NASA/CR-1998-

206785

IN 12 CR

7N-52-CR

OCIT

050070

p2

Progress report - 1 February 1997 to 31 January 1998

Inflammatory and mechanical components of muscle injury.
James G. Tidball, Principal Investigator.
NAG5-4837

1. Progress in the analysis of complement-mediated muscle damage during muscle reloading.

We tested whether the complement system is involved in muscle inflammation and necrosis that result from changes in muscle loading by administering recombinant soluble complement receptor 1 (sCR1) to rats experiencing muscle reloading following periods of unloading. Rat hindlimbs were suspended for 10 days followed by recovery periods of 6 or 24 hours. Thirty minutes prior to reloading, experimental and control rats were intraperitoneally injected with sCR1 (20 mg/kg) or equal volume of PBS respectively. Complement inhibition by sCR1 was maintained in the recovery group by injecting half doses of sCR1 or PBS every 8 hours. Following treatment, soleus muscles were sectioned and labeled with: 1) anti-ED1 and ED2 which recognize antigens specific for monocytes and macrophages, 2) anti-W3/13, which binds to antigen specific for neutrophils. Results showed that complement inhibition through sCR1 significantly reduced the number of neutrophils (-27%) and $ED1^+$ macrophages (-46%) and increased $ED2^+$ macrophages (+23%) after 6 hours of reloading. No significant difference in the concentration of inflammatory cells was found between the two groups at 24 hours of reloading. Also, sCR1 did not significantly affect macrophage invasion of muscle fibers. Together, these data show that the complement system is activated and participates in the early recruitment of inflammatory cells during muscle reloading following periods of unloading. These findings also demonstrate that sCR1 is an effective anti-inflammatory molecule in the early phase of inflammation.

2. Progress in the evaluation of the use of nonsteroidal anti-inflammatory drugs on muscle inflammation and necrosis during reloading following periods of unloading.

Our previous work supported by this NASA grant has shown that increased muscle loading can result in muscle injury and necrosis that is attributable in part to inflammatory cell mediated damage. In this portion of our study, the hypothesis was tested that muscle inflammation and necrosis would be reduced by the application of the non-steroidal anti-inflammatory drug, ibuprofen, to rats prior to increasing muscle loading. Rats were subjected to hindlimb unloading for 10 days, after which they experienced muscle reloading by normal weight-bearing for 24 hours. Quantitative immunohistochemical analysis showed that the concentration of neutrophils, macrophages and necrotic fibers significantly increased in the soleus muscles of animals that received buffer injections only, compared to ambulatory controls. Administration of ibuprofen beginning 8 hours prior reloading caused a large, significant reduction in the concentration of necrotic fibers, but had no effect on the concentration of neutrophils or $ED1^+$ macrophages in the muscle, compared to reloaded animals receiving buffer only. However, administration of ibuprofen caused a 2.6-fold increase in the concentration of $ED2^+$ macrophages. Thus, administration of ibuprofen to animals before muscle reloading following periods of muscle unloading increases muscle inflammation by a specific increase in $ED2^+$ macrophage populations, but causes large decreases in muscle necrosis. These events may be mechanistically related because $ED2^+$ macrophages have been associated with muscle regeneration and repair, and may serve a protective function against muscle injury by other inflammatory cell types.

3. Progress in the investigation of the role of nitric oxide in muscle inflammation and

necrosis in modified muscle use.

The objective of this portion of our investigations was to determine the role of nitric oxide (NO·) in muscle inflammation, fiber necrosis, and apoptosis of inflammatory cells *in vivo*. Specifically, the effects of nitric oxide synthase (NOS) inhibition on the concentrations of neutrophils, $ED1^+$ and $ED2^+$ macrophages, apoptotic inflammatory cells, and necrotic muscle fibers in rats subjected to 10 days of hindlimb unloading and 2 days of reloading were determined. Administration of the NOS inhibitor N^w-nitro-L-arginine methyl ester (L-NAME; N=10) significantly ($p<0.05$, ANOVA) reduced the concentrations of neutrophils, $ED1^+$ and $ED2^+$ macrophages, and necrotic fibers in soleus muscle relative to water treated controls (N=10). The concentration of apoptotic inflammatory cells was also significantly lower for L-NAME treated animals compared with water treated controls. However, the proportion of the inflammatory cell population that was apoptotic did not differ between L-NAME treated and control animals, suggesting that L-NAME treatment did not decrease inflammatory cell populations by increasing the frequency of apoptosis. In conclusion, nitric oxide or one of its intermediates promotes muscle inflammation and fiber necrosis during modified muscle use and plays no more than a minor role in the resolution of muscle inflammation by inducing apoptosis of inflammatory cells.