Inflammatory Response of Kupffer Cells in 3D Bioprinted Human Liver Tissues

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**Abstract**

Hepatic inflammation, mediated by Kupffer cells (KC), can exacerbate hepatic necrosis during drug-induced liver injury. KC are often employed in co-culture with hepatocytes to investigate the potential for inflammation in response to stimuli, such as the prototypical inducer LPS/LPS (LPS), however, such systems seldom include other nonparenchymal cells and fail to recapitulate the complex 3D interactions present in native liver. In the current study, LPS-mediated activation of KC at 24 and 72hrs was investigated in 3D bioprinted human liver tissues (exVivo3D, Organovo, San Diego, CA). Induction of pro- and anti-inflammatory cytokines was measured via electromicroimmunostaining in tissues comprising primary hepatocytes, stellate cells, and endothelial cells (Hep:KC), and compared to tissues containing KC (Hep:KC+). Independent experiments were conducted comparing two KC donors (Hep:KC+D1 (male) and Hep:KC+D2 (male)) in co-culture with hepatocytes to investigate the potential for inflammation in specific response. The current data demonstrate the cytokine release were distinct between KC donors, with greater induction in the Hep:KC+D2 tissues compared to Hep:KC-. To compare donor-specific responses cytokine release were measured via two-way ANOVA with multiple comparisons.

**Results**

**LPS Stimulates Cytokine Release in exVivo3D Liver Tissues (Hep:KC-) and Liver Tissues Containing Kupffer Cells (Hep:KC+).**

**Table 1:** LPS-induced significant release of cytokines in both Hep:KC+ and Hep:KC-D2 tissues with stronger sustained induction to 72 hrs. Cytokines were in Hep:KC+ and Hep:KC-D2 tissues were measured (n=4, standard deviation). To determine the effects of LPS treatment on cytokine levels independently in Hep:KC+ and Hep:KC-D2 tissues, data was analyzed via one-way ANOVA with multiple comparisons. Significant differences due to LPS treatment (Hep:KC+ vs. Hep:KC-D2) within a time point are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). Significant differences in cytokine levels over time (24 hrs vs. 72 hrs) within a treatment group are indicated by daggers (†p<0.05, ††p<0.01, †††p<0.001, ††††p<0.0001).

**CONCLUSION:** LPS-induced cytokine release was significantly greater in Hep:KC+ tissues compared to Hep:KC-D2 tissues at 24 hrs following LPS treatment of Hep:KC+ tissues.

**Figure 2:** Experimental timeline of LPS treatment. Tissues were bioprinted and cultured for 2 days. LPS treatment of Hep:KC+ tissues began on day 3. Media samples to determine cytokine release from tissues were collected at 24 hrs and 72 hrs post-challenge (orange arrow).

**Figure 3:** LPS-mediated cytokine induction was significantly greater in Hep:KC+ tissues compared to Hep:KC-D2 tissues at both 24 and 72 hrs. EXP 1: Hep:KC+ tissues were bioprinted and challenged with LPS (100 ng/mL) for 24 and 72 hrs. Media samples were collected at 24 hrs and 72 hrs post treatment.

**Table 2:** LPS-induced significant release of cytokines in both Hep:KC+ and Hep:KC-D2 tissues with stronger sustained induction to 72 hrs. Cytokines were measured in Hep:KC+ and Hep:KC-D2 tissues (n=4, standard deviation). To determine the effects of LPS treatment on cytokine levels independently in Hep:KC+ and Hep:KC-D2 tissues, data were analyzed via one-way ANOVA with multiple comparisons. Significant differences due to LPS treatment (Hep:KC+ vs. Hep:KC-D2) within a time point are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). Significant differences in cytokine levels over time (24 hrs vs. 72 hrs) within a treatment group are indicated by daggers (†p<0.05, ††p<0.01, †††p<0.001, ††††p<0.0001).

**CONCLUSION:** LPS-induced cytokine release was significantly greater in Hep:KC+ tissues compared to Hep:KC-D2 tissues at 24 hrs following LPS treatment of Hep:KC+ tissues.

**Summary**

- **A robust response to inflammatory stimuli was observed in exVivo3D Liver Tissues containing Kupffer cells.**
- The inflammatory response to LPS was greater in tissues containing Kupffer cells versus tissues devoid of Kupffer cells.
- The LPS-mediated inflammatory response was sustained in exVivo3D liver tissues containing Kupffer cells.
- Consistent with literature reports, donor-dependent effects on pro-inflammatory cytokine production were seen following LPS treatment; induction of TNF-α, IL-6, IL-13, and IL-4 levels was significantly different between donors.
- Additional studies are needed to establish the contribution of factors such as gender and/or donor-to-donor variability in basal and LPS-induced cytokine profiles.