Title: M2 macrophage population persists following injury of denervated muscle

Author(s) and Affiliations:
Alvin Wong, MD, Stony Brook Department of Surgery
Ruixin Xu, Department of Biochemistry and Cell Biology

Faculty Mentor(s):
Addolorata Pisconti, PhD, Department of Biochemistry and Cell Biology

Background:
Muscle fibrosis following direct injury impedes normal regeneration and function. Following injury, macrophages clear debris, facilitate regeneration via coordination of satellite cell activity, and regulate fibrosis. Previous studies have shown that denervated muscle demonstrates fibrosis even in the absence of direct muscle injury, and that infiltrating myeloid-derived TGFβ1 is responsible for development of muscle fibrosis in the setting where direct muscle injury is performed. However, it remains unclear whether macrophages are important drivers of muscle fibrosis in the setting of denervation. Determining whether macrophages in denervated muscle function normally would direct future approaches to treatment of muscle fibrosis in the setting of denervation injury.

Methods:
A 4mm segment of the left sciatic nerve in two C57Bl6 mice was removed. One month post denervation, the tibialis anterior muscle of the denervated leg was injured by percutaneous injection of 30 ul of 1.2% barium chloride ($BaCl_2$). Tibialis anterior (TA) muscles were collected five days and 1 month following injury for immunohistochemical analysis. Control mice underwent $BaCl_2$ injury without denervation injury. Pax7 and MyoD staining confirmed a regenerative response in injured muscle. M1 and M2 macrophages were visualized by staining for CD86 and CD206. Quantification of the number of M2 macrophages per muscle fiber on TA cross-sections was performed by manual counting using ImageJ software.

Results (or Preliminary Results):
Denervated TA muscles demonstrated robust regenerative response to $BaCl_2$ injury as visualized by expression of Pax7 and MyoD 5 days post injury. CD86+ cells were not visualized at five- and 20-days post $BaCl_2$ injury. $CD206^+$ cells significantly increased in number five days following $BaCl_2$ injury in denervated TAs compared with contralateral uninjured TAs (10.2 vs 1.6 CD206+ cells/100 fibers) and remained elevated at 1 month post injury (3.0 vs 1.1 CD206+ cells/100 fibers). When compared to $BaCl_2$- injured innervated TAs 1 month post injury, $BaCl_2$-injured denervated TAs continued to demonstrate a significantly elevated number of CD206+ cells (3.0 vs 0.6 CD206+ cells/100 fibers), $p<0.05$.

Conclusions (or Preliminary Conclusions):
Denervated muscle maintains an intrinsic ability to mount a regenerative response to direct injury. M2 macrophages increase in response to $BaCl_2$ injury in denervated muscle, and this increase does not fully resolve a month after injury. In control muscle in which innervation is uninterrupted, the M2 macrophage population returns to baseline one month post injury when compared to the contralateral, uninjured muscle, indicating resolution of the inflammatory response. M2 macrophages remained increased in number in denervated muscle one month post $BaCl_2$ injury. A persistent inflammatory reaction may play a role in the fibrosis observed following muscle denervation.