Pre-Injury Depletion of Macrophages Results in Increased Acute Joint Inflammation Following Articular Fracture

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Background/Purpose: Posttraumatic arthritis (PTA) is an accelerated form of arthritis that occurs following joint injury. Following articular fracture, C57BL/6 mice showed significant signs of PTA, whereas MRL/MpJ mice, a superhealer mouse strain, exhibited less severe joint degeneration. In order to elucidate the link between macrophages and PTA, a transgenic mouse strain that allows for the specific depletion of macrophages was used in this study. The Macrophage Fas-Induced Apoptosis (MAFIA) mouse strain expresses the inducible Fas-suicide gene, which facilitates apoptosis of macrophages following the administration of AP20187. We hypothesized that locally depleting synovial macrophages in the MAFIA mice would reduce joint synovitis following articular fracture. The objective was to characterize the role of macrophages in acute joint inflammation and synovitis following articular fracture.

Methods: Male MAFIA mice (Jackson Laboratories) were obtained at 6 weeks of age and then aged to skeletal maturity at 16 weeks, at which point the left hindlimb was subjected to a moderate articular fracture as previously reported. Macrophages were locally depleted at 2 days prior to fracture, immediately following fracture, or 2 days post fracture. The AP20187 (n = 6 per time point), which induces macrophage apoptosis in the MAFIA mice, or the carrier solution (n = 3 per time point) were delivered via a single 6-μL intra-articular injection. The mice were sacrificed 7 days post fracture, the limbs were harvested, and serum and synovial fluid were collected and stored at –80° for future analysis. All hindlimbs were formalin fixed and scanned with micro-CT (SkyScan 1176, Bruker BioSpin). The limbs were then processed and paraffin embedded using standard techniques. Histological sections of the joint were stained using hematoxylin and eosin, and synovitis was quantified using a modified synovitis grading scheme for mouse tissue. A multifactorial analysis of variance was used to assess synovitis by group and time, with limb as a repeated measure.

Results: Articular fractures were successfully created in all mice. For synovial inflammation, fracture in the experimental limb led to increased total synovitis scores compared to contralateral control limbs in all groups ($P < 0.05$) Additionally, with predepletion (Day –2), mice that received AP20187 had significantly higher joint synovitis in fractured limbs compared to carrier solution ($P = 0.05$). At the other two time points, where depletion occurred on the day of fracture (Day 0) or 2 days post fracture (Day 2), there was no significant difference in the level of synovitis between AP20187 and the carrier solution. Mice that received a local injection of AP20187 prior to fracture (Day –2) exhibited severe joint inflammation characterized by synovial infiltration with increased cellular density.

Conclusion: The observed changes at all points evaluated did not support our hypothesis; macrophage depletion did not reduce acute joint inflammation following articular fracture. Conversely, when depleted 2 days prior to fracture, joint inflammation increased following articular fracture. The massive influx of inflammatory cells was observed mostly in mac-

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rophage-depleted joints, suggesting that macrophages could be a modulator for recruiting inflammatory cells following injury. Although inflammation has degenerative effects on cartilage, our data suggest that macrophages are important for regulating synovial inflammation and bone maintenance after joint injury. This finding is significant for understanding the role of macrophages in inflammation and joint injury. Our data suggest that macrophages are important to maintain a controlled inflammatory response to joint injury.

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See pages 99 - 147 for financial disclosure information.