Inhibition of TGF-β following contraction-induced muscle injury results in an initial improvement but long term deficit in force production

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Introduction
Transforming growth factor-beta (TGF-β) is a pro-inflammatory cytokine that helps to regulate the response of many tissues following injury. TGF-β promotes the proliferation of fibroblasts and leads to the accumulation of a type I collagen-rich extracellular matrix (ECM). Previous studies in our lab have shown that treating skeletal muscles with TGF-β results in a dramatic accretion of type I collagen, substantial muscle fiber atrophy and a marked decrease in force production.

As TGF-β promotes muscle atrophy and fibrosis, our objective was to investigate whether the inhibition of TGF-β after injury would enhance the recovery of muscle following contraction-induced injury. We hypothesized that inhibiting TGF-β after contraction-induced muscle injury would improve the recovery of force production by limiting the accumulation of fibrotic scar tissue and preventing atrophy of muscle fibers. To test this hypothesis, we induced an injury to the EDL muscle of mice in situ using a series of lengthening contractions and treated mice with either a mouse monoclonal antibody against TGF-β or a sham IgG, and measured the recovery of force production and changes in muscle morphology over time.

Methods
Muscle Contractility: All experiments were conducted with IACUC approval. Mice were subjected to a contraction-induced injury protocol (modified from Brooks 1995) using a custom-modified in situ apparatus (Aurora 809B). The distal tendon of the left EDL muscle of each mouse was attached to a dual-mode servomotor/force transducer and stimulated indirectly via the peroneal nerve using platinum sub-dermal electrodes. Optimal length (L0) was determined using a series of twitches, and maximum isometric force (P0) was measured at L0 before the injury. Contraction-induced injury was caused using a series of 80 lengthening contractions. Following a recovery period of 3, 7 or 21 days, P0 was measured and compared to the pre-injury value. EDL muscles were then removed and mice were humanely euthanized.

Treatment: Mice were given IP injections of either MOPC21, a sham murine IgG, or 1D11, a murine monoclonal bio-neutralizing antibody against TGF-β, immediately after injury and at 3 and 6 days post injury.

Immunohistochemistry: Muscles were sectioned with a cryostat and incubated with DAPI and biotinylated antibodies against collagen I, and avidin-conjugated FITC.

Statistical Analysis: Results are presented as mean±SE. Differences between sham and TGF-β inhibitor treated mice at each time point were tested with Student’s t-test with α<0.05.

Results
Compared with mice that received sham IgG, muscles from mice receiving the TGF-β inhibitor showed a greater recovery in force at the 3 and 7 day time points (Figure 1). However, at the 21 day time point, the mice that received sham IgG had a full recovery of force production while mice that received the TGF-β inhibitor had not fully recovered, and displayed no change from the 7 day time point (Figure 1).

Additionally, compared with mice that received sham IgG, muscles treated with TGF-β inhibitor showed a protection from injury-induced atrophy and fibrosis. At 3 and 7 days, compared to sham IgG treated muscles, TGF-β inhibited muscles exhibited an attenuation of collagen I signal (Figure 2A-D). Additionally TGF-β inhibition protected muscles from atrophy and had more centrally located nuclei, suggesting enhanced early regeneration of muscle fibers. At 21 days post-injury, although the fibers appear to be regenerated and the amount of ECM around the muscle fibers was not different, appearance of the ECM from the TGF-β inhibitor treated muscles appeared mottled and ruffled compared to controls (Figure 2E-F).

Discussion
The results from the current study indicate that inhibiting TGF-β during the initial stages of muscle injury results in a short-term improvement in muscle function, but a long-term deficit in force production. While further studies are necessary, the mottled appearance of the ECM at 21 days suggests that matrix proteins might play an important role in supporting a full regenerative response.

Figure 1: Recovery in force production for the control (Sham IgG) and TGF-β inhibitor cohorts at 3 (A), 7 (B), and 21 (C) days post-injury. *Indicates significantly different from MOPC21

Figure 2: Immunohistochemistry from 1D11 and MOPC21 treated injured muscles imaged at 3 days (A, B), 7 days (C, D), and 21 days (E, F) post-injury. Collagen I, DAPI. Scale Bar = 50 μm

Significance
Improving the treatment of muscle injuries requires further understanding of the mechanisms that regulate the response to injury and subsequent regeneration. This study provided important information on the role of TGF-β in the overall recovery of muscle to injury, and suggests that it might have a dual role in promoting both inflammation and regeneration.

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