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

Rhiannon N. Hardwick, Janice Hampton, Dean Perusse, Deborah Nguyen | Organovo, 6275 Nancy Ridge Drive Suite 110, San Diego, CA 92121

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Abstract

Hepatic inflammation, mediated by Kupffer cells (KC), can exacerbate hepatocellular damage 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 lipopolysaccharide (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 (exVive3D™; Organovo, San Diego, CA). Induction of pro- and anti-inflammatory cytokines was measured via electrochemiluminescence in tissues comprising primary hepatocytes, stellate cells, and endothelial cells (Hep:KC-), and compared to tissues containing KC. Independent experiments were conducted comparing two KC donors (Hep:KC+D1 [male] and Hep:KC+D2 [female]) with all other cell donors held constant. LPS stimulated TNF-α, IL-1β, IL-12p70, IL-10, IL-2, IL-13, and IL-4 levels in Hep:KC+D1 tissues at 24hrs compared to untreated, with sustained induction to 72hrs. TNF-α, IL-10, and IL-8 exhibited greater induction in Hep:KC+D1 tissues compared to Hep:KC-. All cytokines were increased at 24hrs in Hep:KC+D2 tissues treated with LPS compared to untreated, with sustained induction of IL-8, IL-1β, IFN-γ, IL-2, and IL-12p70. IL-8, IL-6, and IL-10 induction was greater in Hep:KC+D2 tissues compared to Hep:KC-. To compare donor-specific responses to LPS, cytokine levels were normalized to untreated Hep:KC-, and the fold change in LPS-treated Hep:KC+ tissues was calculated. Patterns of LPS-induced cytokine release were distinct between KC donors, with greater induction in the female donor. However, IL-1β, IL-10, IL-2, IL-13 exhibited no variation in donor-specific response. The current data demonstrate the ability to measure a robust donor-specific KC response to inflammatory stimuli, thus enabling investigation of immune-mediated drug-induced liver injury in a 3D human liver tissue.

Experimental Design

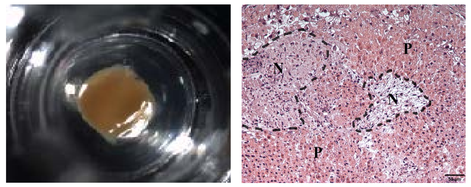

Figure 1: (Left) Representative single image of exVive3D human liver tissue, which measures 2.5 x 2.5 mm with 0.5 mm thickness. (Right) Representative H&E image of exVive3D human liver tissue showing distinct non-parenchymal (N) and parenchymal (P) compartments.

- 3D human liver tissues (exVive3D) comprised of hepatocytes, hepatic stellate cells, and endothelial cells (Hep:KC-) as well as tissues containing hepatocytes, hepatic stellate cells, endothelial cells and Kupffer cells (Hep:KC+) were bioprinted and allow to mature for 3 days.
- Two independent experiments were performed with two Kupffer cell donors. Donor characteristics are shown below.

Kupffer Donor	Age (years)	Sex	TNF-α Baseline (pg/mL)	IL-6 Baseline (pg/mL)
Donor 1	57	M	170.2	46.5
Donor 2	42	F	0.2	0.2

Table 1: Kupffer cell donor characteristics provided by vendor.

- Daily LPS treatment (100 µg/mL) began on Day 3 of culture and continued for 72 hrs.
- Media samples were collected at 24 hrs and 72 hrs of LPS treatment for cytokine analysis via electrochemiluminesce on a Meso Scale Discovery QuickPlex SQ 120 instrument.

Figure 2: Experimental timeline of LPS treatment. Tissues were bioprinted and allowed to mature for 3 days. LPS treatment of Hep:KC- and 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 arrows).

Results

LPS Stimulates Cytokine Release in exVive3D Liver Tissues (Hep:KC-) and Liver Tissues Containing Kupffer Cells (Hep:KC+)

Experiment 1: Kupffer Donor 1

Cytokine (pg/mL ± SD)	Hep:KC-				Hep:KC+D1			
	LPS-	LPS+	LPS-	LPS+	LPS-	LPS+	LPS-	LPS+
	24 hrs		72 hrs		24 hrs		72 hrs	
IL-6	1010.86 ±164.51	1170.75 * ± 90.43	170.78 ±48.06 ††††	249.56 ±8.55 ††††	347.62 ±168.35	702.56 *** ±230.47	66.76 †† ±32.54	214.22 †††† ±50.54
IL-8	2070.94 ±429.90	3825.39 **** ±42.43	1013.73 †††† ±371.66	2658.63 **** ±348.48 ††††	3716.06 ±55.30	3938.74 ±34.15	3420.78 ±286.40	7554.72 **** ±499.07 ††††
IL-10	1.16 ±0.19	4.28 **** ±0.55	0.80 ±0.26	2.72 **** ±0.71 ††††	2.86 ±0.77	11.43 **** ±4.39	1.84 ±0.52	5.56 * ±0.26 †††
IL-1β	2.58 ±0.31	5.31 **** ±0.38	1.98 ±0.58	4.24 **** ±0.43 †††	6.59 ±1.33	12.09 **** ±2.29	4.39 † ±0.19	7.72 ** ±0.58 ††††
IFN-γ	0.00 ±0.00	3.17 **** ±0.69	0.001 ±0.0004	0.004 †††† ±0.01	4.44 ±1.33	14.02 **** ±4.19	0.00 †††† ±0.001	0.02 †††† ±0.01
IL-2	0.48 ±0.13	1.90 **** ±0.43	0.33 ±0.21	1.19 ** ±0.28 ††	2.11 ±0.92	6.30 **** ±1.52	1.47 ±0.59	4.36 **** ±0.68 ††
IL-12p70	0.67 ±0.23	1.36 ** ±0.44	0.17 ±0.22	0.73 † ±0.32	1.52 ±0.54	4.44 **** ±1.24	0.61 ±0.25	2.45 ** ±0.17 †††
IL-13	4.47 ±1.08	7.84 **** ±1.15	4.55 ±0.96	6.37 ±1.04	8.14 ±1.72	14.42 **** ±1.87	6.98 ±1.37	10.60 ** ±2.15 †††
IL-4	1.51 ±0.35	2.01 * ±0.40	0.52 ††† ±0.13	0.92 †††† ±0.09	1.05 ±0.34	2.46 **** ±0.46	0.42 †† ±0.13	1.36 *** ±0.06 ††††
TNF-α	0.69 ±0.10	3.79 **** ±0.60	0.63 ±0.14	2.11 **** ±0.41 ††††	2.38 ±0.93	8.50 **** ±1.89	1.79 ±0.17	5.11 *** ±0.45 ††††

Table 2: LPS Induced a Significant Release of Cytokines in Both Hep:KC- and Hep:KC+D1 Tissues with Strong Sustained Induction to 72 hrs

Cytokine levels in Hep:KC- and Hep:KC+D1 tissues are shown (pg/mL ± standard deviation). To determine the effects of LPS treatment on cytokine levels independently in Hep:KC- and Hep:KC+D1 tissues, data was analyzed via one-way ANOVA with multiple comparisons. Significant differences due to LPS treatment (-LPS vs. +LPS) 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 all cytokines measured at 24 hrs in Hep:KC- tissues with sustained induction of half of those measured at 72 hrs. All cytokines except for IL-8 were induced by LPS in Hep:KC+D1 tissues at 24 hrs, with sustained induction of most at 72hrs. IL-8 was induced later, at 72 hrs, in Hep:KC+D1 tissues.

Experiment 2: Kupffer Donor 2

Cytokine (pg/mL ± SD)	Hep:KC-				Hep:KC+D2			
	LPS-	LPS+	LPS-	LPS+	LPS-	LPS+	LPS-	LPS+
	24 hrs		72 hrs		24 hrs		72 hrs	
IL-6	715.52 ±484.82	632.64 ±451.51	205.20 ±152.82	300.55 ±204.85	696.73 ±396.58	1975.21 ** ±452.23	144.36 ±88.74	616.18 †† ±152.57
IL-8	2175.56 ±1174.40	5789.38 **** ±1088.18	1767.03 ±892.73	4179.36 *** ±795.99 †	2649.95 ±318.65	13858.73 ** ±4100.46	1784.48 ±95.01	6750.48 † ±2159.95
IL-10	2.77 ±1.18	5.97 *** ±1.79	1.46 ±0.56	3.51 * ±1.09 ††	4.24 ±0.44	13.96 ** ±3.51	1.87 ±0.74	4.94 †† ±1.07
IL-1β	5.36 ±2.01	9.28 *** ±1.09	4.22 ±1.32	7.53 ** ±0.98	6.71 ±0.58	13.51 *** ±0.44	4.65 ±0.65	8.81 * ±2.22 ††
IFN-γ	2.27 ±1.46	9.30 **** ±2.07	1.25 ±0.39	5.73 **** ±1.81 ††	5.96 ±2.06	24.77 *** ±6.07	3.23 ±1.58	11.34 †† ±2.25
IL-2	1.30 ±0.54	3.36 **** ±0.70	0.97 ±0.21	2.34 *** ±0.50 ††	2.18 ±0.65	7.67 ** ±1.93	1.97 ±0.92	4.32 † ±0.42
IL-12p70	1.28 ±0.81	3.14 ** ±1.11	0.53 ±0.26	1.97 * ±0.88	1.87 ±0.80	7.43 ** ±1.82	0.57 ±0.18	3.25 †† ±0.89
IL-13	9.07 ±3.01	14.63 *** ±1.43	8.07 ±1.62	12.43 ** ±2.09	10.15 ±2.42	16.39 ±3.19	8.57 ±1.33	13.10 ±2.63
IL-4	2.70 ±1.75	3.02 ±1.59	0.93 ±0.53	1.82 ±1.02	3.38 ±1.27	7.85 ** ±1.12	1.03 ±0.42	3.37 †† ±0.75
TNF-α	2.15 ±1.21	6.59 **** ±1.36	1.33 ±0.53	3.48 ** ±0.98 ††††	2.72 ±0.65	14.03 **** ±2.91	1.50 ±0.40	4.46 ††† ±0.72

Table 3: LPS Induced a Significant Release of Cytokines in Both Hep:KC- and Hep:KC+D2 Tissues with Less Sustained Induction to 72 hrs

Cytokine levels in Hep:KC- and Hep:KC+D2 tissues are shown (pg/mL ± 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 (-LPS vs. +LPS) 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 the release of all cytokines measured except IL-6 and IL-4 in Hep:KC- tissues at 24 hrs with sustained induction at 72 hrs. All cytokines except IL-13 were induced at 24 hrs in Hep:KC+D2 tissues with sustained induction of only IL-1β at 72 hrs.

Cytokine Induction is Significantly Greater in Hep:KC+ Tissues Following LPS Treatment

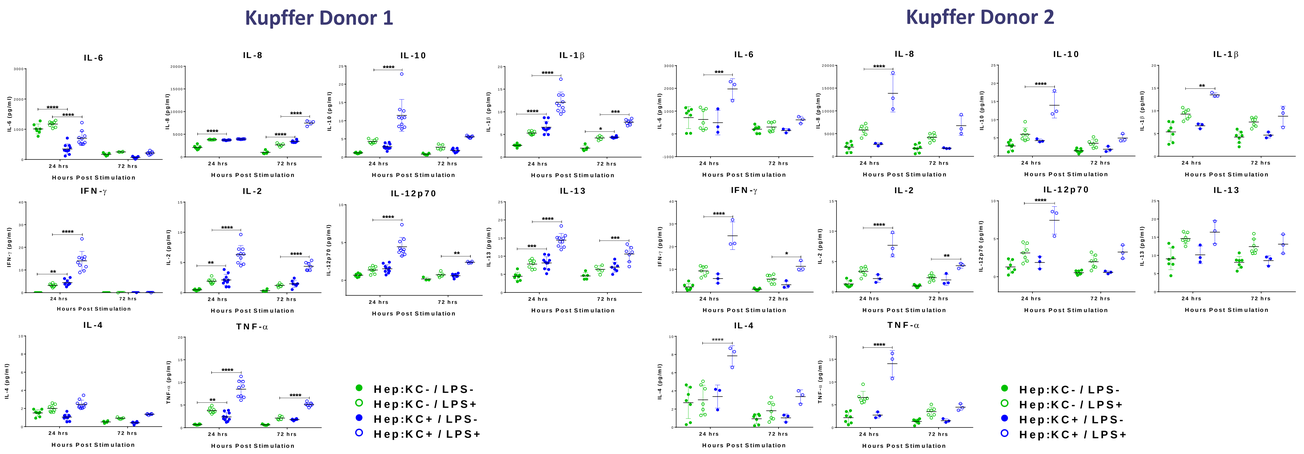

Figure 3: LPS-Mediated Cytokine Induction was Significantly Greater in Hep:KC+D1 Tissues at Both 24 and 72 hrs Compared to Hep:KC-.

To compare the extent of LPS-mediated cytokine induction between Hep:KC- and Hep:KC+D1 tissues, data were analyzed via two-way ANOVA with multiple comparisons. Significant differences in cytokine levels between tissue groups (Hep:KC- vs. Hep:KC+D1) are indicated by asterisks (*p<0.05, **p<0.01,***p<0.001, ****p<0.0001).

CONCLUSION: At 24 hrs, LPS-mediated induction of IL-10, IL-1β, IFN-γ, IL-2, IL-12p70, IL-13, and TNF-α was significantly greater in Hep:KC+D1 tissues compared to Hep:KC-. IL-1β, IL-2, IL-12p70, IL-13, and TNF-α induction by LPS remained significantly elevated versus Hep:KC- at 72 hrs. IL-8 induction was delayed in Hep:KC+D1 tissues and was much greater than that of Hep:KC- tissues treated with LPS.

Figure 4: LPS Treatment Induced the Release of Cytokines to a Greater Extent in Hep:KC+D2 Tissues at 24 hrs Compared to Hep:KC-

To compare the extent of LPS-mediated cytokine induction between Hep:KC- and Hep:KC+D2 tissues, data were analyzed via two-way ANOVA with multiple comparisons. Significant differences in cytokine levels between tissue groups (Hep:KC- vs. Hep:KC+D2) are indicated by asterisks (*p<0.05, **p<0.01,***p<0.001, ****p<0.0001).

CONCLUSION: IL-6, IL-8, IL-10, IL-1β, IFN-γ, IL-2, IL-12p70, IL-4, and TNF-α levels were induced by LPS to a much greater extent in Hep:KC+D2 tissues compared to Hep:KC- at 24 hrs. IFN-γ and IL-2 remained elevated at 72 hrs in Hep:KC+D2 tissues compared to Hep:KC- treated with LPS.

Results

Donor Effects on Pro-Inflammatory Cytokines

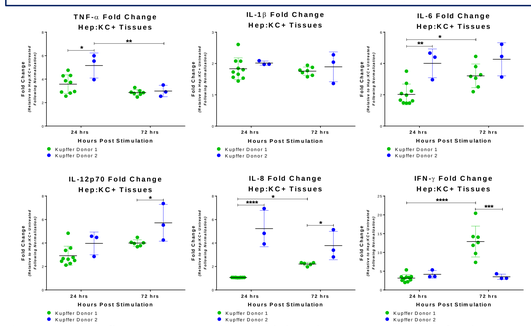

Figure 5: Pro-Inflammatory Cytokines Exhibited Varying Degrees of Induction.

To determine any donor-specific responses to LPS treated, cytokine levels in Hep:KC+ untreated and LPS-treated samples were normalized to untreated Hep:KC-. The fold-change between Hep:KC+ untreated and LPS-treated samples was then calculated and compared between donors via two-way ANOVA with multiple comparisons.

CONCLUSION: Donor response to LPS was significantly different for TNF-α, IL-6, and IL-8 at 24 hrs as evidenced by greater fold induction in Kupffer Donor 2. Additionally, at 72 hrs there was a significant difference in IL-8 IFN-γ, and IL-12p70 release between donors.

Donor Effects on Anti-Inflammatory Cytokines

Figure 6: Regulation of Anti-Inflammatory Cytokines Appeared Conserved Across Assessed Donors.

Cytokine levels were normalized with fold change determined and compared for Hep:KC+ tissues as described in Figure 5.

CONCLUSION: There were no significant differences in the release of IL-10, IL-2, IL-13, or IL-4 between donors at either 24 hrs or 72 hrs.

Summary

- **A robust response to inflammatory stimuli was observed in exVive3D Liver tissues both with and without 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 exVive3D Liver tissues containing Kupffer cells.**
- **Consistent with literature reports, donor-dependent effects on pro-inflammatory cytokine production were seen following LPS treatment; induction of anti-inflammatory cytokines however was similar 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.**