Aging Blunts Anti-Inflammatory Cytokine Responses in Bone, Tendon, and Muscle in the Rotator Cuff

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INTRODUCTION: Muscle stress results in a complex cascade of signaling proteins, both locally and through the bloodstream [1]. Due to the unique anatomy of the rotator cuff, muscle-derived signals released locally into the synovial fluid have the potential to directly influence adjacent tendon and bone. Our lab has been using *in vivo* rodent models and *in vitro* explant models to study mechanisms of local inflammatory signaling in the shoulder [2]. In preliminary studies, we observed the time- and tissue-dependent secretion of anti-inflammatory cytokines (IL-4, IL-15) and IL-6 following downhill running. We posited that these proteins were critical to the resolution of joint stress. In this study, we aimed to investigate further how these signaling molecules affect the health of tendons, muscles, and bones in the rotator cuff, and to determine whether this signaling is age-dependent. We hypothesized that anti-inflammatory cytokines would broadly inhibit pro-inflammatory signaling in all tissues, but that their effect may be diminished with aging based on previous literature.

METHODS: Flexor digitorum longus (FDL) tendon, humeral head bone and supraspinatus muscle explants were harvested from young (4 months old) and aged (24 months old) male C57BL/6J mice (BU IACUC approved). Studies performed in female mice were published earlier. Explants were cultured in stress-deprived conditions with standard culture medium for 24h then treated with exogenous IL-4 at 1pg/mL, IL-6 at 55 ng/mL and IL-15 at 0.1 ng/mL for 24h (n=4/treatment). Concentrations were chosen based on secreted protein levels following downhill running. After 24h, medium from all groups was collected to assess cytokine production using a custom Mesoscale Discovery multi-spot assay, which quantifies protein levels of IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-15, KC/GRO, MCP-1 and TNF-α. Relative gene expression for key tissue-specific markers of injury and turnover was measured via qPCR, normalized to both β-Actin and untreated control explants.

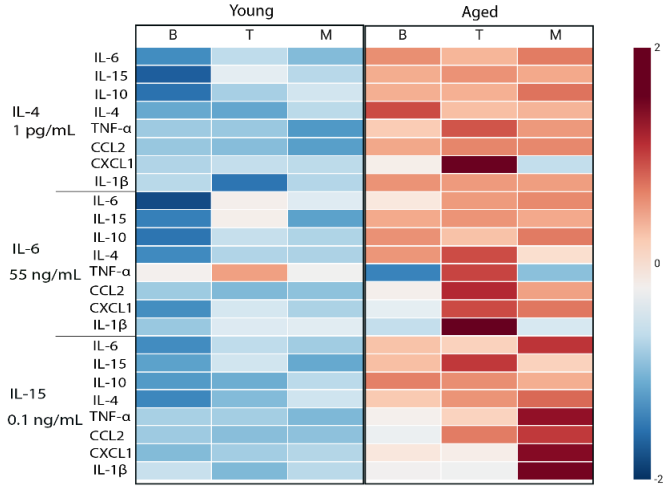

Figure 1: Heatmap showing IL-4, IL-6 and IL-15 -induced modulation of inflammatory and anti-inflammatory cytokines in bone (B), tendon (T), and muscle (M) across age. Rows represent treatments, while columns represent tissues from young (left panel) and aged (right panel) explants. Values are presented relative to untreated controls. The color scale denotes decreased secretion with blue shades and increased secretion with red shades, with intensity reflecting the magnitude of change.

RESULTS: As expected, treatment with all three cytokines largely suppressed both pro- and anti-inflammatory cytokine secretion. However, this was lost in aged tissues (Fig. 1). Aging generally induced a greater impact on the suppression of anti-inflammatory proteins compared to pro-inflammatory mediators, especially in bone. Interestingly, tendon treated with IL-6 and muscle treated with IL-15 both exhibited increased pro-inflammatory cytokine production in aged tissues. IL-6 treatment also reduced TNF-α production in young bone and muscle yet produced the opposite effect in tendon. This phenomenon was even stronger in aged tissues. Tissue-specific markers of turnover were broadly induced by treatment with all three cytokines, especially in tendon and muscle, but these changes were markedly reduced in aging. For example, *Alpl* was differentially expressed with aging bone with all three treatments. Expression of *Il6* was suppressed in aged tendons across all three cytokine treatments. Young tendons also exhibited higher *Tgfβ1* expression relative to aged tendons when subjected to exogenous IL-4. *Igf1* and *Fndc5* were significantly reduced in aged tendons treated with IL-15. Aged muscles also exhibited reduced *Mstn* expression with IL-6 treatment and reduced *Tnf-α* and *Fgf21* expression after IL-4 treatment.

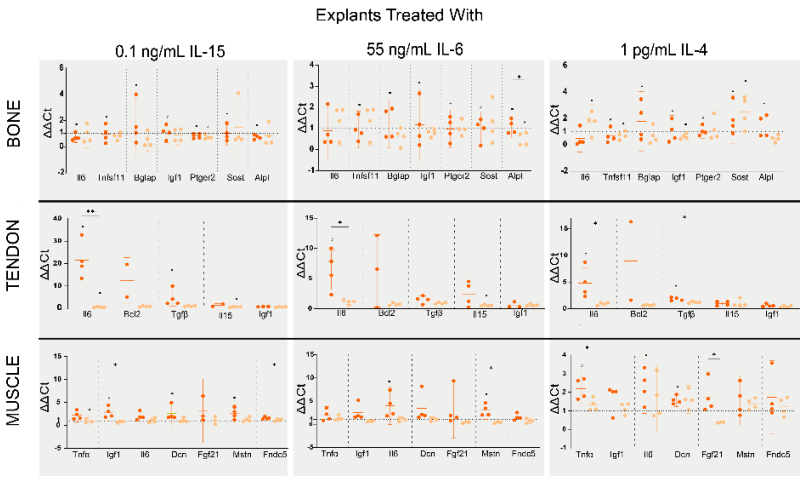

Figure 2: Bone, tendon and muscle explants treated with exogenous IL-15, IL-6, and IL-4 gene expression data. Tissue-specific health markers were chosen. Significance between a specific gene and its control (#$p < 0.05$) and between young male (YM) and aged male (AM) for a specific gene (*$p < 0.05$) is noted. Gene expression values were calculated using the ΔΔCt method, where fold change represents expression in treated samples relative to untreated controls after normalization to housekeeping genes (value of '1' would represent no effect).

DISCUSSION: The response to cytokines in bone, tendon, and muscle is strongly influenced by age. Aged tissues had minimal response to treatment with IL-4 and IL-15, which are both well known for their roles in the resolution of inflammation and transition to the remodeling phase following injury. All three tissues also exhibited reduced tissue-specific markers and genes associated with ECM turnover, adding evidence to a delayed transition to repair. This impaired molecular response may underline the delayed healing commonly observed in aged tissues and could ultimately shift tendons toward degenerative pathways rather than effective repair. Interestingly, some tissues exhibited increased pro-inflammatory cytokine production following treatment with IL-15 and IL-6, suggested a shift toward a pro-inflammatory phenotype under certain stimulatory conditions. This could be support for the theory of 'inflammaging,' where aged tissues exhibit chronic inflammation. At the same time, aged tissues also exhibited increased production of anti-inflammatory cytokines, a sort of positive feedback loop, which could be compensatory. Surprisingly, the response to IL-6 was different in muscle/bone compared to tendon, which underscores tissue-specific inflammatory regulation. IL-6 has been implicated in tendon mechanobiology and exercise-induced adaptation, where it can act as both a pro-inflammatory mediator during acute overload and a regulator of tissue remodeling during repair [3]. Bone exhibited the fewest age-dependent changes, indicating preserved osteogenic phenotype. In contrast, tendon and muscle exhibited pronounced age-related disparities, supporting future work understanding their secretory and metabolic roles in shoulder health. Overall, this work provides new insight into age-related differences in cytokine responsiveness and points to potential pathways that could be targeted to improve rotator cuff healing in the elderly.

SIGNIFICANCE/CLINICAL RELEVANCE: This study suggests that anti-inflammatory cytokine treatments could be progressively less effective with aging.

REFERENCES: [1] Severinsen+2020.Endocrine Rev.,41(4):594–609.,[2] Connizzo+2018. Connect Tissue Res., 59(5):423-436., [3] Docherty+2022. BMC Sports Sci.

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