875

Establishment of Primary Cultures of Human Biliary Epithelium and Induction of Class II Major Histocompatibility Complex Antigens by Interferon Gamma

A J Demetris, B Markus, S Saidman, J Fung, L Makowka, R Duquesnoy, and T E Starz

THE BILIARY epithelium of intermediate and small bile ducts is the target of immunologic damage in liver allograft rejection and primary biliary cirrhosis (PBC).[1-3] The immune damage in both of these disorders is putatively mediated by T lymphocytes. Major histocompatibility complex (MHC) antigens are thought to be the targets of the lymphocytic attack in liver allograft rejection, whereas the target antigens in primary biliary cirrhosis have yet to be defined.[4] Class I MHC antigens are normally expressed on the biliary epithelial cells. Class II MHC antigens while not normally expressed, are induced on bile ducts in pathologic lesions that involve activated lymphocytes such as PBC and liver allograft rejection.[1,5]

Donald et al[6] suggested that the immunologic mediated damage and loss of the small bile ducts in human liver allografts is observed preferentially when there is donor-recipient compatibility at the class II MHC locus. Other groups have found that the loss of bile ducts in liver allografts is associated with an incompatibility at the class II locus.[7,8] Central to Donaldson's hypothesis is the conjecture that the biliary epithelium is capable of acting as an antigen presenting cell thereby promoting a reaction directed at an incompatible class I MHC antigen.[6]

Resolution of some of the discrepancies mentioned above may be more ic... approached by an in vitro system usi... cultures of human biliary epithelium (HB... We have previously shown that primar... c... tures of HBE can be established from n... human liver tissue using a serum supp... mented basal medium combined with ... nective tissue substrate.[9] Also, since act... lymphocytes from liver allograft biopsie... native hepatectomy specimens with PB... be easily expanded in culture with the ... interleukin-2,[10] epithelial-lymphocyte int... tion may be amenable to study. The fol... is a report on primary cultures of h... intrahepatic biliary epithelial cells and p... liminary observations on the inducibi... class II MHC antigens on these cells ... gamma interferon.

MATERIALS AND METHODS

Cell Isolation and Cultures

Biliary epithelial cells were harvested from tw... hepatectomy specimens of donor liver allografts ... transplantation. Techniques for the isolation, cu... and characterization of these cells are reported ... elsewhere.[9] The medium used for the initial ... consisted of CEM 2000 with SGF-7 and SGF-S ... supplements (Scott Laboratories, West Warwick, R... Williams E with L-glutamine. Both media were ... mented with 10% heat inactivated fetal calf serum. ... cells were plated on MATRIGEL (Collaborative Re... Lexington, MA) coated 12 well plastic tissue ... plates.

Three hundred fifty to 500 U/mL of recom... gamma interferon (Cellular Products, Buffa... added to confluent monolayer cultures of biliary ep... hial cells (eight to 12 days after plating). Contr... layers received no interferon supplement to the ... Both control and interferon treated monolayers ... were incubated for five days without further addi... changes to the media. The cells were then harvested

From the Departments of Pathology and Surgery, University Health Center, Pittsburgh.

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

0041-1345/88/2001-1274$3.00/0

f Human Biliary
ss II Major
Antigens

Duquesnoy and T.E. Starz

ve may be more ideal
an in vitro system using cu
n biliary epithelium (HB
sly shown that primary cu
be established from norma
sue using a serum supple
dium combined with a co
strate.[5] Also, since activated
liver allografts biopsies and
y specimens with PBC c
d in cu with the use of
thelial- ite interac
able to stu owing
rimary cult umag
y epithelial cells and pre
ns on the inducibility of
ntigens on these cells by

S AND METHODS

Cultures

were harvested from two partial
of donor liver allografts before
ues for the isolation, culturing,
these cells are reported in detail
m used for the initial isolation
with SGF-7 and SGF-9 growth
tories West Warwick, RI) or
mine. Both media were supple-
activated fetal calf serum. The
RIGEL (Collaborative Res Inc,
12 well plastic tissue culture

to 500 U/mL of recombinant
ular Products, Buffalo) was
ayer cultures of biliary epithe-
s after plating). Control mono-
ron supplement to the media.
on treated monolayer cultures
s without further additions or
cells were then harvested from

(February), 1988 pp 726-730

culture plate using trypsin digestion. Following
digestion, the cells were fixed with cold acetone on
microscope slides using a cytospin for five minutes at
1000 rpm. The cytospin slides were then washed in
phosphate buffered saline (PBS) three times and incu-
bated with monoclonal anti-DR antibodies (Becton-Dick-
inson, Sunnyvale, CA) for 30 minutes. Incubation of the
primary antibody was followed by three washes in PBS
and addition of the fluorescein-labeled anti-mouse anti-
body for 30 minutes. Control slides were incubated with
secondary antibody alone. The slides were then washed
three times in PBS and coverslipped for examination by
fluorescence microscopy.

RESULTS

Establishment of Primary Cultures and Cell Characterization

Initial cell isolates and monolayer cultures displayed morphologic (typical cobblestone appearance, Fig 1) and phenotypic characteristics (prekeratin [AE-1] positive, albumin negative, factor VIII related antigen negative) of intrahepatic biliary epithelium. Ultrastructural examination of initial cell isolates and monolayer cultures revealed easily identifiable intercellular junctions (tight and desmosomal), surface microvilli, sparse cytoplasmic organelles such as small mitochondria, rough endoplasmic reticulin and occasional cytoplasmic lipid droplets. The primary cultures remained viable for up to 4 weeks using a connective tissue substrate.

Induction of Class II Antigens

Addition of 300 to 500 U/ml of gamma interferon to the culture media followed by incubation for three to five days resulted in morphologic and phenotypic alterations in the cultures. Two days after addition of interferon to the culture there was a slight enlargement and spindling of the cells and slight increase in individual cell dislodgement from the plates. No similar morphologic changes were seen in the control culture. Immunofluorescent staining of the cells isolated from the gamma interferon plates revealed positive cytoplasmic and focal surface staining for DR antigens (Fig 2). No similar staining was present in control specimens.

DISCUSSION

The results presented here demonstrate that primary cultures of biliary epithelium can be readily established from whole or fragments of normal human liver tissue. The cells cultured display phenotypic and morphologic characteristics of differentiated biliary epithelium in vitro, and remain viable for up to 1 month. Therefore, this system may be useful for the study of the immunologic properties of biliary epithelium. In fact, the preliminary results presented here suggest that gamma

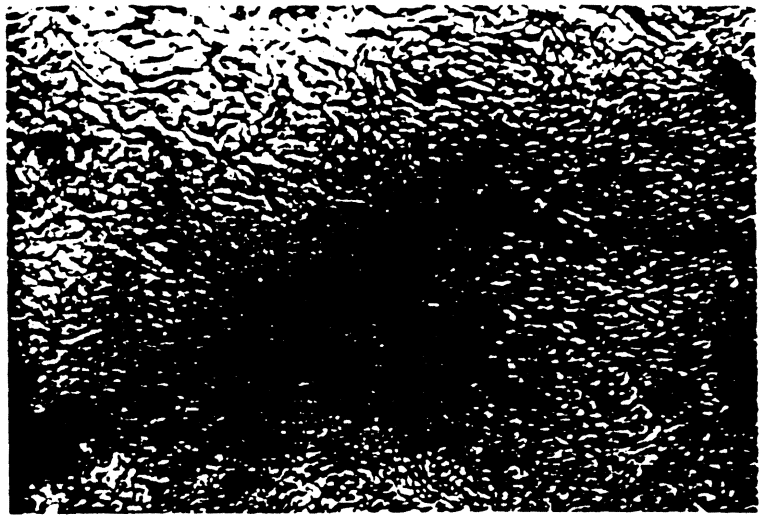

Fig 1. Phase microscopy appearance of confluent layer of primary culture of human biliary epithelial cells (day 10).

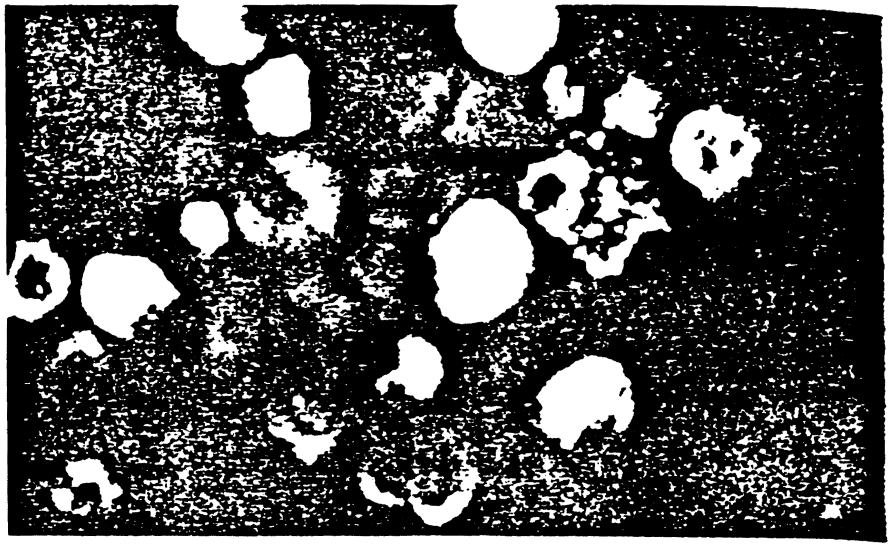

Fig 2 Immunofluorescence microscopy of cytospin preparation of human biliary epithelial cells five days after treatment with gamma interferon. The cells were stained anti-human DR antigen

interferon alone is capable of inducing expression of class II MHC antigens on biliary epithelial cells similar to its effect on endothelial, fibroblast and epithelial tumor cell lines.[11-13] Further studies using dual markers (prekeratin and anti DR) to show the simultaneous expression of phenotypic markers and immuno/electromicroscopy to confirm cell viability are necessary to confirm these findings, and are currently underway. Nevertheless, since the level of contamination with fibroblasts or endothelial cells in this culture is negligible, and up to 40% to 50% of the cells stained positively for class II antigens after interferon treatment, we feel it safe to assume that the bile duct cells are indeed those producing the class II antigens in response to gamma-interferon.

REFERENCES

1 Demetris AJ, Lasky S, Van Thiel DH, et al. Transplantation 40:504, 1985

2 Fennell R. Pathol Ann 2:289, 1981

3 MacSween RNM, Burt AD. Mol Asp Med 8:269, 1985

4 McFarlane IG. Mol Asp Med 8:249, 1985

5 Ballardini G, Mirakian R, Bianchi FB, et al. Lancet 2:1009, 1984

6 Donaldson PT, O'Grady J, Portmann B, et al. Lancet 1:945, 1987

7 Demetris AJ, Markus B, Burnham J, et al. Transplant Proc 19:121, 1987

8 Gronc J, Gouw ASH, Poppema S, et al. Transplant Proc 18:126, 1986

9 Demetris AJ, Markus BH, Fung JJ, et al. In Vitro (in press)

10 Fung JJ, Zeevi A, Starzl TE, et al. Hum Immunol 16:182, 1986

11 Pober JS, Collins T, Gimbrone MA. Nature 305:726, 1983

12 Schwartz R, Momburg F, Moldenhauer G, et al. Int J Cancer 35:245, 1985