Inhibition of TLR adaptor signaling molecule, MyD88 on intervertebral disc homeostasis: in vitro, ex vivo studies

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INTRODUCTION

The cytosolic adaptor myeloid differentiation factor 88 (MyD88) was originally shown to be involved in signaling by the type 1 IL-1 receptor (IL-1R1) and subsequently in signaling by various TLRs. Specifically, TLR-2/TLR-4 ligands drive pro-catabolic chondrocyte responses through MyD88, and are known to be responsible for early cartilage proteoglycan loss as typically observed in early arthritis1,2. In fact, enhanced chondrocyte proteoglycan synthesis was found in the MyD88 gene-deficient mice3 suggesting the crucial role of TLR and the TLR adaptor molecule, MyD88 in arthritis. Despite a relatively large number of reports on TLR-MyD88 axis in the arthritic joint, there is no information on functional role of TLRs and MyD88 in IVD homeostasis. We hypothesize that TLRs and their key downstream cofactor, MyD88 have particular significance in the pathogenesis of spine disc degeneration and in the development of low back pain. We investigated the inhibition of TLR-generated pathways by using our disc degeneration animal model and a cell membrane permeable MyD88 homodimerization inhibitor peptide, which functions as a decoy by binding to the MyD88 TLR domain. No toxicity has been reported by using this inhibitor peptide.

METHODS AND RESULTS: In vitro Studies: The inhibition of LPS- and IL-1 mediated catalytic process by MyD88 peptide inhibitor. Human NP cells in monolayer culture were stimulated with either IL-1 (10ng/mL) or LPS (10μg/mL) in the absence or presence of MyD88 peptide inhibitor (MyD88i, 150 μg/mL) at the concentration of 100 μg/mL. After 24 hrs, the conditioned medium and cells were analyzed for secreted MMP-13 (a), MMP-1 (b), ADAMTS (-4,-5) production by both western blot (WB) and qPCR and enzyme activity by zymography (c). We also investigated altered levels of TLR2 and iNOS (not shown). We used18s rRNA (qPCR) and β-actin (WB) for normalization. Similar results were obtained using bovine disc cells (data not shown). Zymogram of supernatant was performed by using equal volumes of sample on 10 % polyacrylamide gel containing 1 mg/mL gelatin. Band images were digitally captured and the intensity of the Bands (pixels/band) was obtained using the ImageJ densitometry analysis software (www.imagej.nih.gov) and expressed in arbitrary optical density units. Data shown are cumulative of two experiments. P values presented as mean ± standard deviation; Significance was set at p < 0.05.

Ex vivo Studies: Mouse lumbar discs were dissected, and MyD88 peptide inhibitor (MyD88i) at the concentration of 150μg per disc was intradiscally injected en bloc using a 30-guage needle (30 G, 1.5 μL volume). Injected discs were then separated and incubated in DMEM/Ham’s F-12 medium supplemented with 1% mini-ITS. After 24 hrs, the MyD88i-pre-injected discs were challenged with a catabolic factor, either IL-1 (100 ng/mL) or LPS (10 μg/mL) and further incubated for 3 days. Harvested discs were fixed in 4 % paraformaldehyde, and then decalcified in EDTA, which was changed every 5 days. The decalcified discs were paraffin embedded.

CONCLUSION & DISCUSSION: Our data reveal that the TLR adaptor molecule MyD88 plays a central role in the transmission of innate immune cytokine-like signals by promoting catabolic responses in the IVD. MyD88 may potentially modulate disc cell differentiation, disc remodeling and homeostasis, as well as repair responses in the discs. In degenerative human discs, MyD88 itself is not upregulated, but its cognate receptors (e.g., TLR2 and TLR4) are highly increased indicating that the MyD88-TLR axis is pathologically activated in the discs. Based on the present findings, further studies to elucidate the in vivo roles of MyD88 and TLR2/TLR4, in the progression of disc degeneration are warranted.

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