Inflammatory processes in muscle injury and repair

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  Tidball, James G. Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol* 288: R345–R353, 2005; doi:10.1152/ajpregu. 00454.2004.—Modified muscle use or injury can produce a stereotypic inflammatory response in which neutrophils rapidly invade, followed by macrophages. This inflammatory response coincides with muscle repair, regeneration, and growth, which involve activation and proliferation of satellite cells, followed by their terminal differentiation. Recent investigations have begun to explore the relationship between inflammatory cell functions and skeletal muscle injury and repair by using genetically modified animal models, antibody depletions of specific inflammatory cell populations, or expression profiling of inflamed muscle after injury. These studies have contributed to a complex picture in which inflammatory cells promote both injury and repair, through the combined actions of free radicals, growth factors, and chemokines. In this review, recent discoveries concerning the interactions between skeletal muscle and inflammatory cells are presented. New findings clearly show a role for neutrophils in promoting muscle damage soon after muscle injury or modified use. No direct evidence is yet available to show that neutrophils play a beneficial role in muscle repair or regeneration. Macrophages have also been shown capable of promoting muscle damage in vivo and in vitro through the release of free radicals, although other findings indicate that they may also play a role in muscle repair and regeneration through growth factors and cytokine-mediated signaling. However, this role for macrophages in muscle regeneration is still not definitive; other cells present in muscle can also produce the potentially regenerative factors, and it remains to be proven whether macrophage-derived factors are essential for muscle repair or regeneration in vivo. New evidence also shows that muscle cells can release positive and negative regulators of inflammatory cell invasion, and thereby play an active role in modulating the inflammatory process. In particular, muscle-derived nitric oxide can inhibit inflammatory cell invasion of healthy muscle and protect muscle from lysis by inflammatory cells in vivo and in vitro. On the other hand, muscle-derived cytokines can signal for inflammatory cell invasion, at least in vitro. The immediate challenge for advancing our current understanding of the relationships between muscle and inflammatory cells during muscle injury and repair is to place what has been learned in vitro into the complex and dynamic in vivo environment.

  skeletal muscle; macrophage; neutrophil; muscle injury; muscle inflammation

MODIFIED MUSCLE USE or injury typically initiates a rapid and sequential invasion of muscle by inflammatory cell populations that can persist for days to weeks, while muscle repair, regeneration, and growth occur. This relationship between inflammation and muscle repair or regeneration has suggested that they may be mechanistically related and provides the basis for teleological arguments that muscle inflammation after modified muscle use is a functionally beneficial response. However, experimental observations have only recently begun to test that expectation and to distinguish between features of muscle inflammation that promote injury and those that promote growth or repair. As a more mechanistic understanding of the relationship between muscle and inflammatory cells is attained, the interactions are found to be increasingly complex and not wholly beneficial for muscle.

In this review, recent findings are presented that enable us to distinguish between inflammatory processes that disrupt muscle homeostasis and processes that promote muscle repair and regeneration after modified muscle use or injury. In addition, current information concerning muscle-derived mediators that promote or inhibit the invasion of inflammatory cells are discussed. The general picture that emerges from these studies is that neutrophils and macrophages dominate a basic inflammatory response in muscle that follows modified use or injury. Thus far, experimental data show that neutrophils promote muscle damage after modified use, but experimental evidence for a neutrophil function in muscle repair has not yet been attained. Macrophages can promote either muscle injury or proliferation in vitro, although their role in vivo after muscle injury remains poorly understood. Collectively, recent findings show that whether the inflammatory process has an overall beneficial or detrimental effect on muscle function is influenced by the magnitude of the response, the previous history of muscle use, and possibly

injury-specific interactions between muscle and the invading inflammatory cells.

NEUTROPHILS CAN PROMOTE MUSCLE INJURY IN VIVO

Neutrophils are rapid invaders of muscle after increased muscle loading or acute injury. Within 1 h of increased muscle use, neutrophil invasion begins (8, 23), after which their concentrations can remain elevated for periods as long as 5 days (23). Morphological observations indicate that the invading neutrophils may be phagocytic (54), but they also have the ability to release proteases that can help degrade cellular debris that may be produced by muscle damage. However, as part of neutrophil activation that leads to proteolysis and removal of debris, they can also release high concentrations of cytolytic and cytotoxic molecules that can damage muscle or other healthy bystander tissues (91). The extent to which these bystander effects can contribute to muscle damage during modified muscle use or injury is just beginning to be understood.

The role of neutrophils in damaging healthy muscle tissue has been demonstrated in response to several perturbations, although their role in muscle damage that occurs during reperfusion after ischemia has been most thoroughly examined (25, 34, 41). Most inflammatory cell-mediated damage during muscle reperfusion occurs within the first few hours of reperfusion (60), and depletion of neutrophils before ischemia-reperfusion injury (I/R) of muscle can reduce histologically discernible muscle damage by nearly 40% (42, 47, 49). Similarly, muscle activation during restricted blood flow, which produces contractile claudication, apparently causes neutrophil-mediated muscle damage (44). In both of these models, there is release of cytosolic proteins from the muscle, which indicates that the muscle membrane may be a target of injury by neutrophils. However, correlations between the invasion of neutrophils into muscle and the peak occurrence of indicators of muscle membrane injury are not observed in all investigations (56), which may reflect differences in the relative importance of mechanically induced lesions and neutrophil-induced lesions in different models of muscle injury.

Neutrophil-mediated damage to muscle can also cause defects in muscle contractility. This has been most convincingly demonstrated in muscle experiencing I/R injury where neutrophil-depleted animals showed significantly less decrease in peak isometric tension caused by the injury than occurred in nondepleted animals (93). Rat soleus muscle also shows a significant decrease in power when rats are subjected to 10 days of muscle unloading followed by 2 h reloading caused by return to normal ambulation (27), although it is controversial whether neutrophils could also contribute to this defect. The controversy has centered on conflicting conclusions concerning whether neutrophils invade muscle after 2 h of reloading, as has been shown to occur in many other models of muscle injury caused by modified loading (8, 23, 43, 44, 55, 89). Initially, Frenette et al. (26) showed a significant ~60% increase in neutrophil invasion after 2 h of reloading, leading the investigators to conclude that neutrophils could potentially contribute to muscle injury at this early stage of reloading. In that study, the investigators identified neutrophils using a monoclonal antibody for which specificity was confirmed experimentally (26). In a subsequent investigation, Frenette et al.

(27) examined neutrophil invasion in the same experimental model and again sampled at 2 h of reloading but concluded that neutrophils did not invade by that time. However, in this second analysis the investigators used an antibody that recognized all granulocytes, and they analyzed fewer animals and obtained a much larger SE in their data. Nevertheless, this second investigation showed an ~50% increase in the number of granulocytes in the muscle at 2 h of reloading, but the difference did not reach statistical significance, primarily because of a relatively large SE. Thus the preponderant data indicate that neutrophils are present when defects in force production occur in this model; it still remains to be tested whether they contribute to the defects.

Whether neutrophils promote muscle damage that is caused by eccentric contractions is also controversial and may reflect a greater difficulty in resolving the neutrophil's contribution to injury against the extensive mechanical damage that can be produced in muscle that undergoes lengthening contractions. Recent evidence has shown that administration of an antibody that blocks the respiratory burst and degranulation of neutrophils prior to lengthening contractions also produces large reductions in microscopically discernible muscle damage (13). However, a previous investigation showed that there was no difference in muscle injury in the muscles of neutropenic and nonneutropenic mice subjected to eccentric contractions (54). Part of the apparent discrepancy concerning the role of neutrophils in these injuries may result from differences in the treatments used to produce injury; Lowe et al. (54) used 150 lengthening contractions, whereas Brickson et al. (13) used a single eccentric contraction. The more extensive damage that could result from the more extreme protocol (54) could obfuscate neutrophil-mediated injury. In addition, differences in the technique to assay for damage may also produce different interpretations. Previous studies have shown that under at least some conditions, injuries that produce extensive morphological damage may cause little decrement in force production (83), which indicates that assaying for muscle injury by testing for defects in force production may not always be the most sensitive assay. Finally, activation of neutrophils to release cytolytic molecules depends at least to some extent on the type and intensity of exercise, with moderate exercise increasing activation and intense exercise potentially suppressing activation (70, 77).

MECHANISMS OF NEUTROPHIL-MEDIATED DAMAGE TO MUSCLE

Current findings show that neutrophils are capable of direct lysis of muscle cell membranes through a superoxide-dependent mechanism. Cytotoxicity assays in which muscle cells were cocultured with activated neutrophils showed that neutrophil lysis of muscle cells could be largely prevented by addition of superoxide dismutase (SOD) (67). These in vitro observations are consistent with findings using the I/R model of muscle injury, in which systemic administration of SOD can produce large decreases in muscle damage (80). Recent findings also implicate neutrophil-derived superoxide in lysis of muscle membranes that is caused by modified muscle use in the rodent hindlimb muscle unloading/reloading model (68). In that investigation, the effects of null mutation of gp91^{phox} on muscle membrane lysis caused by muscle unloading/reloading

were assessed. Gp91[phox] is the catalytic subunit of NADPH oxidase, which is required for superoxide generation by neutrophils (76). Muscle membrane lysis during muscle reloading was nearly completely prevented by the gp91[phox] mutation, showing that superoxide or a superoxide-derivative was essential for most muscle membrane damage in this model.

Although neutrophil-generated superoxide plays a major role in the lysis of muscle membranes in vitro, after I/R or during modified muscle loading, superoxide itself is not likely to cause direct membrane damage in these models. Superoxide is only a mild oxidant that can be rapidly removed by reaction with other free radicals or by conversion to hydrogen peroxide by SOD (35). Hydrogen peroxide is a stronger oxidant than superoxide and has the capacity to peroxidize lipids and damage cell membranes (35). However, hydrogen peroxide can also be rapidly converted in vivo to more highly reactive free radicals or nonradical oxidants, including hydroxyl radicals and hypochlorous acid, depending on the presence of other reactants, enzymes, and transition metals. Myeloperoxidase (MPO) is particularly important in determining the fate of hydrogen peroxide because it can consume hydrogen peroxide to generate hypochlorous acid more rapidly than hydroxyl radicals are produced from hydrogen peroxide in a competing reaction (99). MPO can also compete successfully with catalase, which converts hydrogen peroxide to benign water and oxygen. Thus modulation of the expression or activity of MPO can play an important role in determining whether superoxide production leads to the production of potentially lytic, nonradical oxidants.

MPO is expressed primarily by neutrophils, and increased muscle loading or injury typically produces an early increase in MPO activity in muscle that reflects neutrophil invasion. Part of the increase in MPO in muscle extracts after exercise or injury may reflect an increase in neutrophils in the vasculature in the muscle, because exercise causes demargination of neutrophils, so that circulating populations increase (65). Exercise or muscle injury also causes increased degranulation of neutrophils, leading to the release of active MPO into the serum (8,14). However, histological evidence shows that much of the increase in MPO concentration in muscle after exercise or injury is attributable to neutrophil extravasation into the muscle. Recent findings have also shown that the level of MPO activity per neutrophil is increased by exercise (85), which indicates increased muscle loading or some associated variable during exercise can elevate the expression of MPO. The net effect of this exercise-induced shift to higher levels of MPO activity could be to shift neutrophils to a more cytolytic, hypochlorous acid-generating state (85). Thus exercise increases neutrophils in circulation and in injured muscle and increases their cytolytic capacity.

Although muscle injury or increased muscle loading can increase the activation, extravasation, and cytotoxicity of neutrophils, the extent to which neutrophils damage muscle is governed by the redox environment in the muscle. Much of the chemistry that affects the relative cytotoxicity of neutrophil-derived free radicals and nonradical oxidants occurs in the extracellular space, where reactants contributed by other cells are also present. The contribution of muscle-derived reactants can be especially important in injuries that result from modified muscle use, because exercise and modified muscle use can increase the release of free radicals by muscle (58). For example, muscle contraction can cause increased release of superoxide (46, 58, 78) and hydroxyl radicals (74), either of which can react with MPO to generate more cytotoxic reactants. Increased muscle use can also increase SOD activity in muscle (38), which could lead to an increase in MPO-mediated cytotoxicity, but can also elevate catalase activity (92), which could compete with MPO to reduce neutrophil-mediated damage. A further level of complexity is added by findings which show that the history of muscle use also affects the level of expression of enzymes that are involved in free radical production. For example, modified muscle use can affect the level of expression of both SOD and nitric oxide synthase (NOS) (38, 45, 66, 73, 90). Thus the extent to which neutrophils promote muscle injury will be determined in part by the history of muscle use, the intensity or severity of the modified use that causes neutrophil invasion, the acute use of muscle at the time of injury, and the numbers and state of activation of the invading neutrophils.

DO NEUTROPHILS CONTRIBUTE TO MUSCLE REPAIR OR REMODELING?

A likely role of neutrophils in muscle repair or remodeling is the oxidative or proteolytic modification of damaged tissue, to allow phagocytosis of debris by neutrophils or macrophages. The extent to which this processing and removal of damaged muscle by neutrophils or other phagocytes is required for muscle repair is not known, although some observations indicate that it may be a significant component of muscle regeneration. Muscle regeneration is slower in older animals, which coincides with slowed phagocytosis by inflammatory cells (32, 102). In addition, mouse strains with slower rates of phagocytic removal of muscle debris show slower rates of muscle regeneration (32). These correlations support the expectation that phagocytosis is a necessary feature of muscle repair, although other age- or strain-related factors could also influence other aspects of the regenerative response. In addition, these correlative findings do not distinguish the contribution of neutrophils from other phagocytes, such as macrophages, that are also present. More recently, muscle regeneration has been reported to be slower after injury by snake toxin if phagocytes were first depleted from the animals by intraperitoneal injections of antisera to neutrophils and monocytes (87). The animals depleted of neutrophils and monocytes also showed more tissue debris in injured muscles, which suggested the possibility that the impaired capacity to remove tissue debris by phagocytes could slow the regenerative process.

Could it be that neutrophil-mediated damage to muscle cell membranes is also a necessary feature of muscle growth or repair? Most assays for muscle injury after muscle injury or modified use have equated muscle membrane lysis with muscle injury. For example, measurements of the loss of muscle cytosolic proteins into the serum or the presence of extracellular marker dyes in the muscle cytoplasm have been used to quantify relative levels of muscle damage, although it is feasible that some active transport of membrane-injury marker molecules may occur. Recently, experimental reductions in neutrophil invasion into injured muscle (44) or neutrophil production of free radical oxidants (68) have shown that neutrophils are directly or indirectly involved in muscle membrane damage in at least some models of muscle injury.

However, teleological arguments have been made to state that neutrophil-mediated disruptions of the muscle membrane may be necessary for muscle growth and adaptation to increased loading. This possibility has been tested recently in the muscle unloading/reloading model. During the reloading of the soleus muscle, neutrophils invade the muscle, and there is an increase in muscle membrane lysis, which is followed by a period of muscle growth as it adapts to reloading. Muscle unloading/reloading of gp91^{phox} null mice showed that muscle membrane lysis was prevented by the null mutation, but the rate of muscle growth during the reloading period was not affected by the absence of membrane damage (68). Thus neutrophil-mediated damage of the muscle cell membrane is not required for muscle growth, at least in this model of modified muscle use. Whether they play a role in muscle repair that is independent of growth or phagocytosis is unknown.

WHAT ARE THE ROLES OF MACROPHAGES IN MODIFIED MUSCLE USE?

Currently, we have a poor understanding of the functions of macrophages that invade muscle after injury or modified muscle use. Predictably, their roles in influencing the course of muscle injury or remodeling are more complex than the role of neutrophils, because macrophages are rich sources of diverse growth factors and cytokines, as well as free radicals. Macrophages are also professional antigen-presenting cells, so they can play important roles in regulating the cellular immune response to injured muscle.

Macrophages can injure muscle cells in vitro and in vivo. Cytotoxicity assays have shown that macrophages lyse target muscle cells by a nitric oxide (NO)-dependent, superoxide-independent mechanism and that their cytolytic capacity is increased by the presence of neutrophils (67). Furthermore, the presence of muscle cells increases NO production by macrophages, suggesting that there may be a positive-feedback mechanism promoting lysis, in which initial muscle damage promotes increased NO-mediated toxicity by macrophages (67). Macrophages also increase muscle membrane lysis in vivo in the mdx mouse model of muscular dystrophy. Mdx mice, which are null mutants for the membrane-associated protein called dystrophin, experience an increased susceptibility to mechanical damage to the cell membrane during muscle contraction, which leads to muscle inflammation and membrane lysis (75). However, depletion of macrophages from mdx mice by intraperitoneal injections of a macrophage-specific antibody resulted in an 80% reduction in muscle membrane lysis in vivo (97). This finding shows that macrophages can play a major role in promoting muscle damage, after muscle injuries that are expected to occur through mechanical damage. Whether macrophages can similarly promote membrane damage in nondiseased muscle has not been demonstrated. On the contrary, macrophage invasion of muscles experiencing reloading after unloading is not necessary for the formation of muscle membrane lesions that occur during the reloading period (89).

Several observations indicate a potentially important role for macrophages in promoting muscle repair and remodeling after injury or modified use. Part of the contribution of macrophages to repair processes may rely on their removal of debris after injury. The first macrophages to invade rat skeletal muscle after injury are a phagocytic phenotype that is present at highest concentrations at sites where tissue damage is most apparent (39, 59) (Fig. 1) and then decline in number at the end of the phagocytic stage (82). Although phagocytic macrophages appear to contribute to removal of cellular debris, it is not known whether removal of debris is required for repair or whether macrophages are important contributors to the removal of debris.

Several findings suggest that macrophages may play a more direct role in muscle repair and remodeling than merely removing tissue debris. For example, muscle regeneration by transplanted myogenic cells can be impaired if the recipients of whole muscle grafts are depleted of monocytes and macrophages by irradiation before transplantation, which has been interpreted as showing a role for macrophages in muscle repair and regeneration in vivo (50). In addition, conditioned media from cultures of peritoneal macrophages or macrophage cell lines can increase the rate of proliferation of myoblasts in vitro as well as increasing the number of MyoD-expressing cells (15, 16, 57, 61); however, the identity of the macrophage-derived factor(s) that induce these changes remains unknown. Identification of macrophage-derived factors that can promote muscle repair or remodeling after injury in vivo is even more difficult, because their times of expression are unknown, their concentrations are likely low, and their identification will require confirming their function in vivo. Expression profiling has provided some initial candidate genes for macrophage-derived factors that may affect muscle repair. For example, transforming growth factor-β (TGF-β), which inhibits differentiation of myogenic cells and represses expression of MyoD (24), was expressed at lower levels in muscle 48 h after eccentric contraction injury (7). In addition, expression of a TGF-β-induced transcript was reduced during the first 3 days
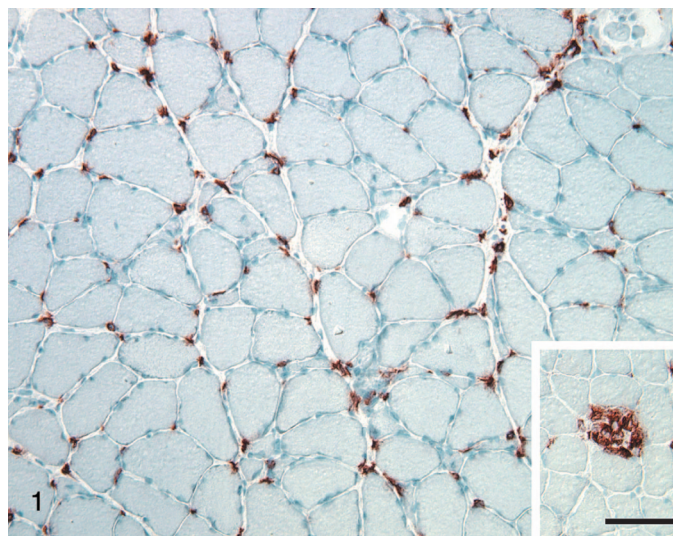

Fig. 1. Cross-sections of rat soleus muscle after a 10-day period of unloading and subsequent 24 h of reloading by normal, voluntary ambulation. Nonphagocytic macrophages that are immunolabeled (anti-ED2) and appear reddish-brown are distributed throughout the connective tissue between muscle fibers. Frequently, the nonphagocytic macrophages appear in close apposition to the surface of muscle fibers in the reloaded muscle, which has contributed to the expectation that they play a role in promoting growth and repair. *Inset*: phagocytic macrophages are immunolabeled (anti-ED1) in reloaded skeletal muscle, some of which have invaded a necrotic fiber. Bar, 60 μm.

after muscle injury by cardiotoxin injection, but expression was then elevated over uninjured controls in samples attained at 5 and 10 days postinjury (101). However, many cells other than macrophages can express TGF-β, and the source of the TGF-β mRNA that was identified in the injured-muscle profiling studies is unknown.

Expression profiling of muscles subjected to injury by a series of tetanic contractions has also revealed that heparin-binding EGF-like growth factor (HB-EGF) may also be a macrophage-derived factor that can influence muscle repair (20). HB-EGF is secreted by macrophages (64) and muscle (19), and it may function to increase muscle cell survival during oxidative stress (40).

Surprisingly, expression profiling of injured muscle has not shown a significant increase in TNF-α, although macrophages are a relatively rich source of TNF-α and there is evidence to implicate TNF-α in affecting muscle repair. TNF-α appears at elevated levels in muscle soon after injury (22, 95). In addition, TNF receptor null mutant mice that were subjected to muscle injury caused by freezing showed significantly lower levels of MyoD expression than observed in injured wild-type muscle (84). The potential functional importance of TNF-α in muscle repair is emphasized by the observation that recovery of muscle strength after freeze injury was significantly impaired in TNF receptor null mice and in mice treated with neutralizing anti-TNF-α compared with injured wild-type mice (95). However, the strength deficits were apparent only at later stages of repair; muscle strength did not differ significantly between wild-type and TNF receptor null mice at 5 days postinjury, but at 13 days, the null mutants were significantly weaker. This delayed influence of the null mutation of the TNF receptor on muscle recovery after injury may reflect that TNF-mediated processes are most important in later stages of repair. Although these reports provide strong evidence that TNF can play a role in muscle repair after injury, no defects in muscle repair or regeneration were reported in TNF-α null mutant mice after a muscle crush injury (22). Furthermore, TNF-α can stimulate muscle wasting through a NF-κB-mediated process (51). In addition, analyses of muscle inflammation and injury in dystrophin-deficient mdx mice that were also null mutants for TNF-α showed that absence of TNF-α increased the rate of necrosis and regeneration in the early stages of the disease (81) but ultimately improved muscle recovery and function at later stages (31). Collectively, these findings show that the role of TNF-α in muscle injury or regeneration may vary with the type, severity, location, and stage of the injury.

Although the identity of macrophage-derived factors that influence muscle growth or regeneration in vivo remains a mystery, indirect evidence continues to accumulate to support the existence of those factors. In a recent interesting investigation, Bondesen et al. (11) found that null mutation of cyclooxygenase-2 (COX-2) or treatment with COX-2-specific inhibitors significantly reduced the rate of muscle regeneration and myoblast proliferation after injury. Although loss of COX-2 activity could possibly affect the regenerative capacity of muscle directly, it also has an anti-inflammatory effect. These investigators observed that COX-2 null mutation reduced the numbers of macrophages in injured muscle during regeneration, which suggested that the reduction in macrophage numbers may underlie the defect in muscle growth and regeneration. These observations and interpretation are consistent with several previous studies in which nonspecific COX inhibitors were reported to slow muscle repair after injury (5, 63, 72).

INTERACTIONS BETWEEN MUSCLE AND INFLAMMATORY CELLS ARE IMPORTANT IN DETERMINING THE COURSE OF MUSCLE INJURY AND REMODELING

Recent investigations have provided new insights into specific mechanisms through which both satellite cells and muscle fibers can regulate extravasation of inflammatory cells and modulate inflammatory cell contribution to muscle injury or growth. Muscle-derived NO appears to be a particularly important regulator of muscle inflammation and muscle damage by invading inflammatory cells (Table 1). In vitro studies have shown that muscle-derived NO reduces neutrophil-mediated lysis of muscle cells and decreases superoxide concentration in the media (67). This protective effect could occur by NO scavenging of superoxide to prevent its conversion to a more cytotoxic oxidant (79) or by inhibiting the activity of NADPH oxidase, so that superoxide production was reduced (21). Muscle-derived NO may also serve to protect muscle from damage by inflammatory cells by inhibiting the expression of adhesion molecules that are necessary for leukocyte interactions with the vascular endothelium (1, 2, 4, 30, 71). At least part of the strong inhibitory effect of NO on leukocyte adhesion to endothelia is attributable to NO inhibition of transcriptional activation of the intercellular adhesion molecule promoter (9), a protein that is necessary for firm adhesion of leukocytes to the vascular wall.

Table 1. *Nitric oxide modulation of muscle inflammation or injury*

Action	Effect	In vitro or in vivo?	Ref.
React with superoxide to form peroxynitrite	Increase cytotoxicity	In vitro	62
React with superoxide to form less reactive intermediates	Decrease cytotoxicity	In vitro	62
Inhibit NADPH oxidase	Decrease cytotoxicity	In vitro	21, 28
React with H_2O_2	Decrease cytotoxicity	In vitro	33
Inhibit neutrophil lysis of muscle cells	Decrease cytotoxicity	In vitro and in vivo	68
Inhibit expression of ICAM	Decrease inflammation	In vitro and in vivo	4
Inhibit expression of E-selectin	Decrease inflammation	In vitro and in vivo	52
Inhibit expression of P-selectin	Decrease inflammation	In vitro and in vivo	48
Decrease AP-1 binding to ICAM promoter	Decrease inflammation	In vitro	9
Increase apoptosis of inflammatory cells	Decrease inflammation	In vitro	3, 10, 94
Increase vasodilation and accelerate repair?	Increase blood supply	In vivo	36, 37, 88, 98

ICAM, intercellular adhesion molecule; AP-1, activating protein 1.

The importance of muscle-derived NO in protecting muscle from inflammation and inflammatory cell-mediated damage has been demonstrated in the mdx mouse model of muscular dystrophy. Mdx mice suffer a secondary loss of neuronal NOS (nNOS) in muscle as a consequence of dystrophin null mutation (12, 17), which leads to muscle inflammation, muscle membrane lysis, muscle wasting, and death. Although the loss of dystrophin is the primary cause of the dystrophy, expression of a muscle-specific nNOS transgene in the mdx muscle greatly reduces mdx muscle pathology (97). NOS transgene expression restores wild-type levels of muscle NO production to the mdx muscle and thereby eliminates most of the muscle inflammation and muscle membrane damage that is characteristic of the mdx pathology (97).

Changes in NOS expression and activity in healthy muscle can also influence muscle inflammation and inflammatory cell-mediated damage. Because NOS expression and activity in muscle are positively regulated by muscle contraction and exercise (6, 90), healthy muscle may be more able to prevent the invasion of potentially cytolytic inflammatory cells. However, removal of normal muscle loading causes a decrease in the expression and activity of nNOS in muscle (90), which could then render the muscle more susceptible to invasion by inflammatory cells. This scenario has received recent experimental support. Expression of a muscle-specific nNOS transgene in mice that were subjected to hindlimb muscle unloading followed by reloading prevented the invasion of neutrophils into muscle during the reloading period, although macrophage invasion was not affected (69). In addition, muscle membrane lesions that occurred in wild-type muscle during reloading did not occur in the reloaded, NOS transgenic muscles. These findings suggest that muscle-derived NO is a negative regulator of neutrophil invasion and also support the view that muscle membrane lesions are caused by neutrophils in this model.

Muscle can also release factors that promote inflammatory cell invasion, particularly macrophages, which may help promote muscle repair. Recently, human satellite cells were shown to release factors that attracted macrophages/monocytes through an endothelial layer in vitro (18). Interestingly, chemoattractiveness of these myogenic cells was highest immediately after they were activated to proliferate and then declined as the muscle cells exited the cell cycle and began to differentiate. In an injury context, this could indicate that activated satellite cells could signal for the rapid, early invasion of macrophages immediately after an injury. In this in vitro model, the investigators showed that nearly 80% of the muscle-derived chemotaxis of macrophages could be blocked by co-application of neutralizing antibodies to monocyte chemoattractant protein-1 (MCP-1), macrophage-derived chemokine (MDC), fractalkine (FKN), vascular endothelial growth factor (VEGF), urokinase type plasminogen-activator receptor (μPAR), and urokinase (μPA). Expression profiling studies of injured muscle have also shown that μPAR-1 (20) and MCP-1 receptor (7) expression are elevated after muscle injury, which supports the possibility of functionally significant signaling via these molecules during muscle inflammation in vivo. The observation that μPAR-1 and its ligand μPA are involved in macrophage chemoattraction to satellite cells in vivo is especially engaging, because μPA null mutant mice show defects in muscle regeneration in vivo that accompany reductions in leukocyte invasion into the muscle (53).
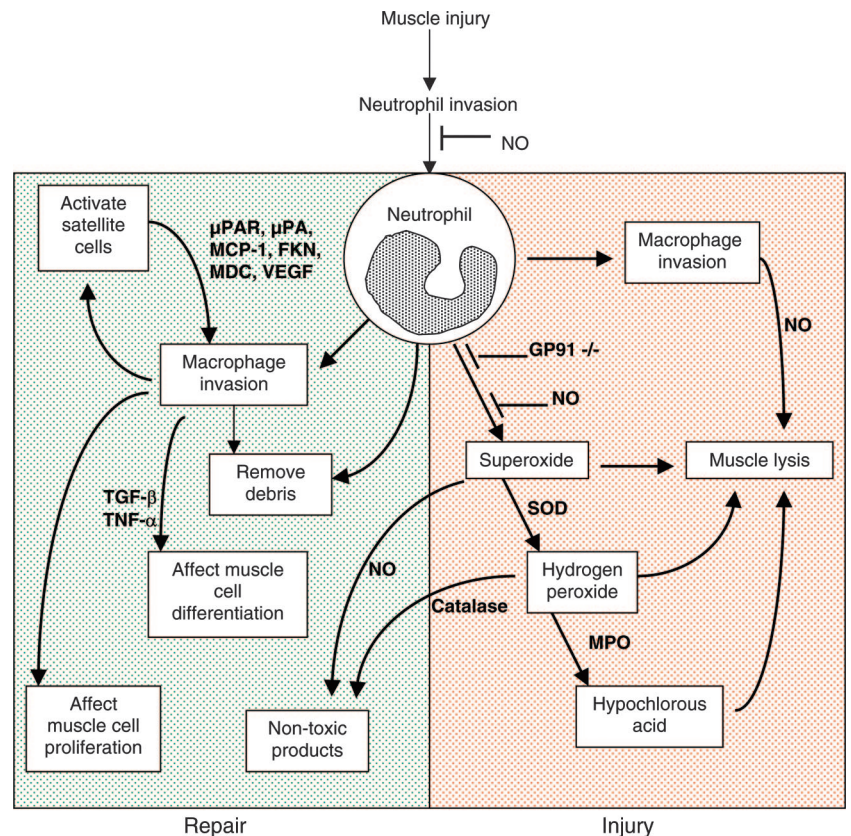
Fig. 2. Schematic of potential mediators of inflammatory cell interactions with injured muscle. Experimental observations that support the potential interactions between neutrophils, macrophages, and muscle are discussed in the text. Neutrophils are shown as having potential role in promoting injury or repair, although their role in promoting repair in injured muscle is speculative. NO, nitric oxide; μPAR, urokinase type plasminogen-activator receptor; μPA, urokinase; MCP-1, monocyte chemoattractant protein-1; FKN, fractalkine; MDC, macrophage-derived chemokine; VEGF, vascular endothelial growth factor; SOD, superoxide dismutase; MPO, myeloperoxidase.

The elevated expression of MCP-1 in muscle soon after injury and its ability to serve as a muscle-derived chemoattractant to macrophages in vitro have contributed to a growing interest in chemokines in regulating communication between muscle and macrophages after injury. Because of overlapping functions of chemokines involved in the inflammatory response, dissecting their individual or synergistic roles in vivo is difficult. However, interesting and promising new work by Simeonova and colleagues (96) has provided strong evidence for a functionally significant role for MCP-1 and other chemokines in muscle repair. Using a freeze injury model, muscle repair and inflammation were studied in mice that were null mutants for chemokine receptor 5 (CCR5) and rendered MCP-1 deficient by injection of neutralizing antibodies to MCP-1. These mutant/depleted animals were found to have a greatly slowed recovery from muscle injury than wild-type animals, using muscle force production as an index of recovery. In contrast with in vitro studies that showed a significant role for MCP-1 signaling in chemoattraction of macrophages to muscle (18), qualitative observations of CCR5 mutant/MCP-1 deficient animals showed no obvious reduction in the concentration of inflammatory cells after injury (96). Thus the role of MCP-1 in regulating interactions between muscle and macrophages may differ between in vitro and in vivo models, or the more complex in vivo environment may provide other factors that compensate for the loss of MCP-1-mediated chemoattraction for macrophages. There is little doubt that the complexity of the systems through which muscle and inflammatory cells communicate will continue to grow as new mediators are identified and as they are analyzed in the complex in vivo environment (Fig. 2).

CONCLUSIONS

Research in the past few years has begun to reveal more specific information concerning the relationships between inflammatory cells and skeletal muscle after muscle injury and modified use. Evidence is now available that identifies specific free radicals and soluble factors that are released by skeletal muscle that can inhibit or promote the invasion of leukocytes. Thus muscle cells can play an active role in regulating their interactions with inflammatory cells. New findings also show that neutrophils can cause muscle injury in vitro and in vivo through the release of free radicals or other oxidants and that this process does not facilitate muscle growth after injury. Nevertheless, neutrophils may also play a role in the repair process, although there are no definitive, experimental data to show this. In particular, are neutrophils essential for the proteolysis and removal of debris that can impede muscle growth and repair? Are they necessary for signaling the subsequent invasion of other inflammatory cells that are needed for muscle repair or regeneration? Perhaps neutrophils release unidentified factors that promote satellite cell activation or differentiation.

Recent experimental findings have also shown that macrophages can promote muscle injury in vivo, at least in muscular dystrophy. However, a large body of in vitro data and in vivo observations associate macrophages with muscle regeneration and growth after muscle injury or modified use. Clearly, macrophages can release factors in vitro that promote muscle growth. They are also present in vivo at sites where repair occurs. However, there are no definitive findings to show which factors, if any, are released by macrophages in vivo that affect the process of muscle repair. If these pro-regenerative, macrophage-derived molecules can be shown to be functionally important in vivo, they may prove to be important therapeutic agents in the treatment of muscular dystrophies, cachexia, or other muscle-wasting conditions.

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