Letter to the Editor

NORMAL HUMAN LIVER ORGAN CULTURE

Dear Editor:

Immune responses within the liver are important for the elimination of infectious agents with liver stages, such as malaria and the hepatitis viruses, and the immune system is also involved in chronic liver diseases, such as primary biliary cirrhosis, primary sclerosing cholangitis, and rejection of liver allografts. The participation of liver cells in the immune response is thought to depend on factors such as the cytokine milieu, which can modulate the expression of various immune cell surface recognition molecules. Thus, there is considerable interest in developing models to study immunological and cytokine-mediated responses in the liver, particularly in the context of cell–cell and cell–matrix interactions of the tissue microenvironment.

Progress in understanding and studying cellular interactions within the liver has been hampered by the relative paucity of normal adult tissue for 72 h, for the assessment of cytokine effects on liver morphology and cell phenotype. Responses to cytokines usually occur at 48-72 h. Thus, the aim of this work was to develop a liver organ-culture system to maintain adult tissue for 72 h, for the assessment of cytokine effects on liver morphology and cell phenotype.

Samples of histologically normal liver were obtained from patients undergoing liver resection for the removal of metastatic tumors. Between 5 and 30 g of liver tissue was collected in transport medium consisting of Hank's buffered salt solution (ICN Flow, Thame, Oxfordshire, U.K.) supplemented with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid buffer, pH 7.6, and 20 KIU/ml Aprotinin ("Trasylol" Bayer Limited, Newbury, Berkshire, U.K.). Samples were processed immediately, and routine histology was used to confirm normal morphology and lack of inflammatory changes. Specimens were dissected into pieces of approximately 25–50 mm³, and for some experiments, thin (approximately 2 mm) sections were cut with a scalpel. The tissues were either cultured submerged in medium in 25-ml conical plastic "Universal" tubes (Falcon, Fahrenheit Laboratory Supplies, Leeds, U.K.) or at an air–liquid interface on 25-mm tissue-culture inserts (3.0 µm pore size, Becton Dickinson, Oxford, U.K.) held inside a 6-well tissue-culture plate (Falcon). Medium was added to the wells just sufficient to moisten the base of the filter insert. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air.

Two types of growth medium were assessed: a 1:1 mixture of Roswell Park Memorial Institute medium 1640 (Sigma Chemical Co., Poole, Dorset, U.K.) and Dulbecco modified Eagle medium (DMEM) (Sigma) (1640/DMEM) or a 1:1 mixture of Ham’s F12 (GIBCO-BRL, Paisley, U.K.) and DMEM (F12/DMEM). Both media were supplemented with 5% fetal bovine serum (Sera Lab, Crawley-Down, Sussex, U.K.). As the successful culture of BEC in F12/DMEM containing supplements has been described (Cruickshank et al., 1998), the effects of epidermal growth factor (5 ng/ml, GIBCO-BRL), hydrocortisone hemisuccinate (0.4 µg/ml, Sigma), insulin (40 µg/ml Sigma), and hepatocyte growth factor (10 ng/ml, R&D Systems, Abingdon, Oxfordshire, U.K.) were assessed individually and in combination. Each culture condition, i.e., submersion versus air/liquid interface, 1640/DMEM versus F12/DMEM, and each combination of tissue-culture supplement were assessed in quadruplicate, using tissue derived from four independent donors with reproducible results.

Cytokines (interferon γ [IFNγ], 200 U/ml; Amersham, Berkshire, U.K.; tumor necrosis factor α [TNFα], 200 U/ml; R&D Systems; transforming growth factor β [TGFβ], 100 U/ml, Genzyme; and IFNγ plus TNFα, 50 U/ml each) were added to supplemented Ham’s F12/DMEM medium at time = 0. The cytokine concentrations were chosen on the basis of previous work on BEC and hepatocyte-derived cell lines (Cruickshank et al., 1998). Organ cultures from four individual donors were used in these experiments with reproducible results.

All cultures were harvested after 72 h and assessed by immunohistochemistry, following fixation in 10% formalin and processing into paraffin wax. Sections (5 µm) were cut and stained in hematoxylin and eosin, or immunolabeled, as previously described, with appropriate antigen unmasking (Southgate and Trejdosiewicz, 1997), and examined blind.

Cytokeratin (CK) 18 (clone CY-90, Sigma) was used to assess the general tissue integrity as it localized to the hepatocytes and BEC. Overall, the liver organ cultures retained good morphology, with the thinnest cultures (2 mm) on tissue-culture inserts being the best preserved. There was little difference in hepatocyte preservation in organ cultures maintained either at an air–liquid interface or submerged in medium. However, CK18 expression was higher in the organ cultures than in the normal tissues (Fig. 1), with a zone of more intense CK18 expression around the portal tracts in the cultures maintained in F12/DMEM.

CK7 (clone LP1K, ICRF, London, U.K.) was used to assess bile duct preservation, taking advantage of its specificity for BEC. (Fig.
There was damage to many of the BEC after 72 h organ culture with spillage of the cell contents into the lumen of the ducts (Fig. 1). However, some of the small bile ducts remained intact and bile duct damage was reduced in the cultures cultured at an air-liquid interface. BEC integrity was also better when cultured with F12/DMEM, compared with 1640/DMEM. Surprisingly, the addition of supplements typically included in BEC growth media, such as the hepatocyte growth factor, epidermal growth factor, triiodothyronine, insulin, and hydrocortisone, did little to improve the survival of BEC when added alone or in combination.

Expression of laminin (antilaminin, Sigma) within the stroma of the portal triad and on the hepatocyte basement membranes suggested that the integrity of the basement membranes was unimpaired in the organ cultures (Fig. 1). The vascular endothelium of the sinusoids and portal triads showed a small degree of cellular disruption, but overall retained good morphology, as judged by CD31 expression (Fig. 1) with antibody JC704 (Dako, High Wycombe, Bucks, U.K.). There was increased proliferation of hepatocytes in the organ cultures, as shown by immunolabeling with the MIB-1 antibody (anti-Ki67 antigen, Immunotech, Coulter Electronics, Luton, U.K.), compared with normal tissues (Fig. 1).

To validate the organ-culture model, the effects of cytokines were assessed. TNFα alone, or in combination with IFNγ, resulted in extensive damage and destruction of both hepatocytes and BEC. Destruction was most evident in the cells adjacent to the portal tracts. IFNγ and TGFβ each reduced the number of proliferating cells, although even these organ cultures contained more MIB-1-positive cells than the matched normal liver in situ. TNFα alone had no obvious effect on proliferation, although when added in combination with IFNγ, resulted in a marked reduction in the number of proliferating cells. The ability of IFNγ and TNFα to reduce cell proliferation in culture, irrespective of the presence of exogenous epidermal growth factor and human growth factor, is consistent with our previous in vitro findings with human BEC and hepatocyte-derived cells (Cruickshank et al., 1998).

Human leukocyte antigen (HLA)-DR expression was visualized with antibody CR3/43 (Dako) within the stroma in the portal triads, and on Kupffer cells within the sinusoids in the normal tissue. Organ culture alone had no effect on HLA-DR expression. However, the addition of IFNγ to the organ cultures, either alone or in combination with TNFα, induced HLA-DR expression on some of the sinusoids and on a small number of hepatocytes, suggesting that the cytokines had indeed penetrated into the tissue. However, only a minority of hepatocytes became HLA-DR-positive, although it is well established that hepatocytes can express the major histocompatibility complex class II in vitro (Cruickshank et al., 1998) and in vivo in diseases such as alcoholic cirrhosis (Chedid et al., 1993). This apparently contradictory finding is reflected in the observation that relatively few hepatocytes and BEC express HLA-DR in diseases such as primary biliary cirrhosis and primary sclerosing cholangitis, although HLA-DR expression is apparent in the sinusoids (Spengler et al., 1997; Cruickshank et al., 1999). Thus, in certain instances, other factors may interact to modulate the cytokine-mediated expression of immune molecules on hepatocytes. A higher or more sustained local concentration of IFNγ and/or TNFα may be required to induce expression of HLA-DR on hepatocytes in vivo.

Our results show that liver histioarchitecture and hepatocyte morphology can be preserved during 72 h organ culture. However, the relative deterioration of the bile ducts suggests that biliary epithelium is more susceptible to damage than hepatocytes, and may require additional factors to maintain full integrity. The relative fragility of the biliary epithelium may underlie their propensity to damage during a local inflammatory response. Increased hepatocyte proliferation together with bile duct damage are also observed in primary biliary cirrhosis and primary sclerosing cholangitis (Nakanuma et al., 1995). Thus, further study of the organ-culture model may provide insights into the mechanisms and also the susceptibility of BEC to damage.

The participation of hepatocytes in immune mechanisms is...