Macrophage Subsets Regulate Ischemic Muscle Regeneration and Repair

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Background and Objective: Acute ischemic injury to skeletal muscle initiates rapid and intensive inflammation. Monocytes infiltrate the ischemic tissue and become macrophages, which may take part in either tissue injury or repair. However, the role of macrophages in controlling the outcome of ischemic muscle injury is not well understood. We aimed to test the hypothesis that minimizing the monocyte/macrophage response to ischemia would modulate the muscle's ability to recover from ischemic injury.

Methods: Balb/C mice were treated with clodronate-liposomes for monocyte/macrophage depletion, or PBS-liposomes for controls. After one day, mice were subjected to hindlimb ischemia by femoral artery ligation and dissection. Laser Doppler perfusion imaging was performed at days 1, 7, 10, 14 and 21 after ischemia. In addition, macrophage subset isolated from the ischemic tissue were characterized by FACS.

Results: Ischemia and necrosis of the limb were followed by a significant increase in relative number of macrophages (from days 3 to 5, p=0.0012). Macrophage subset analysis by FACS revealed that while the initial (days 1-3) macrophage influx was composed of similar percentages of pro-inflammatory M1 (2%) and reparative M2 (1.7%) macrophages, the subsequent (days 3-7) rise was composed of mostly by M2 macrophages (7.45%). Of interest, clodronate-liposome therapy blunted macrophage early and late rise. Compared with macrophage-depleted mice, recovery of perfusion in the ischemic limb was significantly greater in animals with intact macrophages (Figure, p<0.001). Histological analysis revealed that the area of fatty degeneration in the ischemic muscle was significantly greater in animals with macrophage-depletion (11.1 mm2 vs. 2.3 mm2, p<0.001).

Conclusions: Our findings suggest, for the first time, that macrophages are essential for ischemic muscle recovery, and that transition in macrophage phenotypes is a necessary for muscle regeneration following acute ischemic damage.