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Research paper

A method for high purity intestinal epithelial cell culture from adult human and murine tissues for the investigation of innate immune function

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ABSTRACT

Intestinal epithelial cells (IECs) serve as an important physiologic barrier between environmental antigens and the host intestinal immune system. Thus, IECs serve as a first line of defense and may act as sentinel cells during inflammatory insults. Despite recent renewed interest in IEC contributions to host immune function, the study of primary IEC has been hindered by lack of a robust culture technique, particularly for small intestinal and adult tissues. Here, a novel adaptation for culture of primary IEC is described for human duodenal organ donor tissue as well as duodenum and colon of adult mice. These epithelial cell cultures display characteristic phenotypes and are of high purity. In addition, the innate immune function of human primary IEC, specifically with regard to Toll-like receptor (TLR) expression and microbial ligand responsiveness, is contrasted with a commonly used intestinal epithelial cell line (HT-29). Specifically, TLR expression at the mRNA level and production of cytokine (IFNγ and TNFα) in response to TLR agonist stimulation is assessed. Differential expression of TLRs as well as innate immune responses to ligand stimulation is observed in human-derived cultures compared to that of HT-29. Thus, use of this adapted method to culture primary epithelial cells from adult human donors and from adult mice will allow for more appropriate studies of IECs as innate immune effectors.

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1. Introduction

The intestinal epithelium is the largest single site of environmental exposure, immune activation, and tolerance induction in the body (MacDonald et al., 2011). With a surface area of approximately 400 m^2 in humans, a highly dynamic and highly regulated single-cell layer of intestinal epithelial cells (IECs) serves as a first line of defense and mediates an intricate balance between tolerance and host effector responses (Peterson and Artis, 2014). At the individual cell level, IECs exist in a bimodal state — participating in either nutrient absorption or immune activation (Shulzhenko et al., 2011). IECs express a wide variety of molecules crucial for microbial sensing, and as immune educators have the capacity to participate in antigen presentation, co-stimulation, and lymphocyte adhesion and trafficking (Fukata and Arditi, 2013; Nakazawa et al., 1999, 2004; Perera et al., 2007; Mayer, 2000). In addition, IECs are

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a source of a wide array of cytokines and chemokines (Stadnyk, 1994). Together these properties allow IEC to regulate intestinal immune populations, and thereby initiate, propagate, and regulate intestinal inflammation (Mayer, 2005; Shen and Turner, 2006).

There has been recent renewed interest in understanding the environment–IEC–host immune system trialogue (Shulzhenko et al., 2011). Although IECs have the capacity to shape the functional outcomes of these trialogues, investigations of these interactions are hindered by a lack of robust primary cell culture techniques. To overcome this hurdle, epithelial biologists have relied on human or murine-derived cell lines, which are often of tumorigenic origin (Langerholc et al., 2011). While these cell lines have numerous benefits, they may exhibit defects in transcription factors downstream of multiple microbial sensing pathways (Sato et al., 2009). In addition, due to the limited numbers and sources of the cell lines, there is a restriction of those HLA/MHC interactions which can be investigated (Sato et al., 2009; Melmed et al., 2003). Similarly, these cell lines limit the evaluation of disease-specific IEC phenomena. Thus, the establishment of robust primary IEC cultures to evaluate innate immune function under conditions of and disease will facilitate a more intricate evaluation of IEC-specific contributions to disease processes.

Although primary IEC cultures have been periodically described, these systems have largely focused on IEC derived from fetal sources (Perreault and Beaulieu, 1998) or organoid-type studies (Gracz et al., 2012). Similarly, while recent advances have been made in IEC monolayer culture systems from murine colonic sources (Moon et al., 2013), small intestinal and human primary IEC monolayer culture remains a challenge. The development of adult primary IEC culture techniques is important for the analysis of IEC function in disease progression, as well as under conditions of exposure to environmental and microbial diversity, rather than under conditions which lack antigenic experience as is the case with fetal-derived IEC cultures (Perreault and Beaulieu, 1998). The development of methods to study small intestinal epithelial cell innate immune function is particularly important due to the fact that the small and large intestines have been shown to express different levels of innate immune receptors, likely resulting in functional differences between these two populations (Abreu et al., 2003). Thus, in order to most accurately study innate immune function of the intestinal epithelium, it is crucial to work toward developing more robust primary IEC culture methods from a variety of sources, including human adult donor tissues and mouse adult small and large intestines.

Here we present an adapted method which yields high purity IEC cultures from duodenum of adult human organ donors and adult murine duodenum and colon (Booth and O'Shea, 2002). In addition, we compare and contrast the Toll-like receptor (TLR) expression and responsiveness of IEC derived from the duodenum of adult human organ donors to a commonly utilized intestinal epithelial cell line, HT-29 (Fogh, 1975), whereby significant differences were observed. While we have focused on TLR responsiveness of primary IEC cultures, these culture methods will serve as the foundation for future studies to elucidate environment–IEC–host immune system communication and interactions, and can be utilized to evaluate a myriad of innate immune functions.

2. Materials and methods

2.1. Animals

C57BL/6 mice were maintained at the breeding facilities of the University of Florida. All procedures were performed at 10–12 weeks of age, and were conducted in accordance with the guidelines of the University of Florida Institutional Animal Care and Use Committee.

2.2. Human intestinal tissue

Adult human organ donor duodenal tissue was obtained in collaboration with the Network for Pancreatic Organ Donors (nPOD, Gainesville, FL) (Campbell-Thompson et al., 2012). Approximately 8 g (wet weight) of duodenum was received in DMEM/Ham's F-12, Nutrient Mixture with 3.15 g/L glucose, L-Glutamine, Phenol Red, HEPES and Sodium Pyruvate (Thermo Fisher Scientific, Waltham, MA). nPOD cases 6212, 6284 and 6292 were used for imaging and flow cytometric studies while nPOD cases 6230 ("case A") and 6279 ("case B") were used for TLR expression and responsiveness studies. All procedures and protocols were reviewed and approved by the University of Florida Institutional Review Board.

2.3. Primary IEC isolation and culture

Primary IECs were isolated using a protocol adapted from Booth and O'Shea (2002). Human duodenal and murine duodenal and colonic tissues were prepared by removing the longitudinal muscle layer and washing with ice-cold Mg^{2+}- and Ca^{2+}-free Hank's Balanced Salt Solution (HBSS) (Mediatech, Manassas, VA) containing 100 U penicillin, 100 μg ml^{-1} streptomycin (Mediatech), 25 μg ml^{-1} gentamycin (MP Biomedicals, Solon, OH) and 0.5 mM dithiothreitol (DTT) (Thermo Fisher Scientific), hereafter referred to as HBSS wash solution. Tissue was cut into two cm^2 pieces, suspended in 50 ml HBSS wash solution, and inverted vigorously ten times, and the contents were allowed to settle for 1 min. The supernatant was removed and the settled contents were washed an additional four times. After the fifth wash, the settled contents were removed, minced with a sterile surgical scalpel, and suspended in 50 ml of the HBSS wash solution. The resulting suspension was passed over a 1000 $μm^2$ mesh filter. Remaining tissue was digested in 50 ml of a digestion buffer containing 75 U ml^{-1} collagenase type XI (Sigma-Aldrich, St. Louis, MO), 20 μg ml^{-1} dispase neutral protease II (Roche, Indianapolis, IN), 0.5 mM DTT, and 1% v/v fetal bovine serum (FBS) (Thermo Fisher Scientific) in Dulbecco's Modification of Eagles Medium with 4.5 g/l glucose and L-glutamine, without sodium pyruvate (DMEM) (Corning, Corning, NY). The digestion buffer containing the tissue was then evenly divided, placed in a 37 °C incubator and allowed to shake at 180 rpm for 3 h. The resulting digestion mixture was again passed over a 1000 $μm^2$ filter, and the tissue fragments atop the filter were washed with 25 ml complete growth media (DMEM, 8.5 g/l sodium pyruvate [Mediatech], 2.5% v/v FBS, 0.25 U ml^{-1} insulin [Sigma-Aldrich], 100 U penicillin, 100 μg ml^{-1} streptomycin, 25 μg ml^{-1} gentamycin, 5 μg ml^{-1} transferrin [Sigma-Aldrich], and 10 ng ml^{-1} epidermal growth factor [Sigma-Aldrich]) containing 2% w/v D-sorbitol (S-DMEM) (Sigma-Aldrich). Tissue debris remaining in the filter

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