912

Primary Cultures of Human Intrahepatic (Biliary) Epithelial Cells

A.J. Demetris, B.H. Markus, J.J. Fung, L. Makowka, S. Graner, R. Duquesnoy, and T.E. Starzl

THE BILIARY epithelium is a relatively select target in liver allograft rejection.[1,2] An ideal approach to study interactions between immune cells and the biliary epithelium would be the use of an in vitro system. The goal of this study was to establish primary cultures of bile duct cells.

MATERIALS AND METHODS

Source of Tissue

The tissues used were intact normal human livers (unused donor grafts) or large segments of normal liver obtained from liver transplant and primary resection procedures.

Biliary Epithelial Cell Isolation

The liver was placed on a sterile drape under a laminar flow hood with all subsequent steps performed under sterile conditions. The right and left hepatic bile ducts were identified and a silastic cannula (19 × ⅞, 12 inches) was advanced into the duct and sutured in place. A segment (1 to 2 cm) of the lateral portion of the respective lobe was then resected and the biliary tree flushed with HBSS (calcium and magnesium free Hanks' balanced salt solution, GIBCO Laboratories, Life Technologies Inc, Grand Island, NY). The biliary tree was then filled with a 0.1% collagenase intravenous (IV) (Worthington, Diagnostics Systems Inc) solution in HBSS, kept at 37°C, and left for 30 minutes to digest off the cells, which were then collected by reflushing the organ with HBSS.

The fluid containing the cells was transferred into 50-mL plastic centrifuge tubes, spun at 1,200 RPM for ten minutes, and resuspended in a minimal volume of culture medium and plated at a density of 1 to 10 × 10^6/mL on 24-well plastic Falcon tissue culture plates previously coated with MATRIGEL (Collaborative Research Inc, Lexington, MA).

Tissue Culture Medium and Substrata

Basal media used for culturing the epithelial cells (Table 1), included CEM 2000 (Scott Laboratories, West Warwick, RI), Williams E with L-glutamine or a 50/50 (vol/vol) mixture of Ham's F12 and Dulbecco's modified medium (DMEM). Serum supplementation was with 10% heat-inactivated fetal calf serum. Supplements (Tables 1 and 2) consisted of SGF-7 (1 mL/100 mL media), SGF-9 (1 mL/100 mL media, Scott Laboratories), 100 μg/mL endothelial cell growth factor (Collaborative Research), 250 μg/mL Fungizone, 56 μg/mL Gentamycin (GIBCO), and 10 U/mL heparin (O'Neal, Jones, & Feldman, St Louis, MO). The cells were plated on a MATRIGEL (Collaborative Research) substrate.

Follow-Up of Primary NPE Cultures

The plates were washed free of nonadherent cells after two days and these were centrifuged at 1,200 RPM for five minutes. The pellet was resuspended in medium and replated using the previously described techniques.

Half of the NPE culture medium was removed every second day and replaced with fresh complete medium in the previously described formulation. Once the cells were firmly attached (four to five days) the medium was replaced by fresh medium every two to three days.

RESULTS

Bile Duct Cell Isolates: Identification, Purity and Viability

Phase contrast microscopy of eluates obtained after tissue digestion revealed organoid clusters of cells intermixed with single small round cells ranging in size from eight to 12 microns in diameter. Immunoperoxidase staining of the organoid clusters for prekeratin was positive, while factor VIII-related antigen and albumin stains were negative. The organoid clusters and the single cell suspensions possessed from 70% to 90% viability based on trypan blue exclusion.

Effects of Culture Medium

Attempts to establish cell cultures on a MATRIGEL substrate were performed with

From the Departments of Pathology and Surgery, Presbyterian-University Hospital, Pittsburgh, and the Department of Surgery, University of Rochester, NY.

Address reprint requests to A.J. Demetris, Department of Pathology, Presbyterian-University Hospital, DeSoto at O'Hara Streets, Pittsburgh, PA 15213.

0041-1345/88/2002-0005$03.00/0

Table 1. Constituents of Serum Supplemented Basal Media

Constituent	Amount
Basal media	90%
Fetal calf	10%
SGF-7	1 mL/100 mL*
SGF-9	1 mL/100 mL*
Endothelial cell growth factor (ECGF)	100 μg/mL
Fungizone	250 μg/mL
Gentamycin	56 μg/mL
Heparin	10 U/mL

*See Table 2 for a listing of the constituents and their final concentrations.

the following: (1) basal medium alone, (2) basal medium plus SGF-7 and SGF-9, (3) basal medium supplemented with 10% fetal calf serum and, (4) basal medium with serum and growth factor supplements as described in Table 1.

Monolayers could be achieved using any of the basal media, but supplementation with fetal calf serum was required. Serum-free medium, on the other hand, despite the addition of fetuin and growth factor supplements, was unable to support the initiation of cultures.

Morphologic and Phenotypic Characteristic of Cultures

NPE cultures were examined routinely each day using an inverted phase contrast microscope. Cells from the organoid clusters began to spread over the plate within 24 hours and growth to confluence occurred by eight to 12 days. Confluent wells presented as monolayers with a characteristic cobblestone appearance. The individual cells were mostly small and polygonal with smooth intercellular borders and slightly granular cytoplasm. However, as the cultures aged, the cells became larger and squamoid in appearance and detached from the plates at 6 to 7 weeks.

Nearly 100% of the cells comprising the monolayers were uniformly prekeratin positive. Staining for albumin to detect hepatocytic differentiation, alphafetoprotein, and factor VIII-related antigen to detect endothelial cell differentiation were negative.

Table 2. Constituents and Final Concentration of Growth Supplements (SGF-7 and SGF-9) Growth Factor Supplemented Basal Media

Constituent	Concentration
Epidermal growth factor (EGF)	17.5 ng/mL
Transferrin (iron-saturated)	5.0 μg/mL
Insulin (bovine)	1.1 μg/mL
Fetuin	500 μg/mL
Hydrocortisone	750 ng/mL
Selenous Acid	5.6 ng/mL
T3	1.5 ng/mL
Progesterone	1.5 ng/mL
Oleic-BSA and linoleic acid-BSA	500 ng/mL
2-aminoethanol	920 ng/mL
o-phosphorylethanolamine	2.1 μg/mL

Ultrastructural studies of the monolayers revealed numerous intercellular epithelial-type junctional complexes (tight and desmosomal), surface microvilli, and lumenal-like intercellular spaces. The cells contained a few cytoplasmic organelles such as small mitochondria, a paucity of endoplasmic reticulum, ample glycogen, and cytoplasmic intermediate filaments that were recognized as prekeratin by light microscopy immunoperoxidase.

DISCUSSION

Cells isolated by our technique were in all probability derived from biliary ducts. This contention is based on the appearance of histologic sections of the liver after dislodging and harvesting the cells (absence of biliary epithelium in some ducts), the phenotypic characteristics of the isolates immediately after harvesting, and the routine, immunohistologic, and ultrastructural characteristics of monolayer cultures.

Further experimentation is, however, necessary to define more precisely the optimal growth conditions of these cells in culture, and to further characterize the cell type phenotypically, immunologically, and biochemically. Also, we have to date been unable to establish cell lines from these tissues, but rather have extended primary culture viability

to 6 to 7 weeks. Nevertheless, the success to date has enabled us to investigate factors that play a role in antigenic modulation of major histocompatibility complex (MHC) surface antigens in these cells.

ACKNOWLEDGMENT

We wish to express our thanks to B. Lombardi for encouragement and helpful discussions during performance of this work.

REFERENCES

1. Demetris AJ, Lasky S, Van Thiel DH, et al: Am J Pathol 118:151, 1985

2. Snover DC, Sibley RK, Freese DK, et al: Hepatology 4:1212, 1984