Age-Related Differences in the Response to Tumor Necrosis Factor Alpha (TNF-α)

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INTRODUCTION: While chronic tendon disease and injuries disproportionately affect the aging and elderly populations, the reasoning is still unknown. Inflammation contributes to the onset and progression of chronic diseases across multiple organ systems, thought to be due in part to the high levels of circulating inflammatory cytokines and altered immune responses present in aged individuals [1]. Recent studies have shown that inflammatory signaling may also be a key to understanding early tendinopathy [2]. Our group established an *in vitro* secondary joint damage model that is associated with high levels of inflammation leading to tissue damage [3,4]. We previously observed that the response to this model was altered with aging, demonstrating reduced cell death and altered inflammatory responses. The goal of this study was to specifically investigate responses to tumor necrosis factor alpha (TNF-α), which has been found consistently in spent medium from our secondary joint damage model and is a known inducer of apoptosis. We hypothesized that the local cell responses will be altered in aged tendons treated with TNF-α, showing decreased expression of genes associated with inflammation, apoptosis, and matrix degradation.

METHODS: Flexor digitorum longus (FDL) tendon explants were harvested from young (4 months) and aged (22-24 months) male C57BL/6J mice (BU IACUC Approved), as described previously [1]. Explants were cultured in stress-deprived conditions with standard culture medium for 2 days. Then, they were treated with mouse recombinant tumor necrosis factor alpha (TNF-α) at 10 ng/mL and assessed for changes in gene expression, MMP activity and inflammatory cytokine release after 72h. We measured matrix metalloproteinases (MMP-1, MMP-3, MMP-9, and MMP-13), and apoptosis markers (CASP3, CASP8 and CASP9) via quantitative RT-PCR. Expression data for each gene were calculated from the threshold cycle (Ct) value, and normalized to an internal housekeeping gene (β-Actin). Activity of MMPs (1, 2, 3, 7, 8, 9, 10, 13, 14) was determined via analysis of spent culture medium using a commercially available FRET-based generic MMP activity kit (SensoLyte 520 Generic MMP Activity Kit). For assessment of cytokine release, spent medium was analyzed using the Meso Scale Discovery Mouse ProInflammatory 10-Plex multi-spot assay to quantify protein levels of IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, KC/GRO (mouse analog of IL-8), and TNF-α (Meso Scale Discovery, Rockville, MD).

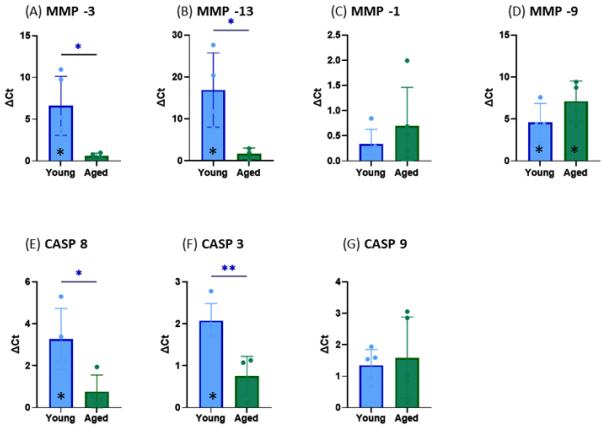

Figure 1. Quantitative PCR data showing relative change in gene expression from young and aged explants after 72 hours TNF-α treatment for (A) MMP-3, (B) MMP-13, (C) MMP-1, (D) MMP-9, (E)CASP 8, (F) CASP 3 and (G) CASP 9. Data is shown as mean ± SD. Statistical significance (*p<0.05) between young and aged is represented by solid bar spanning between groups. Stars inside the bars represent significant difference between control and TNF-α treated samples.

RESULTS: MMPs-3, 13, and 9 were all upregulated with TNF-α treatment (Fig. 1A-D). Treatment caused larger upregulation of MMP-3 and MMP-13 in young compared to aged explants (Fig. 1A-B). There were no significant differences in the magnitude of MMP-1 and MMP-9 upregulation based on age (Fig. 1C-D). Apoptosis markers CASP8 and CASP3 were also upregulated in response to TNF-α treatment (Fig. 1E-F), with more significant changes in young compared to aged tendons. As expected, the expression of CASP9 did not change significantly (Fig. 1G) when challenged with TNF-α in either of the groups. At the protein level, the release of KC/GRO, IL-6, and IL-1β appeared to be increased with TNF-α treatment (Fig. 2). Post-hoc tests revealed that KC/GRO and IL-6 levels were significantly increased with TNF-α treatment in aged tendons only, while IL-1β levels were increased in both age groups. There were no significant differences in any other cytokine measured (not shown). Overall MMP activity was increased in TNF-α treated tendons in both age groups, and the effect appeared to be larger in aged tendons (Fig. 3).

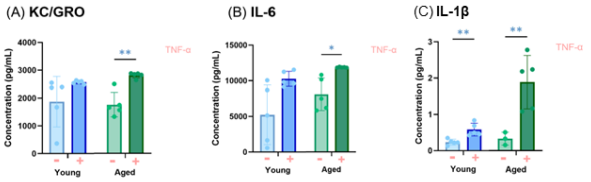

Figure 2. Concentration of pro-inflammatory cytokines KC/GRO (A), IL-6 (B) and IL-1β released into spent medium from young and aged FDL explants. Data shown as mean ±SD. Statistical significance (*p<0.05) between control and treatment is represented by solid bar spanning between groups.

DISCUSSION: Treatment with TNF-α is detrimental to tendon health regardless of age, resulting in apoptosis, matrix degradation and further inflammation. Reduced expression of caspases in aged tendons suggests apoptosis resistance, consistent with our previous work in the secondary joint damage model [3]. Some apoptosis resistance in aged explants could be explained by the presence of a small population of senescent cells, as reported in other tissues [5]. We previously reported senescence-associated beta-galactosidase activity in aged supraspinatus tendons treated with conditioned medium from our secondary joint damage model [6]. Therefore, future studies will potentially aim to clarify the role of senescent cells in the apoptosis resistance of aged tendons. Although we did not find large changes in inflammatory cytokine production, IL-1β and IL-6 were altered with both TNF-α treatment and aging. In addition to inflammation, these cytokines are known to play a role in inter-tissue signaling between muscle and bone and therefore, this could represent altered joint communication in the aged rotator cuff [4]. However, it is not clear whether changes in inflammatory cytokine production are due to local changes in local tenocyte behavior or resident macrophages that may be present in larger numbers in aged tissues [7]. Future studies will focus on identifying mechanisms of altered responses to TNF-α, including lack of receptors or inappropriate signaling, as well as exploring whether other mechanisms of apoptosis are altered in aging.

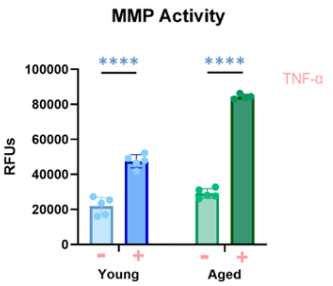

Figure 3. MMP Activity after 72h TNF-α treatment of young and aged FDL explants. Data shown as mean ±SD. Statistical significance (*p<0.05) between control and treatment is represented by solid bar spanning between groups.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates that aging alters the response of tenocytes to inflammation, potentially leading to inflammation-based tissue degeneration.

REFERENCES: [1] Connizzo+2019. *Connect Tissue Res.*, [2]Millar+2017. *Nat Rev Rheumatol* [3] Connizzo+2018. *Connect Tissue Res.*, 59(5):423-436., [4] Connizzo+ 2020. *Trans. Orthop. Res. Soc.*, [5] Liu+2019. *Proc. Natl. Acad. Sci.*, [6] Stowe+2023. *Trans. Orthop. Res. Soc.*, [7] Uchida+2005. *J Biomech.*, 38(4):791-798.,

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