Loss of RAGE Attenuates Intervertebral Disc-specific Immune Cell Recruiting Chemokines, Bone Resorption, and Vertebral Bone Loss Following IVD Degeneration

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INTRODUCTION: Injury to the intervertebral disc (IVD) initiates an inflammatory cascade that directs the degeneration of the IVD and may affect surrounding tissues such as the vertebral endplates. The vertebral endplates provide transport to and from the IVD and maintains the structural integrity of the vertebra; endplate thinning, and ruptures have been shown to contribute low back pain [1]. This tissue crosstalk stemming from IVD degeneration may be mediated by the multi-ligand alarmin and damage-associated-molecular-patterns (DAMPs) receptor, the receptor for advanced glycation end-products (RAGE). RAGE is known to play critical roles in inflammatory diseases and immune responses, and subchondral bone loss has been observed in models of post traumatic osteoarthritis with protection provided by the ablation of the RAGE [2, 3]. Therefore, the objective of this study is to evaluate the effects of RAGE ablation on the vertebral endplate and IVD-specific chemokines using a model of intervertebral disc degeneration.

METHODS: All procedures were done with WUSM IACUC approval. CC4/5 and CC6/7 IVDs in C57BL/6 (WT) mice (n = 10) and RAGE-/- (n = 5-8) were injured with a 30G bilateral puncture confirmed on X-Ray with adjacent IVDs as internal controls. Vertebral endplates were imaged on a vivaCT40 at a 10µm resoluton prior to injury and 2 weeks following injury. Mice were euthanized after the second scan. A subset of the IVDs were immediately fixed in 4% PFA and paraffin embedded; 10 µm sagittal sections were stained with TRAP to measure osteoclast activity and Safranin-O/Fast Green to quantify IVD degeneration [4]. The remaining IVDS were cultured; media was collected every 3 days and day 6 media was run through a multiplex cytokine panel (Eve Technology Assays). Vertebral endplates were segmented using a custom MATLAB GUI. Weighted averages of the caudal and cranial pairs of vertebral endplates are reported. Paired 2-way ANOVAs were used to determine the effect of injury and genotype in Prism 9.4.0.

RESULTS SECTION: Bilateral puncture of caudal IVD results in quick and sustained IVD degeneration in both WT and RAGE -/- mice. IVD injury and subsequent degeneration coincides with bone loss to the vertebral endplate observed at in WT but not RAGE -/- mice (Fig. 1A). Decreased BV/TV was associated with osteoclast resorption. Image registration confirmed that the resorption was occurring at the pores within the endplate. Osteoclast activity was correlated with IVD degeneration, suggesting that the degenerative process by the IVD may be driving osteoclast differentiation and subsequent bone resorption (Fig. 1B). With deletion of RAGE signaling, the endplates are protected from bone loss despite no changes to IVD degeneration. The chemokine production by the injured WT IVD revealed elevated levels of pro-inflammatory factors TNFα [+10%, $p<0.05$) and IL-15 [+20%, $p<0.05$] and immune cell recruitment factors CCL12(MCP-5) [+261%, $p<0.05$], CCL22(MDC) [+164%, $p<0.05$]; CCL20(MIP-3α) [+708%, $p<0.05$] and CCL17(TARC) [+281% $p<0.05$]. RAGE ablation completely inhibited the production of TNFα and IL-15 in the IVDs (both the control and injured) and prevented the injury-mediated increase of CCL20 (Fig. 1D-F).

DISCUSSION: Despite similar levels of degeneration between WT and RAGE-null animals, there is vertebral endplate bone loss in WT animals 2 weeks following IVD injury that is associated with increased osteoclast resorption. In contrast, the RAGE -/- animals are protected from this bone loss, along with reduced osteoclast activity and suppression of immune-cell recruiting chemokines. Enhanced osteoclast activity may be a direct consequence of the inflammatory cascade created by the IVD-secreted chemokines. TNFα and IL-15, among others, are potent factors that promotes osteoclastogenesis [5-8]. A number of these chemokines are significantly lower in the RAGE -/- mice. While CCL20 was at similar levels in the control IVDs of both genotypes, deletion of RAGE prevented increased expression with injury. TNFα, IL-15 and CCL20 produced by the IVD may a play a pivotal role in vertebral endplate bone loss during degeneration and injury of the IVD.

SIGNIFICANCE/CLINICAL RELEVANCE: The caudal IVD injury is a useful animal model to examine multi-tissue cross talk following IVD injury and degeneration, which may provide key insights into the association between IVD degeneration and low back pain. Vertebral endplates are a key tissue in maintaining homeostasis of the IVD as they provide both mechanical and nutritional support and in addition, have been implicated as a source of chronic low back pain.

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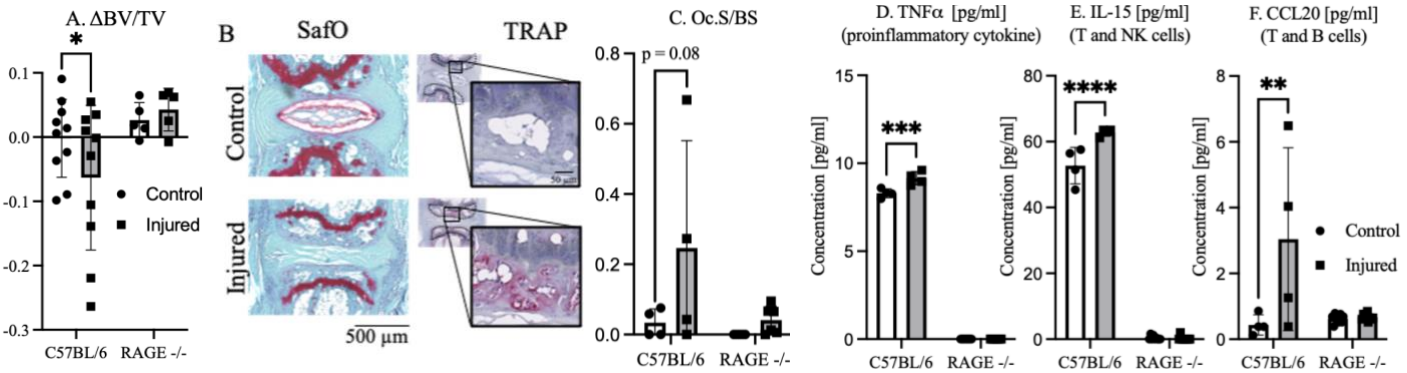

Figure 1: (A) BV/TV following injury normalized to baseline. Bone loss, observed in endplates adjacent to the injured IVD in WT mice, is blunted with deletion of RAGE signaling. (B) SafO and TRAP stained representative histological sections from WT mice demonstrate an association between IVD degeneration and increased osteoclast activity. (C) Osteoclast surface per bone surface in WT and RAGE -/- where we see increased osteoclast activity with injury that is diminished with deletion of RAGE signaling. (D-F) Chemokine production of the IVD 2 weeks following injury where we see an increase in the WT mice of (D) TNFα (E) IL-15 and (F) CCL20 where RAGE deletion reduces expression of (D) TNFα and (E) IL-15 to undetectable levels in the control and injured IVD and prevents upregulation of (F) CCL20.