Zone dependent gene expression following innate immune activation in AML12 hepatocytes

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**INTRODUCTION**

The adult liver has functionally different zones that are metabolically distinct and respond to immune activation (e.g., LPS and downstream TLR4 activation, IL1B/IL6).

In hepatocytes, the main driver of innate immunity is the acute phase response (APR), however, there is little known on whether this response is zonated.

Nuclear translocation of B-catenin is one of the main drivers of liver zonation. CHIR is a GSK3 inhibitor that induces B-catenin nuclear translocation.

We hypothesize that the manipulation of B-catenin, via CHIR, in AML12 hepatocytes will induce a zone specific distribution of APR and immune activating genes after TLR4 activation.

**METHODS**

**Cell culture model:** Cultured AML12 hepatocyte cells

**Cell culture treatments:** Cells were exposed to the following treatments:
- LPS dose: 100ng/ml; IL-1B/IL6 dose: 10ng/ml; 1, 5hr; CHIR 10uM 24,48hr 7,14-day pre Tx

**Outcome measures:** mRNA expression was evaluated by qPCR for tnf, il6, ccl5, crp, saa1, fga, apcs

Immunostaining was achieved to assess CRP expression after 5mg/kg LPS

Statistical analysis was completed using GraphPad prism.

**RESULTS**

*Fig 1: Immunostaining of zone-specific acute phase protein, CRP* Portal Triad + Central Vein (20x)

*Fig 2a: LPS exposure induces cytokine gene expression*

*Fig 2b: LPS exposure induces acute phase response gene expression*

*Fig 3a: IL1B+IL6 exposure induces cytokine gene expression*

*Fig 3b: IL1B+IL6 exposure induces acute phase response gene expression*

**CONCLUSION**

We demonstrated an innate immune response characterized by cytokine and APR activation after LPS and IL1B/IL6 exposures.

Culturing AML12 hepatocytes in CHIR resulted in increased expression of zonated markers, cyp2e1 and axin2.

Future directions will include manipulation of metabolic zonation through nuclear translocation of B-catenin, to better understand the mechanism of how the innate immune response is activated in fully developed liver vs. an undeveloped neonatal liver.