Interactions Between Muscle and the Immune System During Modified Musculoskeletal Loading

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Interactions between the immune system and skeletal muscle may play a significant role in modulating the course of muscle injury and repair after modified musculoskeletal loading. Current evidence indicates that activation of the complement system is an early event during modified loading, which then leads to inflammatory cell invasion. However, the functions of those inflammatory cells are complex and they seem to be capable of promoting additional injury and repair. Recent findings implicate an early invading neutrophil population in increasing muscle damage that is detected by the presence of muscle membrane lesions. Macrophages that invade subsequently serve to remove cellular debris, and seem to promote repair. However, macrophages also have the ability to increase damage in muscle in which there is an impaired capacity to generate nitric oxide. In vivo and in vitro evidence indicates that muscle-derived nitric oxide can serve an important role in protecting muscle from membrane damage by invading inflammatory cells. Collectively, these findings indicate that the dynamic balance between inflammatory cells, the complement system, and muscle-derived free radicals can play important roles in the secondary damage of muscle during modified musculoskeletal loading.

List of Abbreviations Used

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CR-1</td>
<td>complement receptor type 1</td>
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<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule</td>
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<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
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<td>NOS</td>
<td>nitric oxide synthase</td>
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<td>SCR-1</td>
<td>soluble complement receptor type 1</td>
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Glossary

C3b = The larger, activated proteolytic fragment of complement component 3. Primary functions include opsonization of antigen and foreign cells, viral neutralization, promotion of phagocytosis and clearance of immune complexes.

C4b = The larger, activated proteolytic fragment of complement component 4. Primary functions include opsonization of antigen and foreign cells.

C5 = Complement component 5. Its proteolytic fragments contribute to formation of the...
Muscle inflammation is a common, yet poorly understood response to modified musculoskeletal loading. Modified loading that is associated with muscle inflammation can occur after exercise, strain injury, or return to normal musculoskeletal function after periods of unloading. In addition, other, clinically-relevant perturbations in musculoskeletal loading may be expected to lead to muscle inflammation with unknown effects on musculoskeletal function. For example, a feasible but unexplored possibility is that the return to ambulation after prolonged bedrest can result in muscle inflammation that affects recovery, or that surgical procedures that place traction on muscles can induce inflammation that affects recovery. More fundamentally, it is not clear whether inflammation has a net negative or positive impact on muscles that experience modified loading, or whether it is possible to manipulate the muscle inflammatory response to diminish the negative consequences of inflammation, while retaining beneficial effects.

Much of the ambivalence concerning the potential role and importance of inflammatory cells in influencing muscle injury or repair and regeneration is reflected in the literature concerning the effects of nonsteroidal, antiinflammatory drugs (NSAIDs) on experimentally-injured muscle. Nonsteroidal, antiinflammatory drugs clearly can influence muscle recovery from injury. Administration of peroxicam after experimental strain injury reduced the decline in tensile strength of the muscle that occurred in nontreated muscles after injury and increased maximum involuntary force production. In addition, NSAID administration can cause reductions in serum creatine kinase, and reductions in the numbers of injured fibers observed soon after injury. Nevertheless, long-term NSAID administration is associated with a decrease in muscle torque and force generation after increased muscle use. In each case, NSAID administration resulted in a reduction in the concentration of inflammatory cells, and the effects of the NSAID treatment were attributable to inflammatory cell-mediated functions.

Several key observations support the expectation that inflammation can have a physiologically significant influence on muscle function after modified loading. For example, muscle strength after exercise shows a bimodal decline that indicates that simple mechanical damage is not sufficient to explain the reduction in force production, and that the second decline may result from additional impairment of function caused by inflammatory cells. A more recent study also has shown that there is a close relationship between a second decline in eccentric torque production and inflammatory cell invasion in muscles of exercising humans, which also supports the possibility that inflammatory cells can directly or indirectly impair muscle function after modified muscle use.

Few experimental studies have been designed to test whether inflammatory cells pro-
mote muscle damage after modified loading. However, available information concerning the occurrence of muscle inflammation during modified muscle use, and the capacity of inflammatory cells to cause muscle cell damage in other in vivo models and in vitro collectively support this claim. In addition, recent findings have shown that muscle can modulate the activities of inflammatory cells in vivo, and thereby influence the extent of their cytotoxic interactions with muscle. In the current review, some recent findings are presented concerning the interactions between the immune system and skeletal muscle that can influence the course of muscle injury after modified use.

Assessment of Muscle Injury

Interpretations concerning whether inflammatory cells (Fig 1) promote muscle injury after modified musculoskeletal loading rely heavily on the parameters that are used to assess injury. Generally, four types of measurements have been used. In humans, changes in voluntary maximal force production or maximal torque typically are used to test for muscle injury, which has advantages and disadvantages for assessing injury. Although measurements of voluntary force are valuable because they are functionally meaningful assessments, they are not definitive assessments of injury per se, because other factors such as pain could influence the measurements. In animal models, involuntary force production by muscle either in situ or ex vivo has been used to assess injury. Although reduction in involuntary force production muscle provides a clear indicator of muscle damage, it also is feasible that substantial injury could occur and remain undetected in these assays. For example, segmental lesions in muscle fibers can involve extensive damage to the myofibrils, with little decrement in force production. In a third approach to assessing muscle injury, morphologic assays of disruptions of myofibril structure, such as Z-disk streaming, provide valuable and unambiguous assessments of muscle injury. Finally, assays of muscle membrane lesions provide a measurement of muscle fiber damage. Membrane damage can be assessed in vivo by the presence of muscle enzymes in serum, such as creatine kinase, or assessed in muscle tissue samples by measuring the presence of extracellular markers within muscle fibers. In these latter assays,
the entry of large extracellular marker dyes into muscle are assessed microscopically (Fig 2) and the proportion of fibers with lesions sufficiently large to allow marker dye entry is used as an index of injury. The rationale for this assay is that if large membrane lesions are present, there would be a tremendous disruption in fiber homeostasis, and an unregulated transit of ions and other molecules across the membrane. The large influx in calcium into the cell would be sufficient to activate intracellular, calcium-dependent proteases, an occurrence that is expected to be a key early event in muscle damage.5

Although each of these indices of muscle injury can provide a useful measure of perturbations in normal muscle function or homeostasis, muscles can appear injured according to one criterion, but not another. For example, the extent and time course of muscle damage that is measured by histologic damage and by serum creatine kinase levels do not always coincide.16,64 However, assessments of inflammatory cell-mediated damage can be made most directly and relevantly by measurements of muscle membrane damage, because all major cytotoxic mechanisms that inflammatory cells use result in damage to the target cell membrane.

Muscle Inflammation in Response to Modified Musculoskeletal Loading

Most current information concerning inflammatory cell response to modified muscle loading has been based on exercise models in humans, or rodent models in which there is an increase in musculoskeletal loading through exercise or in which hindlimbs are unloaded followed by reloading. Although many of the studies done on humans have relied on assessment of circulating myeloid cell population rather than assessing myeloid cell populations in muscle, a few studies have directly examined inflammatory cell populations in muscle, a few studies have directly examined inflammatory cell populations in muscle after modified use, so that the actual occurrence of muscle inflammation can be addressed.16,36,57 In general, the studies done on humans and rodents have shown that there are stereotypic stages of muscle inflammation after modified loading, regardless of the manipulation causing the change in load. Within hours, the perturbed muscles experience an increase in the concentration of neutrophils in the muscle, which peaks within 12 hours of the perturbation.61 This initial invading population overlaps temporally and spatially with macrophages that remain at extremely high numbers in the muscle for many days after the perturbation. During this latter stage, muscle shows signs of growth, regeneration, and repair, such as satellite cell activation and expression of developmental isoforms of contractile proteins.54 In most cases, this macrophage population in inflamed muscle has been treated as a homogeneous group of cells with a clear capability to phagocytose debris, and potentially to promote repair. However, studies done on rats have shown that the stage of differentiation of macrophages that invade muscle determines, in large part, the functions that they are able to serve.13,37,39 Those monocytes and macrophages that express the ED1 antigen are early-invading, phagocytic macrophages,39 which are replaced by more differentiated macrophages that express the ED2 antigen,13 lose their phagocytic ability, and can release factors that promote muscle cell proliferation.37 These ED2-expressing macrophages have been shown to persist in muscle at elevated concentrations for weeks after increased musculoskeletal loading.54

Neutrophils Have the Ability to Promote Muscle Injury Through Free Radical-Mediated Mechanisms

The time course of muscle invasion by specific populations of inflammatory cells and the bimodal decline in muscle force production that occurs after exercise suggest that the second decline in muscle function during the first 24 hours after exercise could result from the actions of neutrophils or early-invading macrophages.15,35 In particular, close temporal relationships have been observed between the increases in neutrophil concentrations and other indices of muscle damage after modified muscle use. Neutrophil accumulation in muscle after exercise coincides with disruptions in myofibril structure16 and neutrophilia after endurance exercise coincides with elevations of serum creatine kinase.58 Although there are no conclu-
sive data to show whether neutrophils contribute to muscle injury after modified loading, neutrophils clearly are able to cause extensive muscle damage. Muscle damage that is caused by ischemia followed by reperfusion (ischemia/reperfusion) can be reduced largely by depleting circulating neutrophils before reperfusion.10,17,24,27 Furthermore, much of the neutrophil-mediated muscle damage can be prevented in the ischemia/reperfusion model by administration of free radical scavengers.52 Neutrophils are capable of initiating muscle cell injury during reperfusion or after modified loading by the release of reactive oxygen intermediates, which include peroxides, hypochlorite and superoxide. On stimulation, neutrophils have huge increases in O2 consumption, the oxidative burst, which largely results in the conversion of molecular oxygen to superoxide, catalyzed by NADPH oxidase.51 Superoxide then can be converted to other reactive oxygen intermediates and stored in lysosomes or released into the extracellular space.

Several investigators have shown that systemic administration of superoxide dismutase to animals subjected to experimental ischemia/reperfusion can significantly reduce cardiac or skeletal muscle injury.24,25 Although the superoxide radical is cytotoxic, dismutation of superoxide results in the production of hydrogen peroxide, which is more cytotoxic than superoxide. Hydrogen peroxide can be used also to generate hydroxyl radicals that exceed superoxide in reactivity and cytotoxicity. However, dismutation of superoxide in the presence of catalase leads to the conversion of hydrogen peroxide to water. Although it is likely that some of the free radical mediated damage by neutrophils during ischemia/reperfusion is attributable to free radicals that are derived from superoxide, rather than caused by superoxide, the identity of those derivatives is not known with complete certainty.

Neutrophils are the Most Likely Cause of Secondary Muscle Damage Caused by Modified Muscle Loading

Data provided by exercise studies, ischemia/reperfusion studies, and mdx mouse investigations show that mechanical damage, neutrophil-mediated damage, and macrophage-mediated damage all may contribute to muscle injury. Recent attempts to relate the time course of muscle injury to muscle inflammation have indicated that increases in macrophage populations in muscle are not essential for increasing muscle membrane lesions, but that neutrophils may be important in increasing muscle damage.61 Rats that experienced muscle unloading for 10 days followed by reloading through normal ambulation were observed to have a rapid increase in muscle neutrophil populations after 6 hours of reloading and a slower increase in macrophages that became significantly elevated at 24 hours reloading. Muscle...
membrane lesions were observed to increase continuously for the first 24 hours of reloading. Therefore, the data were consistent with the possibility that muscle injury occurred as a result of mechanical damage during reloading, or as a result of damage mediated by inflammatory cells during this period. These two possibilities then were compared by subjecting the animals to reloading for 2 hours followed by return to unloading for 22 hours, and then assessing muscle membrane injury and inflammation. Surprisingly, the extent of muscle injury in animals experiencing continuous reloading for 24 hours did not differ from those that experienced only 2 hours of reloading followed by an additional 22 hours of unloading. Therefore, muscle injury during the 2 to 22 hour period of reloading was not a direct result of muscle loading. Furthermore, the return to unloading prevented an increase in macrophages in the muscle, but neutrophil concentrations in the muscle of animals returned to unloading did not differ significantly from animals subjected to 24 hours of continuous reloading. These findings support the possibility that neutrophils promote muscle injury during the early stages of modified musculoskeletal loading.

Although assessments of muscle fiber injury by measurement of membrane lesions supports the possible role of neutrophils in promoting injury of muscle that experiences modified loading, other indicators of muscle injury are not always influenced by the presence of neutrophils in all injury models. Antibody depletion of circulating neutrophil populations before muscle injury by repeated eccentric contractions of muscle stimulated to tetanus did not affect the involuntary force production by injured muscles compared with injured muscles in mice from which circulating neutrophil populations were not depleted.34

Complement Activation is an Early Event in Muscle Injury That Leads to Muscle Inflammation

The invasion of high concentrations of inflammatory cells into muscles experiencing modified loading indicates that injured muscle may release factors that cause chemoattraction of inflammatory cells. Early speculation focused on the possibility that a wound hormone could be released by injured muscle and serve as a chemoattractant. However, experimental evidence indicates that it is unlikely that chemoattraction occurs through such a direct mechanism in this system.47 For example, extracts of muscles collected immediately after a crush injury showed no chemoattraction for neutrophils or macrophages, although extracts of muscle collected 24 hours after a crush injury were chemoattractive for both cell types.47 The delay between the occurrence of muscle injury and the presence of chemoattractants for inflammatory cells indicates that some other event intervenes between muscle injury and inflammation.

Activation of the complement system is a likely possibility for mediating inflammation after muscle injury. Administration of a truncated, sCR1 to rats experiencing muscle unloading followed by reloading resulted in large reductions in the number of invading neutrophils and macrophages, and a large decrease in muscle fiber necrosis.18 The sCR1 is able to bind C3b and C4b, and thereby can compete with their binding to native CR1, resulting in blockade of the classic and alternative pathways of complement activation.21,33,68 Complement activation also has been shown to contribute importantly to promoting inflammation after skeletal muscle ischemia/reperfusion.30

Although there are strong data to show complement activation contributes to muscle inflammation and damage after either modified musculoskeletal loading or ischemia/reperfusion, the mechanism through which complement activation occurs is less well-understood. However, disruptions in the normal concentrations of reactive oxygen species in muscle may be sufficient to cause activation conversion of complement molecules to forms that are chemoattractant. Exposure of C5 to hydrogen peroxide may cause a change in C5 so that it is structurally similar to C5a,49,65 and functions as a neutrophil chemoattractant.49 There is extensive work showing that disruptions in the normal concentrations of free radicals occur in muscle ischemia/reperfusion.20,26 Muscle that
is subjected to modified loading also experiences changes in free radical concentrations, which feasibly could create conditions that lead to complement activation.

Muscle membrane lesions also have the potential to cause complement activation. Activation of the classic pathway can occur if membrane lesions permit leaking of cytosolic proteins from injured cells that can react with C1, to initiate complement activation. For example, mitochondrial proteins that leak from injured cardiac muscle cells can rapidly cause complement activation through an antibody-independent mechanism. Intermediate filament proteins also have been shown capable of antibody-independent activation of the complement cascade.

**Modified Musculoskeletal Loading Perturbs Muscle Nitric Oxide Production by Muscle**

The preceding sections have noted that free radicals generated by inflammatory cells can cause muscle injury in vivo. However, recent work also suggests that free radical production by muscle fibers can have a protective effect against muscle injury by inflammatory cells, and shows that muscle-derived NO may be particularly important in this role. Previous in vitro investigations have shown that NO can influence myeloid cell functions as either a proinflammatory or an antiinflammatory molecule. The apparently opposing effects of NO in vitro make it difficult to predict the biologic effects of NO because its role may vary with differences in the tissue in which the inflammation occurs and vary with differences in the presence of other proinflammatory or antiinflammatory molecules. In addition, NO concentration or rate of delivery in a tissue also can affect whether it functions as a proinflammatory or antiinflammatory molecule.

Recent findings clearly have shown that muscle-derived NO can reduce muscle inflammation and membrane damage in mdx mice. Most NOS in skeletal muscle is located in the dystrophin protein complex, and NOS concentrations are reduced greatly in null mutants of dystrophin. Muscles from mdx mice show extensive inflammation and muscle membrane damage, which has suggested the possibility that the loss of NOS could contribute to these aspects of pathologic features. Mice lacking dystrophin but which expressed muscle-specific, NOS transgene had levels of muscle NO production that did not differ from wild-type muscle, and the expression of this transgene greatly reduced muscle inflammation and muscle membrane damage. These findings indicate that perturbations in the normal level of NO production by muscle can result in increased inflammation and increased muscle membrane lesions, and thereby show a protective role for NO in muscle. These results also provide additional evidence that perturbations in free radical concentrations can be important in muscle injury. Furthermore, in vitro experimentation has shown that myotubes from mdx mice, which also have low levels of NOS, are lysed more easily by free radicals that can be generated by inflammatory cells. This suggests that at least part of the protective role of muscle-derived NO in vivo may be to scavenge cytotoxic free radicals that are released by invading inflammatory cells.

The correlation between decreased muscle NOS expression and increased susceptibility to free radical-mediated damage in mdx mice suggests that if NOS expression were affected by modified muscle use, then the resulting change in NO production could affect muscle’s susceptibility to injury. Indeed, NOS expression in muscle is influenced greatly by the recent loading history of the musculoskeletal system. Muscle unloading for 10 days results in nearly a 50% reduction in NOS protein and mRNA in muscle, but return to loading leads to a return to normal NOS concentrations in approximately 4 days. Coincidentally, the period of reloading in which muscle NOS levels are low, is the same period of reloading in which muscle inflammation and muscle membrane lysis occur.

Several potential mechanisms exist through which muscle-derived NO could inhibit muscle
inflammation and thereby reduce the extent of muscle damage caused by inflammatory cells. Nitric oxide can scavenge superoxide,48 inhibit NADPH oxidase activity, and thereby reduce superoxide production12 and protect against hydrogen peroxide cytotoxicity.69 Each of these effects would be expected to reduce neutrophil-mediated damage to muscle experiencing modified loading. In addition, NO can inhibit the extravasation of neutrophils and other inflammatory cells by inhibiting the expression of adhesion molecules that are necessary for leukocyte interaction with endothelial cells that precedes extravasation. For example, NO can inhibit the expression of CD11/CD18 and inhibit leukocyte rolling that is dependent on CD11/CD18-mediated adhesion.1,41 Nitric oxide also can inhibit the expression of P-selectin, E-selectin, and ICAM, all of which mediate interactions between leukocytes and endothelial cells before adhesion.3,28,31 As a final, potential, protective mechanism, NO can induce apoptosis of inflammatory cells.2,8,66 In vitro assays have shown that neutrophil apoptosis can be induced by 10 μmol/L NO,8 which is the approximate concentration of NO released by activated, excised muscle into incubation medium. Collectively, these observations indicate that muscle-derived NO has a tremendous capacity to protect muscle against inflammation and inflammatory cell induced damage.

The investigations cited in the current review support a model in which inflammatory cells can promote injury after modified musculoskeletal loading. In this hypothetical model (Fig 3), the initial damage to the loaded muscle results from mechanically-induced injury. This damage then results in activation of the complement system, which leads to chemotraction of neutrophils and macrophages. Neutrophils are the first to invade, at which time they promote muscle damage by the release of free radicals. The number of invading inflammatory cells, and their level of cytotoxicity can be modulated by muscle-derived NO, which can slow leukocyte extravasation, inhibit the production of free radicals, scavenge free radicals, and induce apoptosis in the invading cells.

Fig 3. This diagram shows a hypothetical mechanism through which neutrophils can amplify muscle fiber damage during modified musculoskeletal loading. Mechanical damage can cause activation of the complement system, which would result in neutrophil activation and chemoattraction into the injured muscle. Neutrophils can then further promote muscle damage via free radical-mediated mechanisms. Neutrophil-induced damage can be further exacerbated if the capacity of muscle to generate NO is impaired. PMN = neutrophil

References


44. Petrof BJ, Shragger JB, Stedman HH, et al: Dys-
trophin protects the sarcolemma from stresses developed during muscle contraction. Proc Natl Acad Sci USA 90:3710–3714, 1993.


