Aging May Attenuate Injury-Induced Myeloid Cell Recruitment to Intervertebral Discs by Local Upregulation of *SerpinA1* and Depletion of Systemic Myeloid Cells

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INTRODUCTION: In the US, 17 million older adults (65+ years old) suffer from at least one episode of lower back pain (LBP) annually, and 35% of them experience chronic LBP.[1] Aging promotes a mature cellular phenotype in the intervertebral disc (IVD)[2] and is associated with IVD degeneration,[3] impaired healing and chronic inflammation.[4] This highlights a specific need to study the role of aging in the regulation of discal inflammation. Wnt signaling is responsible for IVD development, cellular differentiation and proliferation, and it declines in cells with aging and injury[5] but beneficially, suppression of Wnt signaling in IVD cells is anti-inflammatory[6]. We previously found that in 1 week of injury, aged (12 mo) IVDs attenuated the magnitude of expression of inflammation-related genes than injury in young-adult IVDs (5 mo). However, it is unclear whether aging regulates short-term inflammatory-related gene expression in response to injury. We hypothesized that aging will either delay or dampen the inflammatory transcriptomic response of the IVD to injury.

METHODS: Male and female C57Bl/6 mice of 5 months (young-adult), 12 months (aged), or 22 months (old) of age were subjected to either 4 hours or 24 hours of catabolic tail compression (TC) as previously described.[2] Tissues were harvested immediately after TC. Two adjacent IVD, that were not compressed, served as internal controls. IVDs were processed for qPCR, ATP6 was used as the reference gene, and the relative expressions are normalized to the 5-month-old male control IVD. Separately, 19-month-old mice female were subjected to 24 hours of tail compression (TC) as previously described.[2] Once de-instrumented, mice received a retro-orbital injection of 89Zr-labeled PERFECTA-HDL at a dose of 100 ± 10 µCi and allowed to circulate for 24 hours prior to PET/CT imaging (Meidso nanoScan).[7] A whole-body CT scan was performed (energy 50 kVp, current 180µA, isotropic voxel size 0.25mm^3), followed by a PET scan. Coincidences were filtered with an energy window 400-600 keV. The voxel size was isotropic, 0.6mm^3 in width, and reconstruction was applied for two full iterations, 6 subsets/iteration. PET data were reconstructed using CT-based attenuation correction and performed using the TeraToma 3D reconstruction algorithm (Mediso Nucline software). All experiments were IACUC approved. A student's t-test was used for qPCR analysis and a paired student's t-test for myeloid tracer intensity was used. A p-value of <0.05 was considered significant.

RESULTS: Neither 4- nor 24-hr of TC in young-adult mice regulated M1 macrophage marker *TLR4* (**Fig. 1A**). By contrast, in old mice, 4hr of TC increased *TLR4* expression by 1.5-fold and 24 hr of TC reduced *TLR4* expression to undetectable levels. 4 hours of TC did not change *IL6* expression in the IVD of any adult, but 24 hours of TC increased *IL6* expression in all ages by over 14-fold in the young-adult group, 5.6-fold in the aged group, and 6-fold in the old group (**Fig. 1B**). Lastly, 24 hours of TC did not increase *serpinA1a* expression in the young-adult IVD but TC increased *serpinA1a* gene expression in both the aged and old IVD by 3.8-fold and 4-fold, respectively (**Fig. 1C**). Next, the PERFECTA nanoparticles target phagocytosis in myeloid cells and the nanoparticle intensity in bone marrow pre- and post-injury was not statistically different and both were greater than the tail IVD pre-injury levels. After 24 hr of TC, the myeloid tracer intensity decreased in the lumbar region by 36% and increased in caudal IVD by 576% (**Fig.1 D, D'**).

DISCUSSION: *SerpinA1* encodes for the protein AAT, which is an anti-protease that is involved in chemotaxis and neutrophil locomotion.[8]AAT also inhibits inflammatory cytokine release and promotes the release of inflammation driven anti-inflammatory agents.[9] After 24 hours of tail compression, *serpinA1a* expression was upregulated in the mature and old IVD, which could potentially explain the attenuated IL6 expression and undetected TLR4 expression in the old IVD. Next, injury increased myeloid cell recruitment to caudal IVDs and reduced myeloid cells in the lumbar region. Myeloid cell recruitment experiments in young-adults is ongoing. Overall, these data suggest that there is a local and systemic mechanism by which IVD recruit myeloid-derived cells to an injury and why aged IVD may recruit less myeloid cells. Locally, old IVD may upregulate *serpinA1a* to repel myeloid cell recruitment and, systemically, life-long siphoning of myeloid cells may deplete the reservoir needed to recruit to the injury site.

SIGNIFICANCE/CLINICAL RELEVANCE: The transcriptomic response of inflammatory genes to injury is differentially regulated by advanced age and treatment for IVD degeneration may need to be age-dependent. Administration of AAT could potentially serve as a therapeutic to mitigate inflammation.

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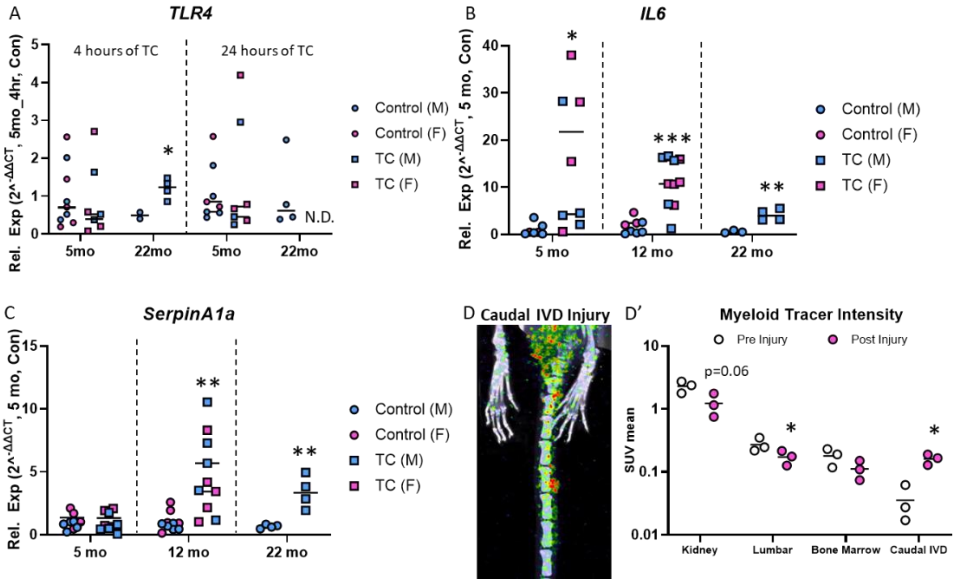

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Figure 1. *(A) TLR4 gene expression between 5 month and 22 month old mice after 4 or 24 hours of TC. (B) IL6 gene expression, (C) Serpina1a gene expression of 5 month, 12 month, or 22 month old mice after 24 hours of TC. (D) Image of PERFECTA nanoparticles in the mouse tail after injury (TC) Green indicates low intensity and red indicates high intensity of nanoparticles. (D') Quantification of nanoparticles from kidney, lumbar, bone marrow, and caudal IVD pre- and post-injury. *p<0.05, **p<0.01, ***p<0.001.*