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Macrophage Regulation of Muscle Regrowth from Disuse in Aging

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Abstract

Skeletal muscle immune cells, such as macrophages, are necessary for proper regrowth following muscle disuse. We suggest that the important role of macrophages concerning muscle regrowth following disuse is divergent compared to young mice (i.e. dysregulated) during the recovery period. Modulation of macrophages may be a promising future therapeutic target to enhance the impaired muscle growth during recovery from disuse in older adults.

Summary:

In this review, the role of macrophages in the recovery of muscle after disuse or injury in aging are discussed.

Keywords

immune cells; polarization; physical inactivity; injury; rehabilitation; aging

Introduction

Aging coincides with a higher frequency of muscle disuse and recovery events (due to illness, surgery, pain) that are likely to lead to rapid loss of muscle mass and strength. Full recovery of muscle mass and function following these disuse / recovery periods may not occur for many of these older adults while others may find recovery much slower than optimal (1). Indeed, several aging studies have reported impaired skeletal muscle recovery in rats (2–4), mice (5, 6) and humans (7–9) following disuse. The concern is that the impaired recovery following muscle disuse events may lead to an acceleration of sarcopenia (10) thereby aiding the development of muscle and functional decline. Several theories as to why aged skeletal muscle recovery from disuse is impaired (2, 5) are just beginning to be investigated. Since macrophages have been demonstrated to have a pivotal role in muscle

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regrowth following disuse in young rodents (11–15) our hypothesis is that dysregulated macrophages (a response divergent from the young adult mouse) in aged skeletal muscle are also a contributing factor to the age-related impairment in muscle recovery following disuse.

Role of macrophages in skeletal muscle injury repair

Research involving rodents describes a complex and well-orchestrated series of events involving a host of different cell types (e.g., immune, satellite cells, endothelial) enable complete skeletal muscle regeneration from robust muscle injury (16, 17). Macrophages are immune cells found in skeletal muscle and they are essential for complete muscle regeneration (18, 19). This critical role has been demonstrated in great detail following macrophage depletion studies (20–22) and experiments blocking macrophage recruitment/migration (23–25) and activity (22–24, 26, 27). Macrophages exist under a spectrum of polarized states but, in general, can be characterized as either pro- (M1-like) or anti-inflammatory (M2-like). In response to injury, granulocytes rapidly migrate to the tissue within 48h. This is thought to be followed by a flux of monocytes/macrophages ($Ly6c^{+}$/$CCL2^{+}$/$CX3CR1^{low}$) and resident tissue macrophages (1–5 days), which function in a pro-inflammatory state commonly thought to remove debris, induce vascularization and stimulate proliferation and fusion of muscle satellite cells. Resident-tissue macrophages are not able to completely handle pronounced skeletal muscle damage and therefore, the circulatory monocytes/macrophages are recruited to muscle where they are differentiated to pro-inflammatory macrophages and potentially re-polarized to a different state later on during the recovery process (20). The pro-inflammatory response is resolved in the 3–7 days following injury by a polarization to macrophages with a predominantly anti-inflammatory phenotype ($Ly6c^{-}$/$CCL2^{-}$/$CX3CR1^{high}$) such that the majority of immune cells residing in muscle throughout the remainder of the recovery is anti-inflammatory, as the total number of macrophages begins to decline. These anti-inflammatory macrophages serve to dampen the initial inflammatory response and stimulate angiogenesis, collagen deposition, satellite cell differentiation and myofiber regrowth (28). A key function of monocytes and macrophages is to release cytokines and growth factors that work in a paracrine fashion to influence muscle regeneration and regrowth (13, 29). For a more comprehensive review of the role of macrophages during muscle injury and regeneration see (28, 30–32).

Muscle disuse vs muscle injury/regeneration models

When examining the role of these immune cells in skeletal muscle of rodents the important distinctions between research models used should be briefly highlighted. Extreme injury and regeneration models (i.e., myotoxin, crush, laceration, freeze, ischemia/reperfusion, eccentric exercise) are not equivalent (33) and some models are more damaging than others (i.e. ischemia/reperfusion is more damaging than myotoxic injury (34)). It is important to note that the damage response caused by ambulatory recovery from muscle disuse is several orders of magnitude less than extreme injury and regeneration models. The injury/regeneration models employ an insult that can ablate whole sections of muscle requiring formation of new myofibers followed by their regeneration. With disuse (e.g., hindlimb unloading, hindlimb immobilization), the myofibers remain intact, but experience myofiber atrophy, weakness and increased susceptibility to injury, especially with aging (35). For example, 2d of reloading after hindlimb unloading in young adult rodents resulted in ~2%

injured/necrotic myofibers, which receded to none being detectable at 4 days following reloading when only 1–2% of centrally-located regenerating myofibers are observed (11). Whereas following 1–4d post-toxin injury, ~10–75% of myofibers are injured/necrotic (20, 24) and typically >50% are myofibers classified as regenerative in the following few days (20, 24, 36). These responses are even more exaggerated following the ischemia/reperfusion injury model because it is more damaging than myotoxic injury (34). As a result, there are also drastic differences in the abundance of macrophages during reloading versus the injury models mentioned above as determined by flow cytometry (Figure 1). The monocyte/macrophage abundance in uninjured mouse tibialis anterior muscle was ~200 cells/mg muscle (20), at 4d post-toxin injury this amount increased in the same muscle to ~15,000 cells/mg muscle (20) and 3d post-ischemia-reperfusion increased to ~34,000 cells/mg muscle in the gastrocnemius (37). We demonstrate that the fast-twitch gastrocnemius or plantaris in young adult mice contained ~50 cells/mg muscle whereas the slow-twitch soleus had ~200 cells/mg in “uninjured” control muscle (38). After 4d of ambulatory recovery following 14d of hindlimb unloading, we observed a relatively minor increase of ~120 cells/mg muscle in the gastrocnemius and ~600 cells/mg muscle in the soleus (Figure 1). Although, the elevated macrophage number during recovery from disuse is far less that during regeneration, they still play an important role during the muscle regrowth process (see below).

Macrophages and recovery from muscle disuse

Myeloid cell responses during ambulatory recovery from muscle disuse (i.e., hindlimb unloading, hindlimb immobilization) have been well examined in young adult rodents (11, 12, 16, 39–41). Like the robust injury models, macrophages undergo a similar sequence of events following recovery from muscle disuse (16, 17, 40). Early studies using pharmacological approaches or genetic models (11–13, 15) have demonstrated the requirement of macrophages or their activity to maximize regrowth of myofibers during the recovery process from disuse in rodents. Indeed, depletion of circulating monocytes (13), all macrophages (11) or cytokines regulating macrophage function (12, 15) impair muscle recovery following disuse in young adult mice. Additionally, we have recently completed preliminary experiments demonstrating that CCL2 KO young adult mice have impaired muscle CSA recovery following disuse (Reidy PT and Drummond MJ, 2019 – unpublished). This further adds credence to the concept that macrophages recruitment are critical for maximum regrowth of muscle following disuse. Although, we have a more comprehensive understanding of the role of muscle macrophages in young adult rodents during recovery from disuse very little is understood in the context of aging skeletal muscle. In particular, growth factors produced by macrophages (e.g. IGF-1) are a key component of muscle regeneration (29) and regrowth (13). Since IGF-1 transcripts are attenuated in aging skeletal muscle following recovery from disuse (42), this could be a likely site of targeted immunotherapies.

Impact of aging on macrophages and muscle injury repair

Evidence suggests that aged tissues have a defective or delayed immune cell response under a variety of conditions (43), such as muscle regeneration following injury (43–46). In general, uninjured aging skeletal muscles have a predominance of macrophages

with a M2-like phenotype skewed toward the development of fibrosis (47, 48). Specific macrophage phenotype polarization (M1 vs M2-like) and time course following injury has not been thoroughly examined in aged skeletal muscle following injury with appropriate methodologies (i.e., flow cytometry and cell sorting), but it is clear that human and rodent aged skeletal muscle has a dysregulated immune cell response in comparison to young muscle. Qualitative histopathological assessments have suggested a delayed (49, 50) or heightened (50, 51) accumulation of pro-inflammatory cells in aged skeletal muscle following injury (49). Other reports indicate elevated and prolonged mRNA expression of pro-inflammatory mediators (in whole muscle) (52), excessive cytokine (53), neutrophil (44, 54, 55) and macrophage abundance (54, 55) in aged skeletal muscle following injury. For example, Patsalos et al. demonstrated a prolonged greater proportion of inflammatory $Ly6C^{high}$ macrophages and a reduced proportions of repair $Ly6C^{low}$ macrophages during recovery from acute sterile injury in old mice compared to young mice (56). The timing of these events from the studies listed above is unclear in aging and confounded by various injury models. Yet, taken together, these reports support a desynchronization of the immune cell response in aging skeletal muscle following injury compared to young in both human and rodent research.

Macrophages and the recovery of muscle tissue following disuse with aging

There are far fewer studies (two to date) describing muscle macrophage responses during the course of muscle disuse and recovery in aged animals. White et al. used a single M2-like macrophage marker, CD163+ cells (via IHC), and demonstrated a trend (non-significant) for young rat soleus muscle to increase the level of M2-like macrophages above both control and late (14d) recovery conditions following 14d of hindlimb unloading (4). Alternately, the old rat muscle showed greater CD163+ cells (vs young) at all time points. CD163+ cells decreased in the old with hindlimb unloading and only returned to baseline after 14d recovery while muscle regrowth remained impaired in aged animals.

Recently, we showed that macrophage recruitment and a shift in M1-like and M2-like macrophage proportions were dysregulated in aging mouse muscle during recovery from disuse in a muscle-specific manner (38). Using flow cytometry, the old gastrocnemius muscle demonstrated a higher level of pro-inflammatory monocyte ($Ly6c^{+}$ cells) infiltration that started during disuse and was maintained throughout the recovery phase. These data were accompanied by a greater C-C Motif Chemokine Ligand 2 (CCL2) mRNA and attenuated interferon regulatory factor 7 (IRF7) mRNA expression during recovery; the coded protein that is responsible to differentiate monocytes to macrophages (57). These data suggest that the old muscle may have an impaired ability to differentiate monocytes to macrophages. When examining the soleus muscle, even greater age-related impairments on macrophages and muscle regrowth were observed compared to the gastrocnemius. We found that the soleus at 4d recovery not only had impaired recruitment of monocytes and macrophages, but also lower abundance of M1-like macrophages. This finding was further confirmed with specific cytokine and growth factor gene expression on sorted CD45+CD11b+ monocytes/macrophage cells which suggested that the macrophages in the old were possibly not shifting their macrophage phenotype similar to young during this M1-like to M2-like transitional time point. Together, these data suggest an altered immune

cell response between old and young rodents during the disuse / recovery paradigm. More intensive studies are needed to confirm the role of muscle macrophages in aging following disuse, specifically in humans, while also examining immunotherapies to assist in muscle regrowth with aging.

Potential therapies to enhance muscle regrowth following muscle disuse in aging

Immunotherapy has more recently been sought as a route to optimize tissue regeneration (26) and regrowth. In brief, the most prominent immunotherapy techniques include: 1) delivery of ex-vivo activated macrophages or 2) delivery of molecules to alter macrophage abundance and/or phenotype. Intramuscular injection of macrophages into injured muscle has been shown to accelerate the regeneration/regrowth process in young adult animals (37, 58, 59). Macrophage immunotherapy has been successful in improving muscle recovery following ischemia-reperfusion (37, 58) and laceration (59) injury. Two studies to date have shown that injection of M1-like macrophages during the early inflammatory phase (58) and M2-like macrophages (37) at the peak of inflammation following injury improved muscle size and function. However, these studies were conducted in young rodents and in a model that does not mimic recovery from a disuse event. Interestingly, injection of macrophage colony-stimulating factor (M-CSF), a cytokine to promote recruitment, proliferation, and maturation of macrophages, into soleus muscle of young mice during disuse, resulted in faster muscle recovery (14). Indeed, our preliminary data (Reidy PT and Drummond MJ, 2019- unpublished) suggests that intramuscular injection of M-CSF (versus PBS) promoted greater regrowth of the soleus muscle of old mice after 2 weeks of reloading. One recent investigation has shown that immunotherapy via supplementation of a macrophage-derived cytokine, growth differentiation factor 3 (GDF3) could improve regeneration in old, but not young, mice following cardiotoxin injury (56). Therefore, these models of immunomodulation may have potential application to improve the recovery of aged skeletal muscle following disuse atrophy.

Immunomodulation may also be applied through cyclic compressive loading (CCL) in skeletal muscle such as massage (60) or even certain types of muscle contraction (61, 62). Massage improves recovery following eccentric exercise in rabbits by reducing edema and macrophage infiltration (63). We showed that the application of CCL to normal muscle facilitated a shift in the expression of genes in the immunity pathways, an increase in muscle macrophages, and a shift in the muscle macrophage pool towards the M2 phenotype, which is supportive of muscle growth (64). Cyclic compressive loading given during 8d recovery from 14d of hindlimb unloading improved recovery in skeletal muscle of young rats and observed a clear trend for elevated M2-like macrophage marker CD163 (65).

Certain types of exercise may also provide immunomodulation. Aerobic exercise in the form of voluntary wheel running (66), can enhance recovery following muscle disuse in young rodents. It is unknown if the improved muscle recovery following disuse with aerobic exercise is mediated via the effects of macrophages, but it is known that a single bout of aerobic exercise is capable of increasing M2-like macrophages in muscle (62). Moreover, an accumulation of muscle macrophages with aerobic exercise training has been associated with muscle growth (61). Future investigations should examine the potential

immunomodulatory impact of aerobic exercise on recovery from disuse atrophy in young and old skeletal muscle.

Conclusions

The reasons for the impaired muscle recovery from disuse with aging are still being explored and macrophages in skeletal muscle are one potential avenue of inquiry. It is clear that macrophage and immune cell responses in aged skeletal muscle are dyssynchronous when compared to young muscles following robust injury, although this is an area in need of further exploration. Using the more generalizable disuse and recovery/regrowth model, a dysregulated skeletal muscle macrophage response is also observed with aging (Figure 2). However, care should be taken when comparing the robust injury models (freeze, ischemia/reperfusion, cardiotoxin) to the disuse/reloading events as the former has a much greater amount of damage, immune cell infiltration, a longer immune response and likely a greater reliance on circulating immune cells than the latter. Future investigations need to utilize the tools of flow cytometry to better characterize the time course, phenotype and role of macrophages in regeneration and regrowth after disuse, and to determine the effectiveness of possible immunotherapies to enhance recovery of aged skeletal muscle.

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Key Point Summary:

- Muscle recovery (size) following muscle injury or a disuse period is impaired or delayed in rodents and humans.
- Macrophage timing and polarization are essential to the recovery process during regrowth
- In aged skeletal muscle, the timing of pro- and anti-inflammatory macrophages is dysregulated, during recovery from injury or disuse.
- Potential therapies such as macrophage replacement, immunomodulation, or exercise may optimize macrophage polarization patterns and possibly enhance muscle recovery in aged muscle

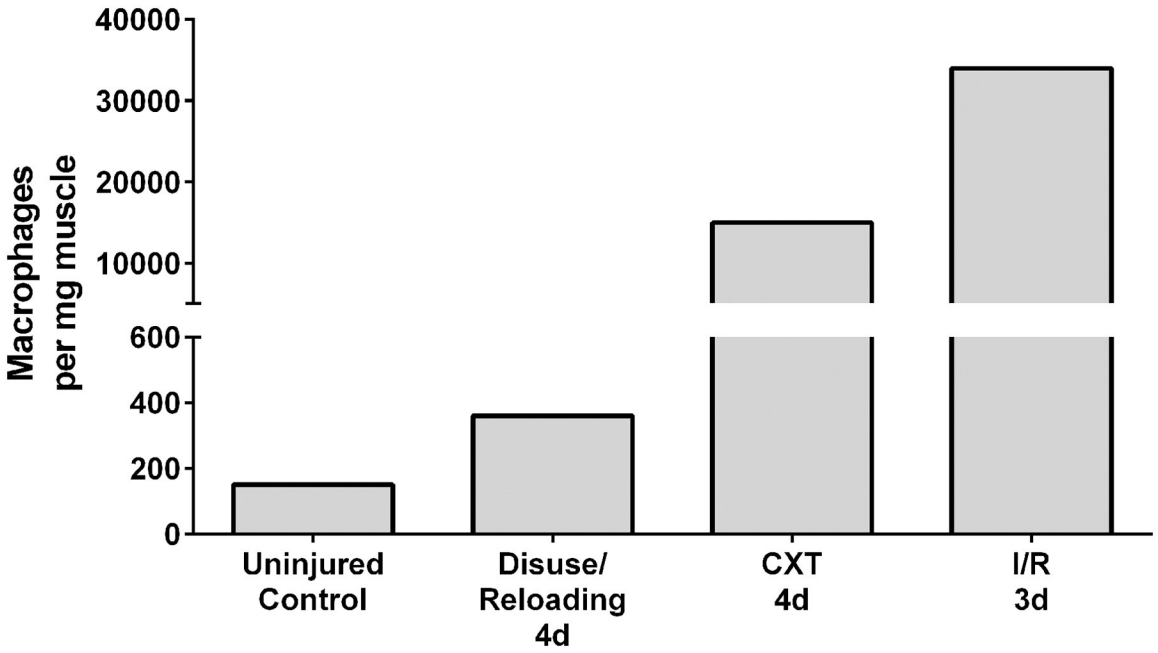

Figure 1.

Representation of differences in mouse macrophage abundance (using flow cytometry) in skeletal muscle from "uninjured" controls, following 4 days reloaded from disuse (38), 4 days post-CXT (cardiotoxin) (20) injection, and 3 days post-I/R (ischemia/reperfusion) (37). Uninjured control was an average of 200 cells/ per mg from the tibialis anterior (20) and our work demonstrating 50 and 200 cells/ per mg from the gastrocnemius and soleus, respectively (38). The muscle disuse/reloading 4d timepoint (representing 14d hindlimb unloading followed by 4d of recovery) was an average from our data (38) demonstrating 120 and 600 cells/ per mg from the gastrocnemius and soleus, respectively.

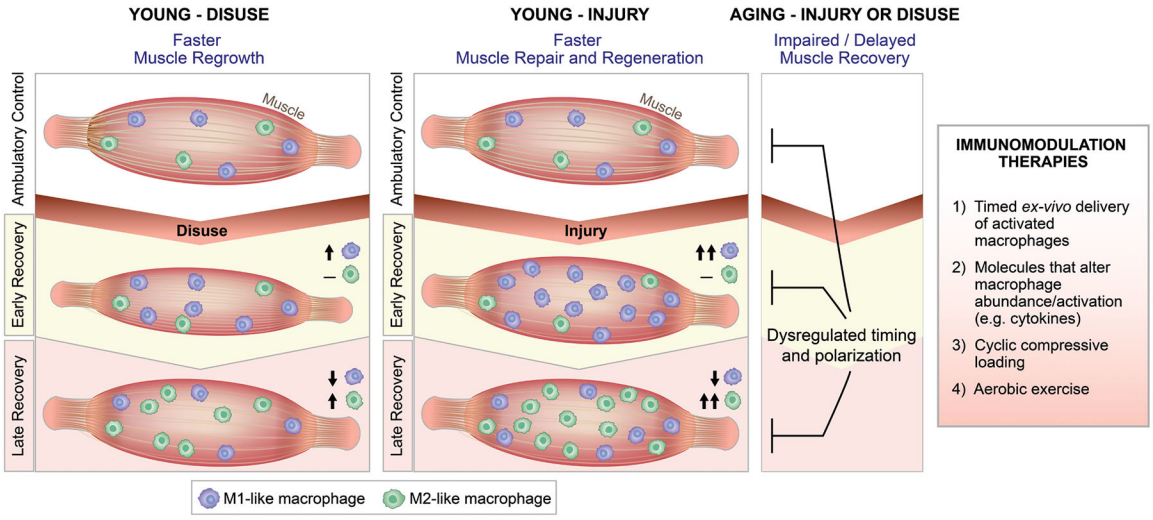

Figure 2.

Hypothetical macrophage immune cell response following muscle disuse or injury in young and old skeletal muscle. During the first few days during ambulatory recovery ("early recovery") following muscle disuse or injury in young adult rodents, the number of pro-inflammatory M1-like macrophages increase within the muscle tissue in order to remove debris and to facilitate the proliferation and fusion of muscle satellite cells. During the latter days of muscle recovery ("late recovery"), M1-like macrophages polarize into M2-like macrophages, thereby increasing the population of these anti-inflammatory macrophages (M2-like). M2-like macrophages serve to induce angiogenesis, collagen deposition, differentiation of satellite cells and myofiber regrowth. During recovery from disuse or injury, aged skeletal muscle is characterized by a dyssynchronous macrophage infiltration and polarization. It is hypothesized that evidence-based immunomodulatory therapies such as injection of activated macrophages or molecules that alter the activate or abundance of macrophages or application of massage or aerobic exercise may be effective to correct the timing and polarization of macrophages and ultimately aide in the restoration of muscle tissue from injury or disuse in aged muscle. An important distinction between the disuse/regrowth and injury/regeneration models is the amount of damage, inflammatory cells, and changes in muscle size. Regarding muscle size, the disuse/regrowth model undergoes atrophy and then regrowth while the injury model does not experience fiber atrophy, but growth of regenerated myofibers. Even with these differences both models require much further investigation using flow cytometry and cell sorting.