Endotoxin Up-Regulates the Proinflammatory Cytokines TNF-α and IL-6 in Freshly-Isolated Human Kupffer Cells

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Introduction

Kupffer cells, macrophages endogenous to the liver, can mediate hepatic inflammation and injury associated with various pathophysiological and toxicological conditions. Pro-inflammatory cytokines, such as TNF-α and IL-6, are secreted by activated Kupffer cells and are important in the pathogenesis of liver disease. To understand the role of Kupffer cells in liver disease, it is critical to be able to isolate and culture these cells from non-transplantable human livers. The present study aimed to examine the effects of treating Kupffer cells with LPS on TNF-α and IL-6 expression in vitro.}

Materials and Methods

Hepatocytes from donor H1152 exhibited typical morphology and culture characteristics. Isolated Kupffer cells were cultured in multi-well plates (5.3x10^5 cells/cm^2, Nunc/Thermo Scientific) in DMEM supplemented with FBS, Pen-Strep, and insulin for up to 11 days (37°C, 5% CO2, 95% relative humidity). Cell morphology and confluency were evaluated by light microscopy. At selected times, cells were treated with up to 50 µg/mL LPS and LPS-treated cells were measured by ELISA according to the manufacturer's instructions.}

Results

- Figure 1: Photomicrographs of Kupffer cells one hour and twenty-four hours post-plating. Cells of donor H1171 at one and twenty-four hours post-plating (A, B). Cells of donors H1160 (C) and H1161 (D) at twenty-four hours post-plating.
- Figure 2: Photomicrographs and immunofluorescent staining of Kupffer cells from donor H1160 five days post-plating. Typical mixed morphology culture observed in vitro (A, B). Circular and elongated cells expressed CD68 (C, green) and CD163 (D, green) markers (100x). Nuclei are stained blue with DAPI (E and F).
- Figure 3: Photomicrographs and immunofluorescent staining of Kupffer cells from donor H1160 eleven days post-plating. Cells cultured to display mixed circular and elongated cell morphologies (A, C and D). CD68 (A) and CD163 (B) markers.
- Figure 4: Photomicrographs of Kupffer cell culture eleven days post-plating. Both circular and elongated cell morphologies were observed in four cultures (100x magnification).
- Figure 5: Photomicrographs and immunofluorescent staining of Kupffer cell present in cultures of fresh and cryopreserved primary plated hepatocytes. Isolated hepatocyte cultures maintained phenotype and displayed both early/circular and mature/elongated cell morphologies (A, B and C). Typical mixed morphology culture observed in vitro (D). Kupffer cells were also observed among cultured cryopreserved hepatocytes from the same donor (E, green). Nuclei are stained blue with DAPI (F and G).

Conclusions

- The method presented here provides a reliable means of isolating human Kupffer cells at plating densities that allow for cell proliferation. The use of LPS in the culture medium facilitates the promotion of pro-inflammatory cell activation.
- Cultures of freshly-isolated human Kupffer cells maintained phenotype and responded to endotoxin in a pro-inflammatory priming stimulus, allowing for the study of cytokine release upon treatment with endotoxin or other inflammatory stimuli.

References