INTRODUCTION:
Rotator cuff tendons heal to bone following surgical repair with an intervening layer of fibrous scar tissue that is mechanically weaker than native tissue. This makes repairs prone to failure, which occurs in 11-94% of cases (1,2,3). The presence of an inflammatory response early in the healing process is thought to be responsible for the formation of this scar tissue. Tumor necrosis factor – alpha (TNF-α) is a major mediator of inflammation and is upregulated in the subacromial bursa of patients with rotator cuff disease. TNF-α also promotes the formation of osteoclasts that may impede bone ingrowth into the interface tissue and prevent healing. PEG-sTNF-R1 (Amgen, Thousand Oaks, CA) is a TNF-α antagonist. The specific aim of this project is to determine if systemic TNF-α blockade can improve the tendon-bone healing in a rat rotator cuff model. Our hypothesis is that a group of rats treated with PEG-sTNF-R1 following rotator cuff repair surgery will heal with less scar tissue at their tendon-bone interface as compared to those who receive saline; and that this will result in improved biomechanical strength at the repaired tendon-bone interface.

METHODS:
120 Lewis rats underwent unilateral detachment and repair of the supraspinatus tendon. Postoperatively, rats were randomized into two groups (60 rats/group). The experimental group received subcutaneous injections of PEG-sTNF-R1 at a dose of 3.8mg/kg every other day for a total of 3 doses (the first dose given on POD1) (4). The control group received injections of a similar volume of saline on the same dosing schedule. 20 animals in each group were sacrificed at 2, 4, and 8 weeks. At each time point, 4 animals were designated for histologic analysis, and 16 for biomechanical testing.

Histomorphometric Analysis:
Four specimens in each group were fixed in formalin, decalcified in Immunocal, embedded in paraffin and sectioned in the coronal plane in 5 micron increments. The amount of new cartilage formation was determined by measuring the area of metachromasia on safranin-O stained slides using ImageJ (NIH). The extent of collagen fiber organization was determined by the level of brightness (Gray Scale) under polarized light microscopy of picrosirius red stained slides with ImageJ (NIH).

Biomechanical Testing:
Sixteen specimens in each group were designated for biomechanical testing. The specimens were preloaded to 0.10 N and then loaded to failure at a rate of 14 microns/sec. The maximum load at failure and the failure site were recorded. This testing protocol has been used in previous studies from our laboratory (5). The material properties of the repaired tendon-bone interface were determined by calculating the stress required for failure. This was done by dividing the ultimate load-to-failure by the cross sectional area of the tendon at the insertion site.

Statistical Analysis:
Statistical analysis was performed with Wilcoxon rank-sum test with significance set at p=0.05. A pre-study power analysis was performed for a primary outcome of ultimate load-to-failure.

RESULTS:
Histomorphometric Analysis:
The amount of fibrocartilage formation at the insertion site was greater in the experimental group compared to controls at both 2 and 4 weeks. There were no differences between groups at 8 weeks. At 2 weeks, the average load-to-failure for the control group was 11.2±2.7N while the experimental group was 13.3±2.6N (p=0.05). At 4 weeks, the average load-to-failure for the control group was 18.5±3.1N and the experimental was 21.7±4.6N (p=0.04). At 8 weeks, the average load-to-failure for the control group was 29.4±6.8N and the experimental was 32.5±7.7N (p=0.33). There were no differences at any timepoint in terms of average cross-sectional area of the tendon at the insertion site, or the calculated stress required for pull-out.

DISCUSSION:
This study shows that TNF-α blockade can improve the biomechanical strength of tendon-bone healing in a rat rotator cuff model at early timepoints. This correlated with modest improvements in histology based on the ability to create more of a fibrocartilaginous transition zone. This adds further evidence that an excessive inflammatory response following surgery may be detrimental to healing, and that by blunting it we can improve the strength of the repair. Further studies are needed with longer timepoints and in larger animals to determine if this therapy can be useful in humans.

REFERENCES:

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