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Acute mobilization and migration of bone marrow-derived stem cells following anterior cruciate ligament rupture

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SUMMARY

Objective: Little is known regarding acute local and systemic processes following anterior cruciate ligament (ACL) rupture. No study has elucidated whether bone marrow-derived mesenchymal stem cells (MSCs) are mobilized into circulation and recruited to the injured joint.
Methods: In Part 1, Lewis rats were randomized to noninvasive ACL rupture (Rupture) or non-injured (Control) (n = 6/group). After 72 h, whole blood MSC concentration was assessed using flow cytometry. Synovial fluid and serum were assayed for stromal cell-derived factor (SDF)-1α and cartilage degeneration biomarkers, respectively. In Part 2, 12 additional rats were randomized and intravenously-injected with fluorescently-labeled allogenic MSCs. Cell tracking was performed using longitudinal, *in vivo* and *ex vivo* near-infrared (NIR) imaging and histology. Synovium SDF-1α and interleukin (IL)-17A immunostaining was performed. Serum was assayed for SDF-1α and 29 other cytokines.
Results: In Part 1, there was a significant increase in MSC concentration and synovial fluid SDF-1α in Rupture. No differences in cartilage biomarkers were observed. In Part 2, Rupture had significantly higher NIR signal at 24, 48, and 72 h, indicating active recruitment of MSCs to the injured joint. *Ex vivo* cell tracking demonstrated MSC localization in the synovium and myotendinous junction (MTJ) of the quadriceps. Injured synovia exhibited increased synovitis grade and higher degree of IL-17A and SDF-1α immunostaining.
Conclusion: ACL rupture induced peripheral blood mobilization of MSCs and migration of intravenously-injected allogenic MSCs to the injured joint, where they localized in the synovium and quadriceps MTJ.

Introduction

Anterior cruciate ligament (ACL) rupture is one of the most common sporting injuries and is associated with an increased risk for developing post-traumatic osteoarthritis (PTOA). Recent literature reports the long-term incidence of PTOA after ACL rupture to be up to 50–90%[1–4] and this incidence does not appear to decrease following surgical reconstruction[1,5–7]. While the pathomechanism of PTOA following ACL rupture is not fully understood, the contribution of both mechanical factors, such as abnormal joint kinematics, and biological factors, such as increased concentrations of inflammatory moieties, have been implicated as exacerbating the disease process[1,5,8,9].

The acute biological phenomena following ACL rupture remain largely uncharacterized. Protein- and gene-level changes in synovium have been implicated as potential contributors to the pathogenesis of PTOA[10]. Mechanical stress sustained by the synovial membrane during the rupture mechanism has been shown to generate a mechanobiologic response, inducing synovitis, by triggering the expression of proteases, cytokines, chemokines, and growth factors[11–14]. Similarly, mechanical stress sustained by the cartilage extracellular matrix activates mechanoreceptors, inducing changes in chondrocyte gene expression and cartilage metabolism and triggering the release of glycosaminoglycans and collagen molecules[8]. Analogous mechanobiologic responses are known to

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occur in the ACL, bone, and the menisci[3,5], and the activation of inter-related, feedback- and feedforward-based pathways perpetuate joint inflammation and trigger abnormal tissue remodeling[5,8,9].

A paucity of data exists on the involvement of local and systemic stem and/or progenitor cells in ACL injury. Morito *et al.*[15] have described a local increase in MSC concentration in human synovial fluid following injury, and these MSCs were found to be synovial-derived rather than bone-marrow-derived, as analyzed by GeneChip. In the same study, Morito *et al.* also concluded that synovium-MSCs exogenously-injected into the knee joint following injury exhibited preferential attachment to the injured ACL, demonstrating that MSCs exhibit affinity for injured intraarticular tissues. In a recent study, Ozeki *et al.*[16] demonstrated that serial, intra-articular injections of synovium-derived MSCs following surgical ACL transection resulted in migration to the synovium where secretion of trophic factors by injected cells were found to inhibit the progression of OA. To the authors' knowledge, no study to date has investigated the involvement of systemically-circulating stem or progenitor cells following ACL injury. Peripheral blood mobilization of endogenous MSCs, the process by which endogenous, marrow-derived cells leave the marrow compartment to enter circulation[17,18], has been described following the acute neurobiochemical stimulus associated with severe bodily trauma such as hypoxia, myocardial infarction, lung contusion, and cerebrovascular ischemia[19–23]. Following mobilization into peripheral blood, MSCs preferentially migrate to the vasculature proximal to the site of injury and transmigrate through the endothelium along a chemokine gradient into the damaged tissue. MSC can then participate in tissue regeneration and repair, via direct transdifferentiation and indirect immunomodulatory processes[18]. While the mobilization of marrow-derived stem and progenitor cells has been described in severe orthopaedic injuries, such as long bone fracture[24], it is to date unknown whether soft tissue injury such as ACL rupture induces systemic MSC mobilization. Furthermore, if stem cell mobilization occurs, it is unknown whether circulating MSCs migrate to the injured joint and to which tissues MSC are actively recruited.

The characterization of the marrow-derived stem and progenitor cell response following ACL rupture can expand current knowledge regarding post-injury mechanisms, which may be related to the onset of PTOA and/or usable for interventional therapies. To this end, the purpose of this study was two-fold: first, to determine whether ACL rupture induces systemic stem cell mobilization and second, to assess whether stem cells actively migrate from systemic circulation to the injured joint.

Methods

Part 1: Systemic mobilization of mesenchymal stromal cells

Treatment groups and noninvasive ACL rupture

After institutional animal care and use committee (IACUC)-approval, 12 female Lewis rats aged 14 weeks, ~200 g (Charles River Laboratories, Wilmington, MA, USA) were acclimated to their environment for 1 week. Rats were then randomized to a control group (Control) or noninvasive ACL rupture (Rupture) using a computer algorithm, n = 6 rats/group, and housed in individual cages in a 12 h light-dark facility with unrestricted access to food and water. One hour prior to anesthesia, rats were subcutaneously administered 5 mg/kg Carprofen, a non-steroidal, anti-inflammatory drug. Anesthesia was induced by intraperitoneal ketamine and xylazine injections and maintained with 0.5%–1.5% inhaled isoflurane. Subcutaneous buprenorphine was administered for post-operative analgesia. Rats in the Rupture group were subjected to a noninvasive ACL injury protocol established for the mouse by Christiansen *et al.*[25] and adapted to the rat by Maerz *et al*[26]. Briefly, animals were positioned prone on a materials testing system utilizing custom fixtures (Insight 5, MTS Systems, Eden Prairie, MN, USA). The right knee was flexed to 100° and positioned in a 3-mm deep trough to restrict medial and lateral translation, and the right paw was mounted in 30° of dorsiflexion. After a preload, preconditioning, and a secondary preload ramp to 15N, a rapid displacement of 3 mm was applied to the paw fixture at a rate of 8 mm/s, which causes anterior tibial subluxation and subsequent failure of the ACL, as previously characterized[26]. ACL rupture was confirmed using an anterior drawer test, which is sensitive to diagnosing complete ACL rupture in this model[26]. This injury has been shown to induce bony remodeling and articular cartilage degeneration representative of the onset of PTOA[27,28]. Control rats received identical anesthesia and analgesia but were not subjected to any mechanical loading. All rats were allowed *ad libitum* cage activity until 72 h post-procedure, at which point they were euthanized via CO_2 asphyxia.

Flow cytometry of mesenchymal stem cells (MSCs) in whole blood

Immediately prior to euthanasia, whole blood was collected via cardiac puncture and transferred to red blood cell (RBC) lysis buffer (5 Prime Inc, Gaithersburg, Maryland, USA). Two samples of 10^6 cells from each animal were stained for cell-surface expression of CD29, CD34, CD45, and CD90 (see Supplementary information). One additional unstained sample and corresponding isotype control sample were utilized, and all samples were analyzed using flow cytometry (FACSCanto II, BD Biosciences, San Jose, California, USA). MSCs were identified by $CD29^+$ $CD90^+$ $CD34^-$ $CD45^-$ expression, a commonly-utilized panel for the identification of BM-MSCs[29–32]. The results of the two stained samples from each animal were averaged and compared between groups.

Serum and synovial fluid analysis

Serum was prepared from whole blood collected via cardiac puncture. To assess whether cartilage breakdown and turnover has been initiated 72 h post-injury, serum was assayed for six biomarkers of cartilage metabolism using enzyme-linked immunosorbent assays (ELISAs): Cartilage Oligomeric Matrix Protein (COMP, MD Biosciences, St. Paul, MN, USA), Aggrecan Chrondroitin Sulfate 846 Epitope (CS-846, Ibex Pharmaceuticals Inc, Mont-Royal, QC, Canada), C-terminal Telopeptide of Collagen II (CTxII, Nordic Biosciences, Herlev, Denmark), Type II Collagen Cleavage Product (C2C, Ibex Pharmaceuticals Inc), Type II Collagen Propeptides (CPII, Ibex Pharmaceuticals Inc), and Types I and II Collagen Cleavage Neoepitope (C1,C2, Ibex Pharmaceuticals Inc). Following blood collection, synovial fluid was aspirated from the right knee using a lavage procedure during which 200 μL of sterile PBS were injected into the anteromedial joint space using a 27-gauge needle, and ~100 μL of synovial fluid lavage was collected via a posteriorly-inserted 23-gauge needle. Collected synovial fluid lavage was immediately stored at −80°C for future quantification of stromal cell-derived factor (SDF)-1α using ELISA (R&D Systems, Minneapolis, MN, USA).

Part 2: In vivo and ex vivo tracking of MSC migration

Treatment groups and ACL rupture loading

Part 2 sought to characterize the systemic trafficking and intra-articular migration of MSCs following ACL injury. For this experiment, allogenic marrow-derived stem cells were optically-labeled, injected intravenously following ACL injury, and characterized via longitudinal near-infrared (NIR) imaging. An additional 12 female, Lewis rats aged 14 weeks (~200 g) were randomized to either