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Do inflammatory cells influence skeletal muscle hypertrophy?

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table of contents

1. ABSTRACT

Most research on muscle hypertrophy has focused on the responses of muscle cells to mechanical loading; however, a number of studies also suggest that inflammatory cells may influence muscle hypertrophy. Neutrophils and macrophages accumulate in skeletal muscle following increased mechanical loading, and we have demonstrated that macrophages are essential for hypertrophy following synergist ablation. Whether neutrophils are required remains to be determined. Non-steroidal anti-inflammatory drugs impair adaptive responses of skeletal muscle in both human and animal experiments suggesting that the routine use of such drugs could impair muscle performance. Much remains to be learned about the role of inflammatory cells in muscle hypertrophy, including the molecular signals involved in calling neutrophils and macrophages to skeletal muscle as well as those that regulate their function in muscle. In addition, although we have demonstrated that macrophages produce growth promoting factors during muscle hypertrophy, the full range of functional activities involved in muscle hypertrophy remains to be determined. Further investigation should provide insight into the intriguing hypothesis that inflammatory cells play integral roles in regulating muscle hypertrophy.

2. INTRODUCTION

A remarkable trait of skeletal muscle is its tremendous capacity for adapting to environmental cues. An example of this adaptive plasticity is the large increase in muscle mass that can be induced by mechanical loading. Resistance exercise is utilized to increase mechanical loading of human skeletal muscle and such exercise elicits responses that lead to muscle hypertrophy. Most research on muscle hypertrophy has concentrated on the responses of muscle cells to mechanical loading, and the pathways by which mechanical signals are transduced (1-5). However, a number of studies also suggest that non-muscle cell types, including inflammatory cells (e.g. neutrophils and macrophages), may contribute to muscle hypertrophy after mechanical loading.

Mechanical loading of skeletal muscle can initiate an inflammatory response, characterized by the accumulation of neutrophils and macrophages in skeletal muscle and the expression of various cytokines. Classically, the function of neutrophils and macrophages has been restricted to the removal of damaged tissue via phagocytosis. However, emerging evidence on their contribution to various physiological responses of skeletal muscle cells both *in vitro* and *in vivo*, indicate that neutrophils and macrophages play a

Table 1. Molecules produced by skeletal muscle cells that can influence inflammatory cells

Chemokines	Cytokines	Growth factors	Proteases
CXCL8 (IL-8)	IL-1α,β	HGF	MMP-2
CXCL9 (Mig)	IL-4	FGF	MMP-7
CXCL10 (IP-10)	IL-6	IGF-1	MMP-9
CCL2 (MCP-1)	TNFα	PDGF	uPA
CCL3 (MIP-1)	IFNγ	TGFβ	
CCL5 (RANTES)		VEGF	
CCL20 (MIP-3)		G-CSF	
CCL22 (MDC)		GM-CSF	
CX3CL1 (fractalkine)			

Molecules included are meant to be illustrative of possible factors secreted by muscle cells during muscle hypertrophy, not a comprehensive list of molecules that can be produced by muscle cells. Data obtained from various sources (17, 103, 104)

far more complex role in skeletal muscle than simply removing damaged tissue.

The objective of this paper is to review the existing literature on the role of neutrophils and macrophages in muscle hypertrophy. To this end, we will first provide a brief overview of the functional activities of neutrophils and macrophages and how these are regulated. This will serve as a foundation for understanding how neutrophils and/or macrophages could contribute to the regulation of skeletal muscle hypertrophy and how the environment of skeletal muscle could influence the function of neutrophils and/or macrophages. We will then proceed to the main focus of the review -- synthesizing data from contemporary studies that have investigated the roles of neutrophils and/or macrophages in models of muscle hypertrophy, as well as other models that may provide additional insight.

3. INFLAMMATION VERSUS INFLAMMATORY RESPONSES IN SKELETAL MUSCLE

Inflammation is typically defined as a response to injury and/or infection characterized by symptoms such as redness, heat, swelling, pain and dysfunction of the organs/tissues involved (6). Physiological responses associated with inflammation include dilatation and increased permeability of blood vessels, increased blood flow, exudation of fluid, and leukocyte migration to the area of injury or infection (7). The terms "inflammation" and "inflammatory response" are widely used in clinical and research settings, and are often used interchangeably. In addition, evidence of an inflammatory response is usually assumed to indicate a response to overt injury. However, emerging evidence indicates that in skeletal muscle, cellular events associated with the inflammatory response, namely those associated with nonspecific (innate) immunity, can occur in the absence of overt injury (8, 9). Such findings have led to questions about the functions of such an inflammatory response, since it occurs without the symptoms typically associated with inflammation.

Furthermore, clinical doctrine suggests that the inflammatory response is detrimental to tissue structure and function and the most common treatment for musculoskeletal injuries is "anti-inflammatory" drugs or modalities in an attempt to alleviate the associated pain/discomfort, swelling, and dysfunction (10). However, recent studies have demonstrated that the inflammatory response may be required for efficient skeletal muscle repair and adaptation and have begun to shed doubt on this dogmatic approach for dealing with inflammation (11-16).

4. Neutrophils and macrophages: what are theIR POSSIBLE FUNCTIONS in Skeletal MUSCLE HYPERTROPHY?

Neutrophils and monocytes/macrophages are inflammatory cells that develop in the bone marrow and are released into the circulation to serve as sentinels of the innate immune system. A variety of molecules can be released from cells residing in skeletal muscle (e.g., skeletal muscle cells, endothelial cells, macrophages and mast cells) that can call inflammatory cells into action by promoting their migration to and within skeletal muscle after mechanical loading (Table 1) (17-19). For example, CXC chemokines (e.g. IL-8, GROalpha,beta) are potent neutrophil chemoattractants, and CC chemokines (e.g. MCP-1, MIP-1alpha,beta) are potent monocyte/macrophage chemoattractants (20, 21). Upon their arrival in skeletal muscle, neutrophils and macrophages could influence muscle hypertrophy via their capacity to perform phagocytosis, and to produce free radicals, cytokines and growth factors.

4.1. Phagocytosis.

Neutrophils and monocytes/macrophages are well known for their ability to perform phagocytosis, and are often referred to as phagocytic cells. Phagocytosis refers to endocytic engulfment of cells and debris, and is thought to be the primary means by which damaged tissue is removed from injured muscle (22, 23). Phagocytosis can be triggered by ligation of membrane receptors, including Fc, complement, scavenger, oxidized LDL, and fibronectin receptors, and can be modulated by different cytokines (24-26). Possible roles of phagocytosis in muscle hypertrophy include removal of damaged extracellular matrix as well as the removal of damaged, necrotic and/or apoptotic cells from skeletal muscle.

Neutrophils and macrophages are also capable of other functions, including the regulation of other cells by generating free radicals, producing numerous cytokines and growth factors, and by cell-cell contacts. These functions can occur in concert with phagocytosis or independently from phagocytosis. Interestingly, phagocytosis of apoptotic cells can modify the function of mononuclear phagocytes, turning "inflammatory" monocytes/macrophages (those that produce high levels of TNFalpha, IL1beta and iNOS) into a "non-inflammatory" phenotype (those that produce high levels of arginase and TGFβ and lower levels of TNFalpha, IL1beta and iNOS) (25, 27). The ability of neutrophils and macrophages to perform functional activities that are not contingent upon phagocytosis indicates that these cells could do more in skeletal muscle than simply remove tissue debris.

Table 2. Molecules produced by neutrophils and macrophages that could influence skeletal muscle hypertrophy

Chemokines	Cytokines	Growth factors	Proteases
CXCL1 (GROα)	IL-1α,β	HGF	MMP-1
CXCL5 (ENA-78)	IL-4	FGF	MMP-2
CXCL8 (IL-8)	IL-6	IGF-1	MMP-7
CXCL9 (Mig)	IL-12	PDGF	MMP-9
CXCL10 (IP-10)	TNFα	TGFβ	uPA
CCL2 (MCP-1)	IFNα, γ	VEGF	
CCL3 (MIP-1α)	IL-10	G-CSF	
CCL4 (MIP-1β)	IL-1ra	GM-CSF	
CCL5 (RANTES)			
CCL20 (MIP-3)			
CCL22 (MDC)			
CX3CL1 (fractalkine)			

Molecules included are meant to be illustrative of possible factors secreted by neutrophils during muscle hypertrophy, not a comprehensive list of molecules that can be produced by neutrophils. Data obtained from various sources (20, 44-48).

4.2. Free radicals.

Both neutrophils and monocytes/macrophages are capable of producing free radicals such as superoxide, hydrogen peroxide, hypochlorous acid, and hydroxyl radical (28-30). Although it is well known that inflammatory cells of mice and other species can produce nitric oxide, whether human inflammatory cells produce this radical remains controversial (31-33). Neutrophils and monocytes/macrophages differ in their capacity to produce free radicals; human blood neutrophils produce substantially more oxygen radicals compared to monocytes and macrophages (28, 30). In addition, hypochlorous acid production from macrophages is lower relative to monocytes and neutrophils because of the reduction in myeloperoxidase that occurs during macrophage differentiation (29).

The production of free radicals by inflammatory cells in skeletal muscle could have multiple functions. In the context of phagocytosis, free radicals released into the phagolysosome aids in the degradation of endocytosed material. In addition, the release of free radicals from inflammatory cells into the extracellular fluid may also cause “collateral damage” to adjacent healthy tissue (34, 35). Indeed, free radicals produced by inflammatory cells are known to damage different cell types, including skeletal muscle cells (36-38). Downstream products of hydrogen peroxide (e.g., hypochlorous acid and hydroxyl radical) appear to be most injurious to differentiated skeletal muscle cells (36).

In addition to the destructive role of free radicals, they could serve as signals to trigger redox-sensitive physiological processes (39). Free radicals have been shown to induce cell signaling in skeletal muscle and have been suggested to contribute to beneficial adaptations induced by exercise (40). Interestingly, transgenic mice with reduced levels of selenoproteins exhibit enhanced muscle hypertrophy (41). Many selenoproteins, including glutathione peroxidase, are potent antioxidants, and one mechanism by which reduced selenoprotein levels could enhance muscle hypertrophy is through increased activity of redox sensitive signaling pathways. Additionally, inhibition of nitric oxide synthesis inhibited muscle hypertrophy in rats, suggesting that nitric oxide promotes muscle growth in response to mechanical loading (16, 42, 43). These data indicate that redox signaling may be important during muscle hypertrophy.

4.3. Cytokines and growth factors.

Neutrophils and monocytes/macrophages can produce a vast array of cytokines and growth factors which have multiple physiological actions (Table 2) (44-48). The capacity for cytokine production varies considerably between neutrophils and monocytes/macrophages. Depending on the cytokine, human monocytes possess 10-20 times more RNA per cell and synthesize 10-300 fold more cytokine than neutrophils (48). This evidence serves as a basis for the idea that macrophages are a primary source of cytokines during tissue inflammatory responses. However, other non-inflammatory cell types that are found in skeletal muscle (e.g., endothelial cells, fibroblasts, and skeletal muscle cells) can also produce cytokines and little is known about the cellular sources of cytokines during mechanical loading.

Some cytokines produced by neutrophils and macrophages amplify the inflammatory response by either inducing the expression of leukocyte adhesion molecules on endothelial cells (e.g., TNFalpha, IL-1beta and IFNgamma), causing migration of neutrophils (e.g., IL-8 and GROalpha,beta) and monocytes (e.g., MCP-1 and MIP-1), or by stimulating hematopoiesis (e.g., G-CSF and GM-CSF) . Inflammatory cells can also produce IL-1 receptor antagonists (IL-1ra) and IL-10 that may contribute to the resolution of the inflammatory response.

Many factors produced by inflammatory cells are also known to have biological functions that are not directly related to the inflammatory response per se. For example, a number of growth factors (e.g. IGF-1, FGF, HGF, VEGF, TGFbeta) influence the proliferation, migration and metabolism of different cells, including those that contribute to muscle hypertrophy. Indeed, soluble factors produced by monocytes/macrophages are known to induce proliferation and differentiation of skeletal muscle cells (49-51). However, the identity of the soluble factor(s) involved has not been determined, and the majority of factors produced by inflammatory cells have not been studied for an influence on skeletal muscle cells. Furthermore, little is known about the factors that are produced by inflammatory cells *in vivo*, particularly during muscle hypertrophy.

4.4. Regulation of inflammatory cell activity.

Functional activities of inflammatory cells are regulated via complex cell-signaling events initiated and/or modulated by a vast array of molecules. Many of these molecules can be produced by skeletal muscle cells (Table 1). Different molecules can prime or activate neutrophils or macrophages for different functional activities, or induce their deactivation. Priming agents generally do not induce functional activity by themselves, but instead enhance activity of cells exposed to an activating agent. For example, IL-8, TNF-alpha, GM-CSF or HGF prime neutrophils for free radical production when they are stimulated with a bacterial peptide (e.g., fMLP) or when they are adherent to

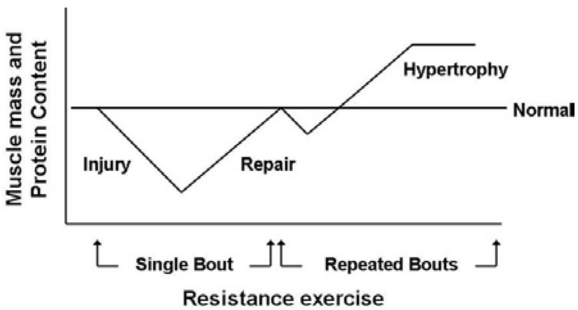

Figure 1. Adaptive responses of skeletal muscle to resistance exercise. An initial bout of resistance exercise can lead to muscle injury, especially if it involves high-force eccentric contractions. Injury typically leads to a decrease in muscle mass and protein, and is followed by a repair response that restores muscle mass and protein. Repeated bouts of the same resistance exercise elicit less injury and loss of protein, and eventually results in increased muscle mass and protein.

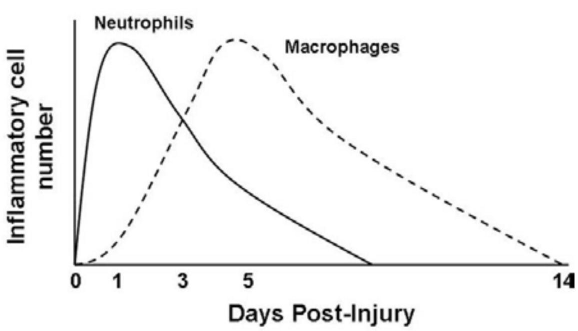

Figure 2. Skeletal muscle injury or increased mechanical loading results in accumulation of neutrophils and macrophages. Neutrophil accumulation is evident within hours and peaks around 1 day following the initiating event. Macrophage accumulation typically occurs later, reaching a peak when neutrophil levels are decreasing.

extracellular matrix proteins (52, 53). Also, exposure of macrophages to low levels of IFNgamma enhances production of free radicals and "inflammatory" cytokines when activated by higher levels of IFNgamma, TNFalpha or IL-1beta (27). In addition, deactivating agents can induce signaling that leads to decreased functional activity. For example, IL-10 reduces free radical production and production of "inflammatory" cytokines in both neutrophils and macrophages (54, 55). Many of these priming, activating or deactivating agents can be produced by neutrophils and macrophages (Table 2) in addition to muscle cells, and products released by one cell type can influence the functional activity of the others. Thus, the cells and molecules present in the micro-environment of skeletal muscle after mechanical loading likely dictate whether phagocytosis, free radical production and/or cytokine release is performed. Much has to be learned about the factors produced in skeletal muscle during muscle hypertrophy to better understand how the environment of skeletal muscle after mechanical loading regulates functional activities of inflammatory cells.

Monocytes and macrophages are renowned for their phenotypic diversity, and great strides have been made recently in understanding how the diverse functions of these cells are regulated. Classical activation of macrophages is induced by IFNgamma and leads to the production high levels of "inflammatory" cytokines (e.g. TNFalpha, IL-1beta), reactive oxygen species, and iNOS (27, 55). Alternative activation of macrophages is induced by IL-4 and IL-13, and leads to production high levels of certain growth factors (e.g. PDGF, TGFbeta, IGF-1) and arginase instead of "inflammatory" cytokines. In conditioned medium experiments, classically activated macrophages enhanced proliferation of primary myoblasts, but did not stimulate proliferation of muscle fibroblasts (49-51). In other experiments, alternatively activated macrophages stimulated myoblast differentiation (49). Since satellite cells may contribute nuclei to muscle fibers undergoing hypertrophy, macrophage stimulation of satellite cell proliferation may play a role in this process. However, the factors that influence macrophage activation during muscle hypertrophy, and whether classically or alternatively activated macrophages predominate, remain to be determined.

5. EVIDENCE FOR A Role of inflammatory cells in Skeletal Muscle Hypertrophy

Resistance exercise can elicit responses in skeletal muscle that can be categorized as those associated with injury, repair/regeneration, and/or hypertrophy (Figure 1). Resistance exercises that emphasize eccentric contractions are more likely to cause muscle injury than those that emphasize concentric contractions. Muscle injury, characterized by altered sarcolemma permeability, cytoskeletal disruption, and muscle dysfunction, is usually associated with reductions in protein content of injured muscles (56, 57). Cellular and molecular events associated with muscle repair serve to restore muscle protein content and the normal structure and function of injured muscles. With repeated bouts of resistance exercise (i.e. training), the magnitude of the injury induced by each successive bout is reduced, and skeletal muscle eventually adapts by increasing mass and force production (58-60). Whether overt injury is required to induce hypertrophy has been questioned, as progressively increased mechanical loading is less likely to induce injury and can still produce gains in strength (61).

Muscle injury and repair are accompanied by an inflammatory response characterized by the accumulation of neutrophils and macrophages (Figure 2). We and others have also reported the accumulation of neutrophils and macrophages in models of hypertrophy. The focus of this section is the evidence for neutrophil and macrophage accumulation in skeletal muscle after mechanical loading and how these cells influence muscle injury, repair, and hypertrophy.

5.1. Evidence from human studies.

There are scant data in the literature on the role of inflammatory cells in human skeletal muscle hypertrophy. A few studies have examined the accumulation of inflammatory cells in human skeletal muscle after a bout of resistance exercise. Previous investigators have reported an elevation in the concentration of neutrophils in human skeletal muscle in the hours to days after resistance exercise (62-66) and downhill running (67, 68). In these studies, neutrophil accumulation was measured using either radioactively labeled cells introduced into the blood or immunohistochemical detection of neutrophil markers (e.g. CD15, myeloperoxidase). Other studies using immunohistochemistry reported no evidence of neutrophil accumulation following eccentric exercise (65, 69, 70). These conflicting observations may be attributable to differences in exercise protocols, sampling time points, and/or specific techniques used to quantify neutrophils in skeletal muscle (71).

Macrophages have been more consistently reported to be elevated in the days following resistance exercise. In these studies, macrophage accumulation has been measured using immunohistochemical detection of different macrophage markers. Following resistance exercises involving eccentric contractions, CD68+ macrophages were increased in the quadriceps muscle group at 24 hours after exercise (65, 66, 70). Following standard weight training exercises involving both eccentric and concentric contractions, CD11b+ and CD163+ macrophages increased at 3 days after exercise in young but not old men, and the majority of macrophages found in muscles were CD163+ (72). The authors associated CD11b+ and CD163+ macrophages with classically and alternatively activated macrophages, respectively. These data highlight the possibility that both classically and alternatively activated macrophages may play roles in hypertrophy.

The kinetics of neutrophil and macrophage accumulation in human muscle after injury is experimentally difficult to ascertain. When using immunohistochemical assessments of inflammatory cells in muscle biopsies, either large numbers of subjects are required for cross-sectional studies, or multiple biopsies are required for longitudinal studies. The biopsy procedure itself can elicit an inflammatory response (69), and data from studies in which multiple biopsies have been obtained from the same muscle must be interpreted with caution. However, other studies have utilized contralateral muscles for non-exercised controls, and thus one can be more confident that the changes in inflammatory cell numbers observed were in fact due to the exercise protocol. In addition, the biopsied sample represents a very small fraction of a whole muscle and the reported large variability in quantifying macrophages in biopsied muscles (73) contribute to the difficult in determining the kinetics of inflammatory cell accumulation in human muscle after resistance exercise.

Studies on the influence of anti-inflammatory drugs on skeletal muscle responses to resistance exercise have produced intriguing results. Ibuprofen is routinely prescribed to treat symptoms of inflammation following exercise, and has been found to impair prostaglandin production following resistance exercise, and to blunt the increase in protein synthesis that is normally induced by such exercise (14, 74). Ibuprofen had no effect on inflammatory cell accumulation following resistance exercise, but whether their functional activity was impaired by ibuprofen treatment was not determined (70). In a prior study, 5 days of ibuprofen pre-treatment inhibited the ability of blood neutrophils to produce free radicals prior to eccentric contractions; however, continued dosing failed to blunt blood neutrophil responses (75). Another anti-inflammatory drug, indomethacin, was shown to reduce satellite cell activity following distance running (76); however, similar data has not yet been reported for resistance exercise. In total, although the mechanisms have yet to be elucidated, human studies indicate that use of non-steroidal anti-inflammatory drugs may be detrimental to the adaptive response to resistance exercise. Future studies will need to determine whether non-steroidal anti-inflammatory drugs inhibit functional activities of neutrophils and/or macrophages and/or other components of the inflammatory response within skeletal muscle.

5.2. Evidence from animal models of skeletal muscle injury.

A number of studies have investigated the role of neutrophils and macrophages in different animal models of skeletal muscle injury. These models involve mechanical loading (e.g. eccentric contractions (8, 77-79) or reloading of atrophic muscle (80-82), chemical injury (57, 83, 84) or crush or freeze trauma (85, 86) in mice, rats and rabbits. Despite differences in the nature, kinetics, and magnitude of the injury, studies using the aforementioned models demonstrate that neutrophils and/or macrophages accumulate in the hours to days after the injury.

The time course of neutrophil and macrophage accumulation in skeletal muscle injured by mechanical loading (or otherwise) resembles the classic response to infection. Specifically, we and others have reported that neutrophils begin to accumulate in skeletal muscle within 2 h, reach their peak concentration at 1 d, and return to control levels within 7 d after injury induced by either controlled eccentric contractions or mechanical loading of atrophic muscle (8, 9, 79-82, 87) (Figure 2). Macrophages on the other hand, generally appear in injured skeletal muscle after the arrival of neutrophils and remain elevated while neutrophil concentrations are diminishing. Mast cells, which reside in connective tissue of skeletal muscle, may play a role in regulating the accumulation of neutrophils and macrophages following mechanical loading. Inhibition of mast cell degranulation had a modest effect in reducing neutrophil accumulation during reloading of atrophic muscle, but resulted in increased accumulation of macrophages (88).

Recent studies have attempted to manipulate neutrophil and macrophage function in an effort to determine their role in muscle injury and repair. Some of these studies indicate that inflammatory cells can exacerbate mechanically-induced muscle injury. In a recent study, we used mice with a hypomorphic allele for CD18, which is part of the Mac-1 receptor complex used by neutrophils to adhere to endothelial cells during tissue infiltration (79). Following an eccentric contraction protocol, CD18 mutant (CD18-/-) mice exhibited reduced neutrophil accumulation in damaged muscle at 1 and 3 days post-injury, but no change in macrophage accumulation compared to wild-type mice (79). The reduced neutrophil accumulation was associated with reduced muscle force deficits, decreased morphological damage and decreased carbonyl formation, a marker of oxidative stress. Similarly, systemic treatment using an antibody against CD11b, another component of the Mac-1 receptor complex, reduced neutrophil accumulation in muscle subjected to an eccentric contraction and reduced morphological evidence of damage (77). In another study, neutrophils from mice deficient in gp91phox (NOX-2) were less potent killers of muscle cells *in vitro* and these knockout mice exhibited reduced muscle damage during mechanical loading of atrophic muscle compared to wild-type mice (89). NOX-2 is a subunit of NADPH oxidase which produces superoxide radicals. Although NOX-2 can be expressed by many different cell types, these data are also consistent with a role for neutrophils in promoting muscle damage via oxygen radicals. In addition to neutrophils, macrophages have been reported to injure muscle cells *in vitro* (37). However, this did not occur during reloading of atrophic muscle *in vivo* (81). Taken together, the evidence suggests that neutrophils can exacerbate damage to injured skeletal muscle *in vivo*. Whether any damage that may be induced by inflammatory cells influences muscle hypertrophy following mechanical loading remains to be determined.

Emerging evidence indicates that neutrophils and macrophages can also influence the restoration of normal structure and function in skeletal muscle following injury. We have compared markers of muscle repair in wild-type and CD18-/- mice after contraction-induced muscle injury (79). To our surprise, reduced neutrophil accumulation in muscle of CD18-/- mice was associated with faster restoration of muscle force production. Our functional observations were corroborated by a higher myofiber expression of embryonic myosin heavy chain (a marker of regeneration), and a larger cross-sectional area of regenerating myofibers in CD18-/- relative to wild type mice. These preliminary observations indicate that neutrophils may delay some of the events associated with restoring structure and function to skeletal muscle injured by exercise. In contrast, mice treated with an antibody against the antigen Gr-1 demonstrated reduced accumulation of both neutrophils and monocytes/macrophages following chemically-induced muscle injury (84). In this study, reducing both neutrophil and macrophage accumulation resulted in deficient muscle repair.

Further studies have confirmed a role for macrophages in promoting muscle repair following different types of injury. Treatment of muscle transplants with macrophage inflammatory protein (MIP)-1beta increased macrophage infiltration and satellite cell activity in these transplants, and when the host was treated with radiation, regeneration was impaired in the transplant (90). Although these data are suggestive of important roles for macrophages in muscle repair, both MIP-1beta and irradiation may have non-specific effects on different cell types in addition to macrophages. Liposomes containing clodronate have been used extensively as a method for selectively depleting macrophages *in vivo* and have been used to study repair of different tissues. Macrophage depletion using this method resulted in impaired muscle regeneration following injury induced by freeze damage or by cardiotoxin (91, 92). In addition, transgenic mice have been developed that allow specific, conditional ablation of macrophages (93). These mice express the human diphtheria toxin (DT) receptor under control of the CD11b promoter; the mouse form of the receptor binds DT poorly, and expression of the human form makes macrophages sensitive to killing by DT in these mice. Macrophage depletion in these mice resulted in impaired healing following notexin injection (49). Finally, in a recent study focused on muscle reloading following hindlimb suspension, repeated injections of an anti-F4/80 antibody resulted in decreased macrophage accumulation at 4 days, but not 2 days after reloading, and appeared to impair the regenerative response during reloading (94). In total, these studies demonstrate that macrophages are required for efficient muscle repair following injury.

5.3. Evidence from animal models of non-injurious mechanical loading and hypertrophy.

We reported the surprising findings that inflammatory cells accumulate in skeletal muscle after mechanical loading that did not result in overt muscle injury. Controlled passive stretches and isometric contractions are generally thought not to induce muscle injury, and we found no evidence of overt muscle fiber damage following such manipulations of the mouse extensor digitorum longus muscle (8). However, we did observe accumulation of neutrophils following both passive stretches and isometric contractions at a level that was approximately one-half of the concentration observed after injurious eccentric contractions. We extended these observations by reporting that controlled concentric contractions of rat soleus and plantaris muscles elevated both neutrophil and macrophage concentrations in the absence of histological abnormalities (9). Repeated bouts of exercise using this rat model has been demonstrated to induce muscle hypertrophy (58-60), leading to the question of whether inflammatory cells played any role in the hypertrophic response.

In other animal models of skeletal muscle hypertrophy, there is also evidence of an inflammatory response in skeletal muscle in the absence of overt muscle injury. Surgical removal of all but one muscle of a functional group in the rat or mouse hindlimb increases the mechanical loading on the remaining synergistic muscle and can produce a doubling of muscle mass and protein content within two weeks (41, 95-97). In this synergist ablation model of muscle hypertrophy, increased mechanical loading of the plantaris muscle promoted the vascular phase of the inflammatory response as indicated by an increase in muscle water content (edema) during 24 hours of muscle loading (98). The majority (95%) of the increased muscle mass observed throughout 24 hours of muscle loading was attributable to edema and not to increased protein mass.

A number of studies also reported evidence of inflammatory cell accumulation during synergist ablation-induced muscle hypertrophy. In histological observations made using light microscopy, muscle hypertrophy has been associated with accumulation of nuclei in the muscle interstitium, epimysium, and interfasicular spaces (98). Although the authors suggested that these nuclei predominantly belong to neutrophils, other cell types could

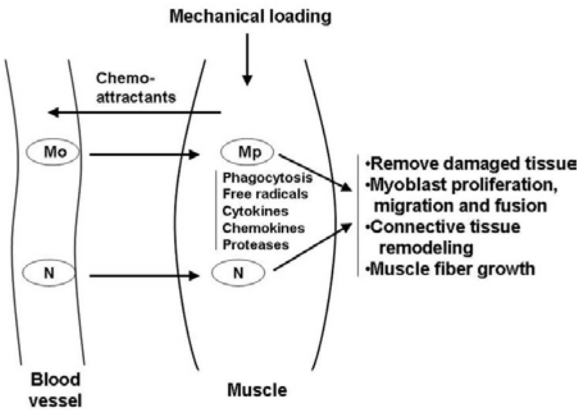

Figure 3. Model of neutrophil and macrophage activities during muscle hypertrophy. Increased mechanical loading is thought to induce release of chemoattractants that call neutrophils and monocytes from the blood into the muscle. After their arrival, muscle-derived factors can further regulate the functional activities of neutrophils and monocytes/macrophages. In addition, neutrophil and monocyte/macrophage derived factors can either promote or inhibit the further accumulation of inflammatory cells. Functional activities such as phagocytosis, release of free radicals, cytokines, chemokines, growth factors and proteases can promote repair responses following mechanical load-induced injury as well as adaptive process that are required for muscle hypertrophy.

not be excluded. In observations made using electron microscopy, muscle hypertrophy has also been associated with an increase in macrophages and fibroblasts, although the increase in these cells was not quantified (99). Finally, immunohistochemical analysis of macrophage-specific antigens indicated that both ED1+ and ED2+ macrophages, subpopulations of rat macrophages, were increased during muscle hypertrophy (16, 100). Differences in opinion have existed as to whether the accumulation of inflammatory cells is due to surgical procedures or to increased mechanical loading following synergist ablation. In our experiments and others, sham surgery did not induce accumulation of inflammatory cells, indicating that increased mechanical load was likely responsible for the accumulation of inflammatory cells (16, 95, 99).

Data from studies using anti-inflammatory drugs are consistent with a role for inflammatory cells in muscle hypertrophy. Administration of ibuprofen resulted in reduced muscle hypertrophy in rats following synergist abation (16) and a specific cyclooxygenase-2 inhibitor reduced muscle recovery following atrophy in mice (101). These data are also consistent with similar studies showing that anti-inflammatory drugs impair muscle repair following injury (11-16). Although these studies indicate that inflammatory cells may be involved in muscle hypertrophy, the drugs utilized may affect many different cells, including muscle cells.

We recently tested the hypothesis that macrophages are required for muscle hypertrophy using the synergist ablation model (95). In our studies, we first tested the hypothesis that the urokinase type plasminogen activator (uPA) promotes muscle hypertrophy in part by promoting macrophage accumulation following synergist ablation. uPA has been shown to be required for macrophage accumulation in models of muscle repair (57, 102), likely through effects on macrophage migration. Using mice deficient in uPA and the synergistic ablation model, we found that uPA promotes macrophage accumulation during muscle hypertrophy and is essential for hypertrophy.

Next, we tested the hypothesis that macrophages are required for muscle hypertrophy (95). We used clodronate liposomes to specifically deplete macrophages in wild-type mice and found that clodronate liposomes had the intended effect of sustained reduction in macrophage accumulation, but also produced a delayed, transient reduction in neutrophil accumulation. We also found that treatment with clodronate liposomes blunted the hypertrophic response. We interpreted these data to indicate that macrophages are required for compensatory hypertrophy. Whether neutrophils directly influence muscle hypertrophy remains to be determined. We also reported that macrophages isolated from muscle during hypertrophy expressed factors that are known to stimulate muscle growth, including uPA and IGF-1. The full range of factors produced by macrophages during muscle hypertrophy remains to be determined.

6. SUMMARY/CONCLUSIONS

Neutrophils and macrophages perform many functions that could contribute to physiological processes required during muscle hypertrophy, including phagocytosis, production of free radicals, cytokines and growth factors. The literature indicates that neutrophils and macrophages accumulate in skeletal muscle following increased mechanical loading, and the precise functions of these inflammatory cells during hypertrophy are likely dictated by the micro-environment of the muscle induced by mechanical loading (Figure 3). Macrophages are required both for efficient muscle repair following injury, and for muscle hypertrophy during the adaptation to chronic loading. Whether neutrophils are required for hypertrophy remains to be determined. Non-steroidal anti-inflammatory drugs have been shown to impair the adaptive response of skeletal muscle to resistance exercise in both human and animal experiments suggesting that the routine use of such drugs could impair muscle performance. Much remains to be learned about the role of the inflammatory response in muscle hypertrophy, including the molecular signals involved in calling neutrophils and macrophages to skeletal muscle following mechanical loading as well as those that regulate their function after their arrival in the muscle. In addition, although we have demonstrated that macrophages produce growth promoting factors during muscle hypertrophy, the full range of neutrophil and macrophage functions involved in muscle hypertrophy remains to be determined. Further investigation into these questions will provide insight into the intriguing hypothesis that the inflammatory response plays an integral role in regulating muscle hypertrophy.

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