n = 19), or (3) closed ACL rupture followed by delayed ACL reconstruction at 7-days post-injury ("delayed" group, n = 15). Thirty-three of the mice were sacrificed at 10 days post-injury or post-surgery, 16 of the mice were sacrificed at 3 days, and 6 mice were sacrificed as uninjured controls. For the group of mice sacrificed at 10 days, half of the mice underwent 1 week of daily treadmill running for 40 minutes (n = 6 in the immediate group, n = 6 in the delayed group, and n = 6 in the closed group), with the other half remaining as a free cage activity group (n = 6 in the immediate group, n = 6 in the delayed group, and n = 3 in the closed group). Draining ipsilateral iliac lymph nodes (iLN) to assess local immune responses and spleens to assess systemic responses were harvested and processed for flow cytometry. Unpaired, two-tailed t-tests were used to evaluate for differences in total lymph node cellularity between groups. In addition, the Osteoarthritis Research Society International (OARSI) scoring system was used to evaluate the degree of articular cartilage degeneration in each mouse.

Results: Compared to uninjured normal mice, mice undergoing closed ACL rupture with or without ACL reconstruction had increased total iLN cellularity. The greatest increase in cellularity (over 8-fold) was observed in the 10-day immediate ACL reconstruction group with post-operative physical activity having little effect. There was a similarly large increase in cellularity in the 10-day delayed group only observed in mice that underwent daily treadmill running (Figure 1, p < 0.001). These differences were reflected in the absolute B cell and CD4+, CD8+, and T regulatory cell counts. Monocyte, macrophage, resident dendritic cell (DC), migratory DC, and neutrophil counts also mirrored this trend. In contrast, differentiated plasma cells (PCs) were increased only in the treadmill running groups regardless of surgical timing. Compared to normal uninjured mice, total spleen cellularity was increased only in 10-day immediate, and 10-day delayed running groups. Splenic monocyte and neutrophil counts were increased in these groups as well. The B/T cell ratio was elevated in only the 3-day groups.

Discussion: Postoperative physical activity in the setting of delayed ACL reconstruction is associated with increased local and systemic immune responses, reflecting either increased inflammation to clear the site of injury or an aberrant, prolonged inflammatory response. Postoperative physical activity played a smaller role in the setting of immediate ACL reconstruction. We hypothesize that excessive physical activity may delay or prevent resolution of the post-injury inflammatory process. The greater increase in immune response in the delayed ACL reconstruction group may be due to further stimulation of inflammation secondary to knee instability following the ACL injury. While inflammation is part of the normal response to injury, unresolved or excessive inflammation may impair graft healing due to stimulation of fibrosis and may also contribute to the development of PTOA. Our ongoing histologic, gene expression, and biomechanical studies will provide further insight into the biological relevance of such immune responses.

Significance/Clinical Relevance: The present study highlights the impact of both surgical timing and postoperative physical activity on the local and systemic immune and inflammatory cytokine profile following ACL injury and ACL reconstruction. Both the timing of surgery and postoperative physical activity likely impact tendon-bone healing and patient recovery following ACL reconstruction.

Images and Tables:

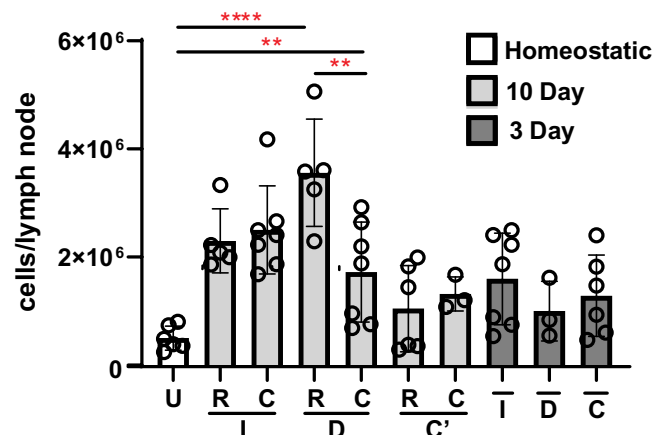


Figure 1. Total number of cells per iliac lymph node in uninjured control (homeostatic, U), immediate ACL reconstruction (I), delayed ACL reconstruction (D), and closed rupture only (C') groups undergoing either: (1) daily treadmill running (R) or (2) free cage activity (C) only. The greatest increase in cells per iliac lymph node was seen in the immediate and delayed ACL reconstruction groups compared to the uninjured control group. ** denotes significance at p < 0.01, *** denotes significance at p < 0.001.