Journal of Immunological Methods xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Precision-cut human liver slice cultures as an immunological platform

Xia Wu[a,*], Jessica B. Roberto[a], Allison Knupp[a], Heidi L. Kenerson[b], Camtu D. Truong[a], Sebastian Y. Yuen[a], Katherine J. Brempelis[a], Marianne Tuefferd[c], Antony Chen[c], Helen Horton[c], Raymond S. Yeung[b], Ian N. Crispe[a]

[a] Department of Pathology, University of Washington, Seattle, WA 98195, USA
[b] Department of Surgery, University of Washington, Seattle, WA, USA
[c] Infectious Diseases and Vaccines, Janssen Research and Development, B-2340 Beerse, Belgium

ARTICLE INFO

Keywords:
Liver slice culture
Innate immunity
Hepatocyte
Antiviral

ABSTRACT

The liver is the central metabolic organ in the human body, and also plays an essential role in innate and adaptive immunity. While mouse models offer significant insights into immune-inflammatory liver disease, human immunology differs in important respects. It is not easy to address those differences experimentally. Therefore, to improve the understanding of human liver immunobiology and pathology, we have established precision-cut human liver slices to study innate immunity in human tissue. Human liver slices collected from resected livers could be maintained in *ex vivo* culture over a two-week period. Although an acute inflammatory response accompanied by signs of tissue repair was observed in liver tissue following slicing, the expression of many immune genes stabilized after day 4 and remained stable until day 15. Remarkably, histological evidence of pre-existing liver diseases was preserved in the slices for up to 7 days. Following 7 days of culture, exposure of liver slices to the toll-like receptor (TLR) ligands, TLR3 ligand Poly-I:C and TLR4 ligand LPS, resulted in a robust activation of acute inflammation and cytokine genes. Moreover, Poly-I:C treatment induced a marked antiviral response including increases of interferons *IFNB*, *IL-28B* and a group of interferon-stimulated genes. Therefore, precision-cut liver slices emerge as a valuable tool to study human innate immunity.

1. Introduction

The liver is critical in innate and adaptive immunity in humans (Crispe, 2016; Heymann and Tacke, 2016). The human liver contains diverse populations of liver cells, the majority of which are the parenchymal cells, or hepatocytes. In addition, the resident non-parenchymal cells include liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), and hepatic macrophages (*i.e.* Kupffer cells), as well as vascular endothelial cells and bile duct epithelial cells. Furthermore, trafficking monocytes, dendritic cells (DCs), natural killer (NK) cells and NK T cells are also present in the human liver (Crispe, 2016). These cell populations together form a complex immunological network.

Despite the recent advances in liver research, liver diseases continue to be a major cause of morbidity and mortality worldwide (Blachier et al., 2013; Wang et al., 2014). In 2010 alone, 31 million people globally were afflicted with liver cirrhosis, and one million of those patients succumbed to the disease (Mokdad et al., 2014). Likewise, liver cancer is one of the leading causes of cancer deaths globally, accounting for > 600,000 deaths each year. According to statistics available from the American Cancer Society, liver cancer cases have been rising on average 2.7% each year over the last 10 years, and death rates have been rising on average 2.6% each year from 2005 to 2014. Therefore, improved understanding and treatment of liver diseases is urgently needed.

One approach to study liver biology that is gaining popularity is the precision-cut liver slice culture (PCLS) (de Graaf et al., 2010; Lagaye et al., 2012). The tissue preparation and culturing conditions for PCLS have steadily evolved (Olinga et al., 1997; Graaf et al., 2007). It is likely to be important that slices are established in culture within three hours of the *in vivo* excision, because the viability of hepatocytes in conventional cell culture is strongly associated with the time delay between hepatectomy and liver cell isolation (Bhogal et al., 2011). Compared with other liver research methods, including cell monocultures and patterned tissue cultures, the PCLS method has the advantage that hepatocytes and the major subsets of non-parenchymal cells are cultured simultaneously, enabling the analysis of liver cell function in the context of diverse liver cell types. Gene expression profile of PCLS was found to have a higher degree of similarity to intact liver compared to mono cell cultures of primary hepatocytes and cell lines after 24 h of *ex*

* Corresponding author at: Department of Pathology, University of Washington, 1959 Northeast Pacific Street, Seattle, WA 98195, USA.
E-mail address: xiawu2@uw.edu (X. Wu).

https://doi.org/10.1016/j.jim.2018.01.012
Received 23 October 2017; Received in revised form 17 January 2018; Accepted 24 January 2018

vivo culturing (Boess et al., 2003). Furthermore, since each set of liver slice cultures originates from an individual donor, over time a diverse human population is sampled using the PCLS approach. This advantage will facilitate the development of precision medicine and personalized therapy for liver diseases in the era of personal genomics (Sitia, 2015; Li and Wang, 2016). To date, the PCLS method has been developed to study the metabolism (Janssen et al., 2015; Ijssennagger et al., 2016; Starokozhko et al., 2017), toxicology (Graaf et al., 2007; Hadi et al., 2013; Karim et al., 2013) and development of liver cells (Kasper et al., 2005; Westra et al., 2016). However, to our knowledge there has been little exploitation of PCLS to study human immunology.

In the present study, we used the PCLS approach to investigate innate immunity in human liver tissues. First, we developed a modified culture protocol that does not depend on elevated oxygen concentration in the incubator. Second, we documented the innate immune response in liver slices associated with tissue slicing. Third, we analyzed the antiviral response in liver slices induced by the Toll-Like Receptor 3 (TLR3) pathway.

2. Materials and methods

2.1. Liver samples, preparation and culturing

Fresh liver tissues were obtained from patients undergoing liver resection at the University of Washington Medical Center (Seattle, WA, USA). All patients in this study prospectively consented to donate liver tissue for research under the Institutional Review Board protocols #31281 and #51710. Clinical information regarding the patients is provided in Table S1. All patients in this study were free from active hepatitis and cirrhosis, which was confirmed by molecular or histologic examination of core needle biopsies of the liver. Liver cores of 6 mm diameter were excised from the resected liver tissue using a biopsy punch (Integra Miltex, York, PA, USA), stored in BELZER-UW solution (Bridge to Life Ltd., Columbia, SC, USA), and transferred to the research laboratories typically within 1 h of tissue excision.

Liver slices of 250 μm thickness were cut using a vibrating microtome, Leica VT1200 S (Nussloch, Germany), using Dulbecco's Modified Eagles Medium (DMEM) as the cutting medium (de Graaf et al., 2010; Lagaye et al., 2012). Liver slices were cultured individually on 0.4 μm millicell organotypic inserts in 24 well plates (Millipore Corporation, Billerica, MA, USA). The culturing medium comprised 1 × advanced-DMEM medium, 5% Fetal Bovine Serum (FBS), 1 × GlutaMAX, 0.5 × Penicillin-Streptomycin, 1 × Insulin-Transferrin-Selenium supplement and 15 mM HEPES (pH 7.2–7.5) (all from Gibco, Grand Island, NY, USA). The liver cultures were maintained on a rocking platform at 17 rpm in a humidified incubator at 5% CO_2 and atmospheric concentration of O_2 at 37 °C. The medium was renewed every two to three days. An experimental schematic of the liver slice culture procedure is provided in Fig. S1.

2.2. MTS cell viability assay

Individual slices were placed in a 48-well plate with four hundred microliters of DMEM medium and eighty microliters of MTS assay reagent (Promega, Fitchburg, WI, USA). MTS is a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt]. MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium. The absorbance of the formazan product at 490 nm can be measured directly from 96-well assay plates without additional processing. The conversion of MTS into the aqueous soluble formazan product is accomplished by dehydrogenase enzymes found in metabolically active cells. Reactions were incubated at 37 °C for 1 h with shaking. The $OD_{490\ nm}$ of the supernatants was measured, using a Synergy H1 microplate reader (Biotek, Shoreline, WA, USA).

2.3. Liver perfusion and isolation of liver cells

Human liver cell isolation procedures were adapted from several sources (Bhogal et al., 2011; Mohar et al., 2015). Cell isolation was performed on wedges of resected tissue rather than cores. Perfusion buffer contained 1 × Hank's Balanced Salt Solution (HBSS, without Ca ++, Mg++, or phenol red, from Gibco), 10 mM HEPES (pH 7.2–7.5) and 0.5 mM EDTA (pH 8.0). Collagenase buffer contained 1 × HBSS (Gibco), 5 mM $MgCl_2$, 5 mM $CaCl_2$, 5 mM HEPES (pH 7.2–7.5), 0.5% w/v Collagenase IV (Sigma-Aldrich, St. Louis, MO, USA), 0.25% w/v Protease (Sigma), 0.125% w/v Hyaluronidase (Sigma), 0.05% w/v DNase I (Sigma). Fresh aliquots of enzymes were added to the buffer on the day of the perfusion experiment. Forty mL of Perfusion buffer and 20 mL of Collagenase buffer were used for each 10 g of liver tissue. Buffers were pre-warmed to 37 °C prior to the perfusion step.

Before the perfusion step, liver tissue samples were rinsed with PBS (pH 7.4) until the fluid ran clear. Liver tissue wedges were sequentially perfused with Washing buffer (1 × HBSS and 10 mM HEPES, pH 7.2–7.5), Perfusion buffer, Washing buffer, and the recirculating Collagenase buffer at a flow rate of 12 mL/min (Gilson's MINIPULS 3, Middleton, WI, USA). The perfused liver tissue samples were gently mashed with a sterile syringe plunger through a sterile mesh strainer in ice-cold DMEM medium. Cell extracts were filtered through a 100 μm sterile strainer and centrifuged at 50 × *g* at 4 °C for 3 min to enrich for hepatocytes in the pellets and non-parenchymal cells in the supernatant. The supernatants were transferred to a new tube and kept on ice. The pellets were washed three times with ice-cold DMEM medium, and were pelleted each time at 50 × *g* at 4 °C for 3 min. To further purify the live hepatocytes, cell pellets were resuspended with 5 mL of ice-cold PBS, overlaid with 10 mL of 25% Percoll gradient solution. The mixture was centrifuged at 1400 × *g* (no brake) at 4 °C for 20 min. The pellet contained the purified live hepatocytes. The viability of the isolated hepatocytes was determined with the trypan blue exclusion assay (Thermo Fisher Scientific). If the viability was > 50%, the isolated hepatocytes were stored for RNA extraction.

For the non-parenchymal cells, the reserved centrifuged supernatants were further centrifuged at 500 × *g* at 4 °C for 7 min. The pellets were resuspended in 5 mL of ice-cold PBS, and overlaid on top with 50% and 25% Percoll gradients (10 mL layers each), and were centrifuged at 1400 × *g* (no brake) at 4 °C for 20 min. Cell layers were collected into 20 mL PBS each, and centrifuged again at 500 × *g* at 4 °C for 7 min. The pellets were resuspended with the ice-cold Flow buffer containing 1 × PBS, 2% FBS, and 1 mM EDTA.

2.4. Flow cytometry and cell sorting

The isolated non-parenchymal cells were stained with the antibody mixture that included eBioscience (San Diego, CA, USA): anti-CD45 (Cat. No. 15-0459-42); BioLegend (San Diego, CA, USA): anti-CD3 (Cat. No. 317330), anti-CD11b (Cat. No. 553310), anti-CD14 (Cat. No. 301834), anti-CD31 (Cat. No. 303120), anti-CD32 (Cat. No. 303206), anti-CD68 (Cat. No. 333814), and anti-CD271 (Cat. No. 345110). In addition, cells were also stained with LIVE/DEAD Fixable Far Red Dead Cell Stain Kit (Cat. No. L10120, Life Technologies, Carlsbad, CA, USA). The incubation mixture was kept at 4 °C for 30 min in the dark on a rocking platform. The mixture was centrifuged with 500 × *g* at 4 °C for 7 min. Cells were washed once with Flow buffer.

The antibody-labeled cells were sorted with a BD Aria III (BD Biosciences, San Jose, CA, USA). Analysis of cell populations was performed using FlowJo software, version 9.8.5 (FlowJo, LLC, Ashland, OR, USA). Kupffer cells were selected as the CD45+, CD3−, CD14+, CD68+, CD32+ populations (Alabraba et al., 2007; Ikarashi et al., 2013). LSECs were selected as CD45−, CD31+, CD11b− (Elvevold et al., 2008). HSCs were selected as CD45−, CD271+, autofluorescence positive with the emission wavelength at 460 nm (Buhring et al., 2007; D'Ambrosio et al., 2011) (Fig. S2).