Introduction:
Damage to soft connective tissue results in scar formation and inferior mechanical properties compared to normal tissue. Scar development may be attenuated by modulating the immune cells and their cytokines in a temporal and spatial manner. Cytokines regulate wound healing by modulating the inflammatory response and cellular activities. Interleukin 1 receptor antagonist (IL-1Ra) is an inflammatory cytokine that competitively blocks binding to the IL-1 cell surface receptors. An imbalance between IL-1 and IL-1Ra results in inflammatory disease and tissue damage. Previous reports indicate that administration of neutralizing antibodies to IL-1Ra results in exacerbated arthritis, colitis, and bacteria-induced hepatitis whereas, delivery of recombinant IL-1RA prevents or reduces these diseases. Ligament healing also involves the balance of inflammatory factors in a time-dependent manner. We hypothesized that inhibition of IL-1 at a specific time will stimulate a more regenerative healing response, reducing scar formation. Our objective was therefore to determine the influence of IL-1Ra on early wound healing.

Materials and Methods:
The study was performed according to a protocol approved by the University of Wisconsin Institutional Animal Use and Care Committee.
To determine the in vivo influence of IL-1Ra on medial collateral ligament (MCL), mature male Wistar rats were bilaterally transected and received either 1) IL-1Ra i.p. at the time of surgery and 30 minutes and 3 hours post surgery, 2) IL-1Ra s.c. over the MCL 18-24 hours post surgery, 3) PBS i.p. at the time of surgery and 30 minutes and 3 hours post surgery or 4) PBS s.c. over the MCL 18-24 hours post surgery. At 5 days post surgery, ligaments were collected, snap-frozen and used for immunohistochemistry to detect M1 and M2 macrophages, T-lymphocytes, and endothelial cells.

Statistics:
To determine differences between PBS and IL-1Ra treatments, data were log-transformed and Kruskal-Wallis ran sum tests were performed.

Results:
Endothelial Cells: Ligaments injected with IL1Ra at the time of surgery resulted in an increase in granulation tissue-localized endothelial cells 5 days post-injury (p = 0.03; Fig. 1). Injections of IL1Ra after 24 hours post rupture, did not have an influence (p > 0.05; Fig. 1).
M1 and M2 Macrophages: M1 macrophages tended to decrease within the epilagmen after IL1Ra injection at the time of surgery (p = 0.07; Fig. 2). IL1Ra Injection after 24 hours did not influence M1 macrophage numbers (p > 0.05). M2 macrophage numbers did not change with IL1Ra injection, regardless of time (p > 0.05; Fig. not shown).
T-lymphocytes: Although few T-lymphocytes are found within the healing ligament, treatment of IL1Ra at the time of surgery significantly increased cell numbers within the healing region (p < 0.013; Fig. 3). IL1Ra injection 24 hours post-surgery, did not significantly influence T-lymphocytes (p > 0.05).

Discussion:
We hypothesized that administration of IL1Ra in a time-controlled manner stimulate a more regenerative healing response, reducing scar formation after ligament healing. In a typical healing scenario, injury initiates an immediate neutrophil response. Macrophages follow thereafter and peak at day 5 post-injury. A paucity of T-lymphocytes appear during the first 2 weeks of healing. Blood vessels predominate infiltrate the healing ligament between days 7-11 post-injury. Treatment of IL1Ra at the time of injury significantly increased endothelial cells, T-lymphocytes, and to some degree, the M1 macrophages. These results suggest 1) IL1Ra stimulates a cellular response during MCL healing and 2) IL1Ra injection affects the MCL in a time-dependent manner. IL1Ra is a factor that affects cells involved in scar formation. It therefore may have a therapeutic role in regenerative ligament healing.

Significance:
IL1Ra may alter the cells involved in wound formation

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