The Response of MyD88-Transduced Human Bone Marrow Mesenchymal Stem Cells to Lipopolysaccharide and Interleukin-1-β Stimulation In Vitro

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Introduction
Mesenchymal stem cells (MSCs) are somatic, multipotent stromal cells with potent pro-regenerative and anti-inflammatory properties. MSCs can be isolated from a patient’s bone marrow tissue, expanded in vitro, and ultimately re-administered as a therapeutic agent. There is great potential in utilizing these properties to treat autoimmune diseases, like Inflammatory Bowel Disease (IBD), in humans and veterinary species.

IBD (Crohn’s Disease and Ulcerative Colitis) affects millions of people worldwide and is also prevalent in dogs and cats. It is characterized by damage to the gut epithelial barrier, resulting in acute and chronic or recurrent inflammation. MSCs transplanted into laboratory animal species have shown great promise in treating models of IBD,7,8 prompting several human clinical trials.²⁻⁴

MSCs are activated by inflammatory mediators. Binding of lipopolysaccharide (LPS) and interleukin-1 (IL1ß) to MSC receptors prompts a signaling cascade through the adapter protein MyD88. This pathway results in nuclear factor kappa B (NF-κB) translocation from the cytoplasm to the nucleus. NF-κB is a transcription factor that leads to expression of an array of inflammatory genes. In MSCs, NF-κB translocation through MyD88 recruitment is believed to promote transcription of key pro-regenerative and anti-inflammatory genes.¹⁻³

Hypothesis
Downregulation of MyD88 will enhance MSCs response to LPS and IL1ß, increasing NF-κB nuclear translocation and transcription of the anti-inflammatory genes (Cox2, IOD, and IL1ß).

Methods
Experimental Design
8001L, 8011L, and 8013L, three human bone-marrow mesenchymal stem cell lines (hBM-MSC), were transduced with a vector carrying the MyD88 gene. Cells were confirmed to be expressing higher levels of MyD88 than their non-transduced counterparts (Figure 2D).

Immunofluorescence
• Stimulated cells for 2 hours with: 10 ng/mL IL1ß, 200 ng/mL LPS, and RNA-free MSC media without additives (control).
• Fixed cells with 4% formaldehyde.
• Stained with primary antibody against NF-κB and secondary antibody, and DAPI.
• 2D-imaged the stained cells using the Leica TCS SP8 STED 3X confocal microscope at the UC Davis Health Sciences District Advanced Imaging Facility.
• Quantified ratios of nuclear to cytoplasmic fluorescence intensity using Imaris software.

qRT-PCR
• Stimulated cells for 6 hours with: 10 ng/mL IL1ß, 200 ng/mL LPS, and RNA-free MSC media without additives (control).
• Used cells and RNA extraction methods from CSKZ, IOD, ELRA, and calculated ΔCt values for each stimulated sample. We added TNFα and IL1ß pro-inflammatory genes for further research.

Results

Figure 2: There is increased NF-κB fluorescent signal (red) in the nucleus of the stimulated cells (B-D) compared with non-stimulated cells (A). To accurately quantify nuclear NFκB translocation and compare nuclear-cytoplasmic fluorescence intensity between non-transduced and transduced cell lines, we used Imaris. D. GFP (green) is evident, indicating successful transduction. The MyD88 plasmid contained a GFP tag.

Figure 3: Mean nuclear to cytoplasmic fluorescence intensity ratios on the red channel using Imaris.

Figure 4: Relative RNA transcript copies of genes for each MyD88 transduced and non-transduced cell line.

Conclusions
• The data indicate that MyD88 overexpression in MSCs enhances subsequent transcription of anti-inflammatory mediators.

Future Research
We expected increased transcription of anti-inflammatory mediators to follow the increased use of NF-κB nuclear translocation, yet the data did not fit this hypothesis. The NF-κB signaling pathway is complex, and it is possible that there truly is a lack of a relationship between increased MyD88 expression and transcription of inflammatory mediators.

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References

Figure 3: Fold-change values for IL1ß and LPS-stimulated control and MyD88-transduced cells, graphed by cell line. A. ΔCt sample control. B. ΔCt: stimulated control and ΔCt: overexpressed ΔCt changes (ΔCt = Ct stimulated - Ct control).

Figure 4: Relative RNA transcript copies of genes for each MyD88-transduced and non-transduced cell line.