Sex-dependent spinal expression of SERPINA1/Alpha-1 antitrypsin may predispose women to inflammation

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SIGNIFICANCE/CLINICAL RELEVANCE: Low expression of spinal alpha-1 antitrypsin (AAT-1) may partially account for the heightened frequency of inflammation and back pain in women.

INTRODUCTION: Women exhibit stronger inflammation-driven pain responses compared to men, suggesting a distinct interplay between sex and inflammation; potentially indicating a need for unique treatment options for women1. Several bulk- and single-cell bioinformatics approaches2-3 have identified SERPINA1 (human) serpinA1a-e (mouse) as a prominent gene in intervertebral disc (IVD) cells during IVD degeneration, but the role of SERPINA1 in the IVD and adjacent bone are unclear. SERPINA1 (SERine Protease Inhibitor A1) is a gene that encodes AAT-1 protein, which suppresses recruitment of myeloid-derived cells (macrophages, neutrophils) to the site adjacent of injury4 and is a protease inhibitor of elastase. AAT-1 deficiency exhibits a greater occurrence and prevalence in males, yet it is associated with a lower mortality rate compared to females5. We find by RNA-sequencing that IVD injury activates more inflammatory pathways in female mice than male mice (unpublished). Therefore, we hypothesized that SERPINA1/AAAT-1 is differentially expressed in spinal tissues/cells by sex of mice and humans.

METHODS: This study is approved by IACUC. Tissues (liver, lungs, IVD, bone) were harvested from 4-month-old male and female mice (n=5/sex). The tissues were subsequently pulverized, and processed into mRNA, which was subsequently converted into cDNA to determine serpinA1a gene expression by qPCR. Lumbar L1-L3 was used for histological staining of SafraninO (IVD degeneration score) and AAT-1 (immuno-fluorescent), L3-L5 IVDs for gene expression, L6-S1 for IVD mechanics and L6 vertebra for μCT. Bone marrow was extracted from tibia, femur, and ilium of C57BL6 mice and used as a cell source for osteoclast induction. While M-CSF facilitated the differentiation of marrow cells into macrophages, the combination of M-CSF+RANKL promoted osteoclast formation, and M-CSF+RANKL+IL-1β supported both osteoclast formation and differentiation6. Ten human IVDs from male (n=5) and female (n=5) individuals within the age range of 28-83 years, were collected. The IVDs were categorized based on their degeneration level using the Thompson grading system, ranging from 1 (healthy) to 5 (most degenerated). Subsequently, these IVDs were processed and stained for AAT-1. A 2-way ANOVA was used to compare serpinA1a expression by tissue and sex or osteoclast expression of serpinA1a expression by osteoclast-differentiation by treatment (RANKL, RANKL+IL-1β) and sex. A Student's t-test compared sex for all other comparisons, with statistical significance defined at a p<0.05.

RESULTS: SerpinA1a expression is differentially expressed across mouse tissues, where the order from most to least expression of the tissues harvested is liver, lung, IVD and bone (Fig. 1A). Further, female tissues expressed less serpinA1a than males in lung, IVD and bone cells (mostly osteocytes) by 6-fold, 4-fold and 2-fold, respectively. Compared to male murine IVDs, females expressed 97% less AAT-1 protein (green color) in the IVD, particularly annulus fibrosus (AF) cells (Fig. 1B, B'). Next, we focused on bone marrow-derived osteoclast differentiation because the histological expression of AAT1 was intense in the bone marrow of vertebrae (data not shown). In males, treatment to induce osteoclast differentiation (RANKL or RANKL+IL-1β) reduced serpinA1a gene expression by ~0.4 fold but, in females, the treatment-induced osteoclast differentiation inconsistently reduced serpinA1a expression (interaction: p<0.05) (Fig. 1C). In human IVD, the AAT-1 protein expression in male IVD is less than that of women by 76% (Fig.1D, D'). Investigation of the remaining outcomes is currently ongoing.

DISCUSSION: Males consistently exhibited higher serpinA1a/AAT-1 expression levels than females in various tissues. Notably, the annulus fibrous of the IVD and bones expressed less serpinA1a/AAT-1 among all tissues tested, which could promote greater recruitment of neutrophils and macrophages as occurs in the lungs of people with a genetic variant in SERPINA19. In human IVDs, AAT1 protein expression was greater in male IVDs than female IVDs, highlighting a conserved sexual dimorphism across species. In bone, we harvested a predominately osteocyte-rich tissue for qPCR and the sexual disparity in the expression of serpinA1a was mild. However, the expression of serpinA1a with osteoclast differentiation was strikingly different between biological sex. In myeloid-derived osteoclasts, RANKL promotes osteoclast differentiation and the presence of RANKL downregulated serpinA1a in males and the inclusion of IL-1β further promoted differentiation and suppression of serpinA1a. By contrast in bone marrow-derived female macrophages, RANKL appeared to upregulate serpinA1a during osteoclastic differentiation and IL-1β downregulated serpinA1a with further differentiation. Overall, the sexual dimorphic expression of serpinA1a in the spine and regulation of serpinA1a during osteoclast differentiation suggest that inflammatory signaling may yield greater inflammatory-related consequences to the musculoskeletal system of women than men.

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Fig. 1. (A) Gene expression of serpinA1a in male (n=5) and female (n=5) mouse tissues (Liver, Lung, IVD, bone), (B) Representative immunofluorescent images of AAT-1 (magnification 63x) in CON male (n=5) and female (n=5) IVDs. Blue color (DAPI) represents cell nuclei while green is the AA1 protein expression, L6

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