DOES OVEREXPRESSION OF MYD88 ENHANCE MSC RESPONSE TO STIMULATION?

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Mesenchymal Stem Cells and Inflammatory Disease

- Multipotent stromal cells
- Immunomodulatory
- Treatment of autoimmune disease
  - IBD in humans, dogs, cats
  - Success in laboratory models, less efficacious in clinical trials
- Need for research into pathways
NUCLEAR FACTOR KB (NF-KB) PATHWAY

LPS → TLR-4 → MyD88 → NF-KB → IL1R, IL1β

MyD88

NF-KB

IL1R, IL1β

Cox2, IDO, IL1RA, TNFa, IIlb
Hypothesis

- Increased expression of MyD-88 by human bone marrow-mesenchymal stem cell (hBM-MSC) will enhance their response to lipopolysaccharide (LPS) and Interleukin-1-b (IL1b) stimulation in vitro.

Specific Aims

- Determine NFκB nuclear translocation in MyD-88-transduced hBM-MSC in response to LPS and IL1b stimulation in vitro.
- Determine COX2, IDO, IL1RA, TNFa, and IL1b transcription levels in MyD-88-transduced hBM-MSC in response to LPS and IL1b stimulation in vitro.
**METHODS**

<table>
<thead>
<tr>
<th>3 cell lines naïve hBM-MSC</th>
<th>3 cell lines MyD88- transduced hBM-MSC</th>
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<tbody>
<tr>
<td>No stimulation</td>
<td>No stimulation</td>
</tr>
<tr>
<td>10 ng/mL IL1β</td>
<td>10 ng/mL IL1β</td>
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<tr>
<td>200 ng/mL LPS</td>
<td>200 ng/mL LPS</td>
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- Immunofluorescence (IF)
- Quantitative Real Time Polymerase Chain Reaction (RT-PCR)
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- Plated MSCs on chamber slides
  - Poor adherence
  - Switched to glass coverslips in 24-well plates (Thanks Dr. Woolard!)
- 2 hour stimulation followed by formalin fixation
- Stained with NF-KB p-65 primary antibody
- Donkey anti-rabbit secondary antibody conjugated with Alexa fluor 594
- DAPI nuclear stain
- Data Acquisition and analysis- Confocal Microscope and Imaris software
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8013 + MyD88 unstimulated

8013 + MyD88 200 ng/mL LPS
RT-PCR

- Plated cells into 6-well plates
- 6 hour stimulation
- Cell lysis and RNA extraction with Qiagen RNeasy Kit
- cDNA synthesis
- RT-PCR of cDNA samples to Calculate Ct values
  - Allows determination of relative amounts of RNA transcripts at the time of lysis
RESULTS

- Immunofluorescence - nuclear translocation

![Graph showing Nuc/Cyto ratio for different conditions](image)
RESULTS

- RT-PCR - Gene Transcription
CONCLUSIONS

- Increase in NFkB nuclear translocation appears to correlate with MyD88 transduction
  - One cell line evaluated
- MyD88 overexpression and immunomodulatory gene expression do not seem correlated
  - Lack of conditional testing for MyD88 transduced?
  - Effect of transduction?
  - Real relationship?


THANK YOU!

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