Examining the Role of CCR2 in Persistent Intervertebral Disc Inflammation

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INTRODUCTION: Low back pain is the leading cause of years lived with disability and intervertebral disc (IVD) degeneration (IDD) is a known contributor.[1,2] IDD is characterized by an inflammatory microenvironment with persistently elevated pro-inflammatory cytokines and chemokines.[2] The production of chemokines in the IVD has been shown to promote macrophage infiltration during degeneration, but the role of inflammation during IDD and crosstalk with other immune cells is unknown.[3] Previous work from our lab using an inducible conditional *Ikk2ca* mouse model (IKKβCA) to induce persistent disc inflammation showed severe IDD including extracellular matrix degradation, disruption of nucleus pulposus (NP) and annulus fibrosus (AF) structure, and upregulation of pro-inflammatory cytokines and chemokines.[4] Interestingly, this model showed increased immuno-positivity of pan-macrophage marker F4/80 in the outer AF, suggesting a macrophage-mediated mechanism contributing to the progression of severe IDD. Notably, a recent study showed that knocking-out *Ccr2*, a chemokine receptor found on monocytes and macrophages, is protective against injury-induced IDD, highlighting CCR2 as a potential therapeutic target.[5] To investigate this mechanism further, this study aimed to target macrophage infiltration during inflammation through CCR2. We hypothesized that infiltrating macrophages express CCR2, and that partial deletion of CCR2 will be protective against IDD in the IKKβCA mouse model.

METHODS: Experiments were performed on male and female mice with IACUC approval. **IKKβCA;*Ccr2$^{RFP/+}$* Mice:** Inducible conditional *Ikk2ca* mice were bred with *RFP:Ccr2* knock-in/knock-out reporters to create a mouse exhibiting persistent localized inflammation by constitutively activating canonical NF-κB pathway in *Acan*+ cells with global partial deletion of *Ccr2* (*AcanCre$^{ERT2/+}$;Ikk2ca$^{fl/fl}$;Ccr2$^{RFP/+}$*, referred to as IKKβCA;*Ccr2$^{RFP/+}$*). Cre-mediated recombination was induced in 3-month-old IKKβCA;*Ccr2$^{RFP/+}$* mice via intraperitoneal tamoxifen injections daily for 5 consecutive days. *Acan$^{+/+}$;Ikk2ca$^{fl/fl}$;Ccr2$^{RFP/+}$* (Control;*Ccr2$^{RFP/+}$*) mice were used as controls. Mice were euthanized at 2 months post recombination. **Gene Expression:** NP (n=3-5 mice/genotype) and AF (n=4-5 mice/genotype) tissue from caudal discs were isolated separately and RNA was extracted. Relative gene expression was quantified normalized to ribosomal protein s29 (*Rps29*) gene using ΔΔC$_T$ method. **Disc height index (DHI):** Fluoroscopic images of caudal spine in sagittal plane at coccygeal (Co) 7-8 and Co8-9 levels were captured for DHI assessment using ImageJ (n=4-5 mice/genotype).[6] **Histology:** Motion segments Co7-8 and Co8-9 were harvested, cryoprotected in 30% sucrose, cryoembedded, and cryosectioned using cryofilm adhesive. 10 μm-thick sections were stained with Safranin O/Fast Green (SafO) (n=4-5 mice/genotype). **Immunofluorescence:** Sections were fixed in 10% formalin, blocked in 3% bovine serum albumin, incubated with antibodies against F4/80 and CCR2 (both 1:200), and incubated with secondary antibodies. Slides were mounted using a mounting medium with DAPI and imaged (n=4-5 mice/genotype). **Statistics:** Two-way ANOVA (GraphPad Prism 10) with Fisher's LSD post-hoc test determined significance $p<0.05$; data is displayed as mean with standard deviation.

RESULTS: Gene expression of pro-inflammatory chemokine *Ccl2* increased in IKKβCA;*Ccr2$^{RFP/+}$* NP and AF tissue compared to Control;*Ccr2$^{RFP/+}$*, with NP exhibiting a greater level than AF (Figure 1A). Gene expression of chemokine receptor, *Ccr2,* and macrophage marker, *Adgre1* (encoding F4/80), were significantly upregulated in the AF, but not in the NP of IKKβCA;*Ccr2$^{RFP/+}$* mice compared to Control;*Ccr2$^{RFP/+}$* (Figure 1A). DHI was significantly decreased in IKKβCA;*Ccr2$^{RFP/+}$* mice compared to Control;*Ccr2$^{RFP/+}$* mice at both levels (Figure 1B). Histomorphological examination of SafO-stained sections exhibited degenerative changes in IKKβCA;*Ccr2$^{RFP/+}$* mice including loss of NP cells, disruption of the NP/AF boundary, and areas of increased cellularity in the endplate and AF periphery (Figure 1C). Immunostaining of F4/80 (green) and CCR2 (red) showed increased immuno-positivity and co-localization in the endplate and AF periphery regions of IKKβCA;*Ccr2$^{RFP/+}$* mice compared to Control;*Ccr2$^{RFP/+}$* (Figure 1D-F).

DISCUSSION: The goal of this study was to examine the role of CCR2 in macrophage infiltration during persistent disc inflammation. From our previous study, we showed that IKKβCA caused a degenerative phenotype in the disc.[4] Interestingly, partial deletion of *Ccr2* in the same inflammatory model was not protective and continued to exhibit evidence of degeneration. IKKβCA;*Ccr2$^{RFP/+}$* disc tissue showed increased gene expression of *Ccl2*, the primary ligand of CCR2, as seen previously.[4] Our findings of increased macrophage-associated gene expression (*Adgre1* and *Ccr2*) suggest that partial deletion of *Ccr2* does not prevent macrophage recruitment to inflamed IVD. Dense clusters of F4/80+ and CCR2+ cells in the AF periphery and endplate regions further support this. Additionally, decreased DHI and histological evidence of a degenerative phenotype in IKKβCA;*Ccr2$^{RFP/+}$* mice point to a lack of protection from IDD by partial *Ccr2* deletion. In a recent injury-induced herniation mouse model study, partial *Ccr2* deletion also failed to recover DHI to similar levels as sham controls, whereas the full knock-out of *Ccr2* was found to be protective.[5] Future work will investigate a constitutive CCR2 knock-out (full knock-out) to further examine the role of CCR2 in macrophages during persistent disc inflammation and IDD.

SIGNIFICANCE/CLINICAL RELEVANCE: Insight into immune signaling pathways responsible for immune cell recruitment during persistent inflammation informs development of targeted immunomodulatory therapeutic strategies for IDD.

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REFERENCES: [1] Buchbinder, R. et al., (2013). *Best Pract Res Clin Rheumatol.* [2] Silva, J. et al., (2019) *Front Immunol.* [3] Dou, Y. *et al*, (2025) *Bone Res.* [4] Burt, K. *et al*, (2024) *Sci Adv.* [5] Jin, L. *et al*, (2025) *OA & Cart.* [6] Tajerian, M. *et al*, (2011) *Molecular Pain*

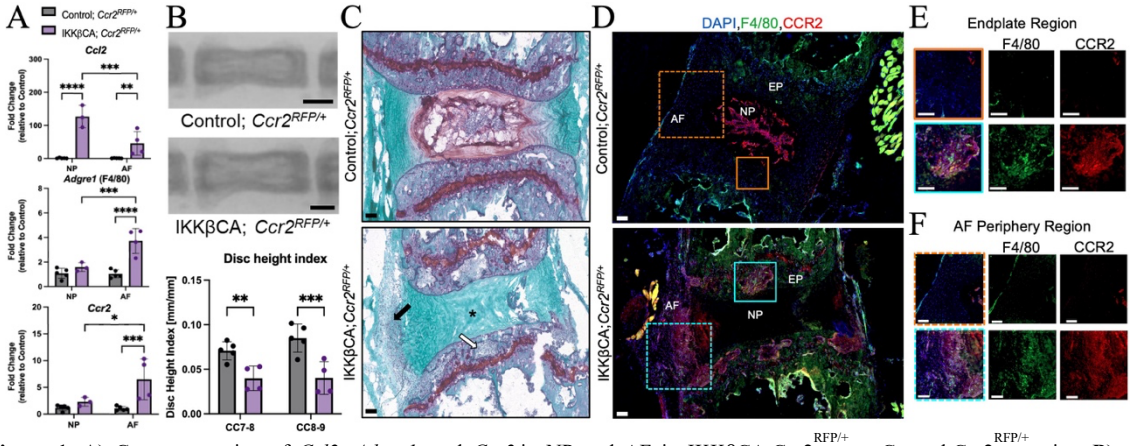

Figure 1. A) Gene expression of *Ccl2*, *Adgre1*, and *Ccr2* in NP and AF in IKKβCA;Ccr2$^{RFP/+}$ or Control;Ccr2$^{RFP/+}$ mice. **B)** Representative fluoroscopic images and disc height index quantification. **C)** Representative images of Safranin O/Fast Green staining. (Black arrow: cellularity in AF periphery, White arrow: cellularity in endplate (EP), Black asterisk: loss of NP cells) **D-F)** Representative images for DAPI (Blue), F4/80 (green), and CCR2 (red) staining in **(D)** whole disc and regions of interest in the endplate (**E**) and AF (**F**) periphery regions. Scale bar = 100μm. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001