Immunomodulation using Macrophage Colony Stimulating Factor or Voluntary Wheel Running Rescues Aged Skeletal Muscle Function following Disuse Atrophy

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ABSTRACT

Introduction: Aging impairs muscle regrowth and function following a period of disuse. Poor recovery of muscle size and strength with aging coincides with a suppressed macrophage response and increased fibrosis. Immunomodulation in the form of exercise and cytokine injections has been demonstrated to improve skeletal muscle regeneration. However, it is unclear if these macrophage-promoting approaches would improve skeletal muscle recovery following disuse in aged animals. Therefore, the purpose of this study was to examine the effectiveness of macrophage colony stimulating factors (MCSF) or voluntary wheel running (VWR) on the recovery of muscle and strength following disuse atrophy in aged mice. **Methods:** Aged (20-22 mo) male C57BL/6 mice were assigned to be an ambulatory control, or undergo 14 days of hindlimb unloading or 14 days of hindlimb unloading followed 14 days of reloading. Mice in the recovery group received a single injection of MCSF or PBS in both triceps surae while a second group of recovery mice underwent 14 days of VWR. **Results:** We found that MCSF and VWR at the initiation of reloading enhanced soleus muscle regrowth and rescued grip strength above ambulatory control and PBS injection. Moreover, following MCSF and VWR, soleus muscle cross sectional area and the ratio of BHP/COLIV (indicative of decreased fibrosis) was also elevated above control and PBS. **Conclusions:** Overall, these results suggest that immunomodulating approaches such as MCSF and VWR during the recovery phase from disuse atrophy restore aged skeletal muscle mass, myofiber size, and grip strength while also reducing muscle fibrosis.

INTRODUCTION

Impaired muscle regrowth following a period of disuse in aged skeletal muscle is a widely recognized and studied phenomenon [1,2]. Aged skeletal muscle often fails to achieve the muscle quality that it had prior to disuse atrophy [3]. Poor muscle recovery following disuse events may contribute to age-related muscle and functional decline (i.e., sarcopenia) [4-5]. Unfortunately, there are numerous gaps in our understanding of the cellular and molecular processes during the recovery phase of aged skeletal muscle.

An important contributor to skeletal muscle recovery is a properly coordinated immune response triggered by extracellular and intracellular cues that recruit circulating immune cells to the area of damage [6]. Macrophage Colony Stimulating Factor (MCSF) is believed to be a critical recruiter of macrophages to skeletal muscle during a remodeling response. Macrophages that are recruited during the early phase of recovery are referred to as M1 (or classically activated) and exhibit pro-inflammatory functional properties secreting chemokines and cytokines such as Interleukin-1β (IL-1β), Tumor necrosis factor α (TNF-α), and C-C Motif Chemokine Ligand 3 (CCL3) [7]. Conversely, M2 macrophages are considered anti-inflammatory and are involved in tissue repair and promote collagen synthesis [7].

Our laboratory and others have recently demonstrated that aged skeletal muscle presents with impaired M1 macrophage recruitment during the early phase of recovery from disuse atrophy. This is also coupled with an elevated M2 response [6] . Furthermore aged skeletal muscle presents with impaired muscle mass and increased fibrosis during recovery from disuse [6]. The lack of macrophage recruitment in aged skeletal muscle has recently become an interesting investigative target. The use of therapies such as cytokine injections and exercise have been explored in the field of regeneration, but significant gaps remain in our understanding of these therapies in the context aging and muscle recovery from disuse atrophy.

PURPOSE

The purpose of this study was to examine the effectiveness of macrophage colony stimulating factors (MCSF) or voluntary wheel running (VWR) on the recovery of muscle and strength following disuse atrophy in aged mice.

METHODS

Animals:. Aged (20-22 mo) male C57BL/6 mice were assigned to be an ambulatory control, or undergo 14 days of hindlimb unloading or 14 days of hindlimb unloading followed 14 days of reloading. Mice in the recovery group received a single injection of MCSF or PBS in both triceps surae while a second group of recovery mice underwent 14 days of VWR. To verify MCSF treatment a subset of aged mice underwent hindlimb unloading and received a single injection of MCSF or PBS and were collected after 4 days of reloading (initial macrophage immune response time).

Muscle Collection: The hindlimb muscles were extracted at 20 weeks of age, frozen in liquid nitrogen cooled isopentane and stored at –80°C for immunohistochemistry. The right soleus muscles were used for ex-vivo force measurements.

Immunofluorescence : Soleus muscles were cryosectioned and stained for CSA (Laminin FITC), Fibrosis (BCHP mCherry/COLIV CY5) , and macrophages (CD68 mCherry, CD163 CY5, DAPI). Sections were imaged at 10-20X on a Nikon TI Eclipse microscope and analyzed using FIJI ImageJ.

Ex-Vivo Force : Maximal force production was assessed in soleus muscles using an *ex vivo* small animal muscle contraction apparatus (Aurora Scientific). Soleus muscles were carefully excised from live unconscious mice and attached to the anchor and force transducer of the apparatus and submerged in Krebs-Henseliet buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.25 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose). Muscles were then set at an optimal length for maximal contraction and then stimulated at increasing frequencies until maximal force production was achieved.

RESULTS

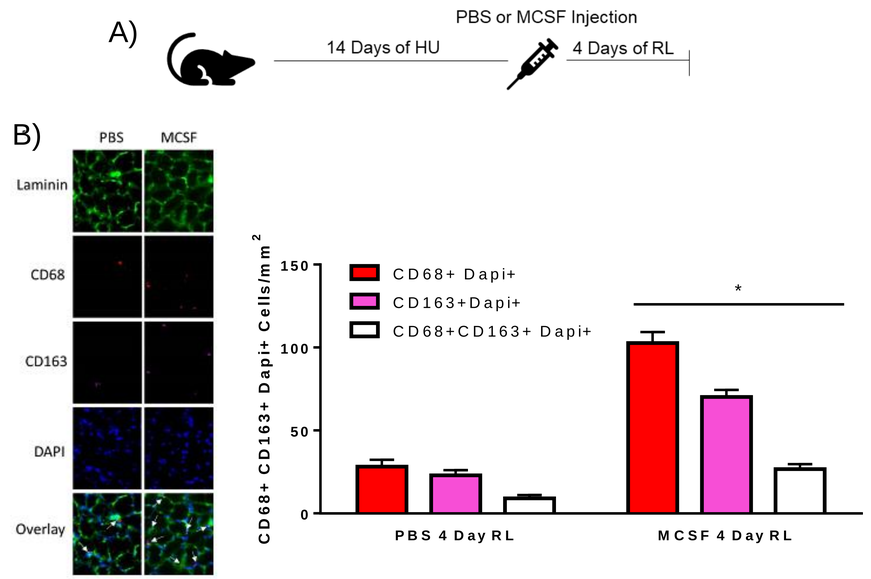

Figure 1: MCSF Increases Macrophage Content in Aged Solei Muscles During Initial Reloading Phase. A) Experimental Design figure depicting mice undergoing 14 days of hindlimb unloading followed by an MCSF injection and 4 days of reloading. B) Representative images of Laminin, CD68 (M1 Macs) CD163 (M2 Macs) , DAPI, and an overlayed image with quantification of macrophages per mm2. Values are means ± SEM. Significance was set at $p<0.05$. * Signifies different from each respective PBS population..

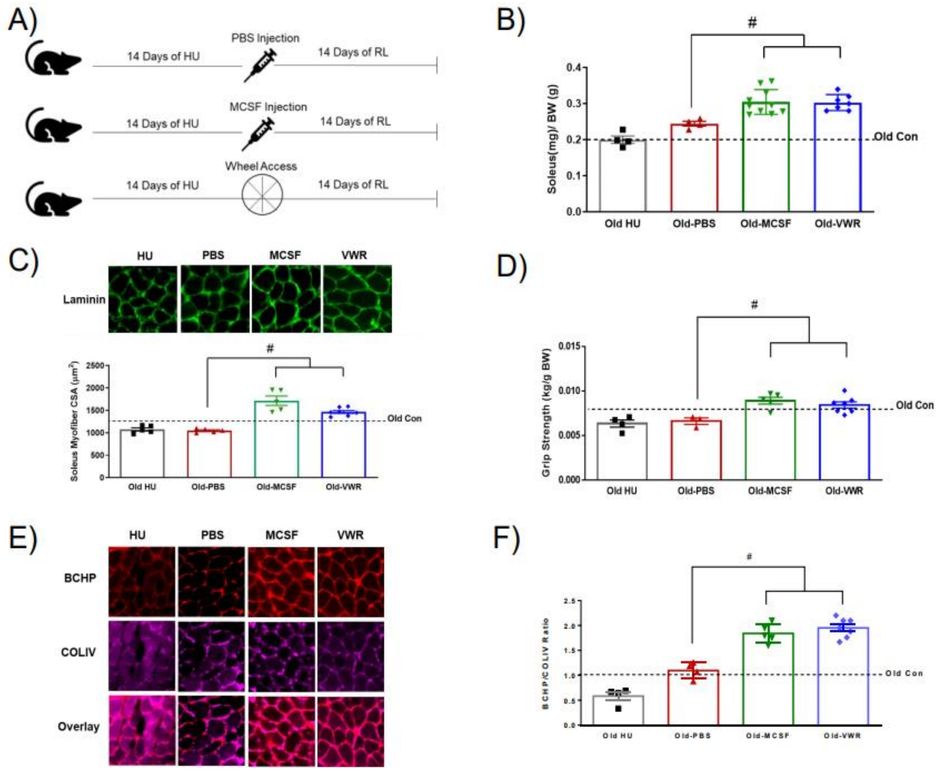

Figure 2. MCSF and Voluntary Wheel Running Improve Aged Muscle Recovery Following Disuse Atrophy. A) Experimental design depicting treatment groups for PBS, MCSF, and VWR. B) Soleus Muscle mass to bodyweight ratio in HU,PBS, MCSF, and VWR mice. C) Laminin stain and quantification of cross sectional for HU, PBS, MCSF and VWR mice. D) Grip strength normalized to bodyweight for HU, PBS, MCSF and VWR mice. E) BCHP and COLIV stain for HU, PBS, MCSF, and VWR mice. F) Quantification of BCHP/COLIV ratio in HU, PBS, and VWR mice. Values are means ± SEM. Significance was set at $p,<.05$. # denotes different from PBS injection

Results

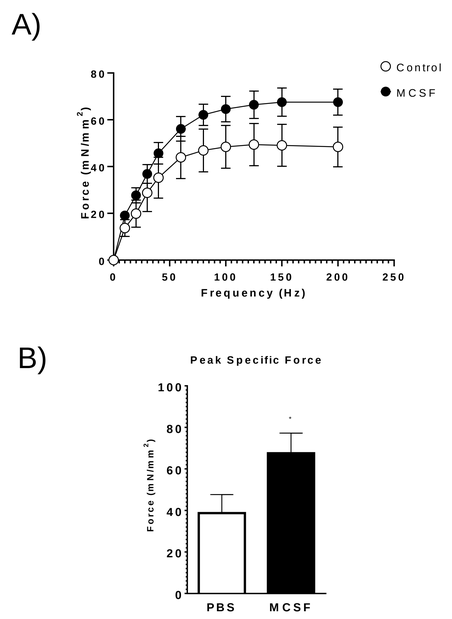

Figure 3: MCSF Improves Soleus Muscle Specific Following 14 Days of Recovery. A) Specific force frequency curve of PBS control and MCSF mice following 14 day reload. B) Peak specific force in PBS and MCSF mice following 14 day reload. Values are means ± SEM. Significance was set at $p<0.05$. Values are means ± SEM. * Signifies different to PBS

CONCLUSIONS

•A single MCSF injection increases early macrophage recruitment in the soleus muscle of old mice (4 day reload).

•MCSF and VWR improve soleus muscle to bodyweight ratio following 14 days of reloading after disuse atrophy. MCSF and VWR also restored soleus muscle cross sectional area and grip strength following 14 days of reloading after disuse atrophy.

•MCSF and VWR improve the ratio of BCHP/COLIV demonstrating improved fibrosis and improved peak specific force when compared to PBS treated mice during a 14 day reloading period.

•Overall, a single injection of MCSF as well as wheel access during recovery improve aged skeletal muscle recovery following a 14-day period of disuse atrophy.

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