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HUMAN HEPATIC ENDOTHELIAL CELLS AND HEPATOCYTES IN CULTURE: MORPHOLOGICAL FEATURES, AND PRODUCTION OF VON WILLEBRAND FACTOR AND FIBRINOGEN. R. Harrison. Department of Pathology, The University of Texas Medical Branch, Galveston, TX, U.S.A.

Liver cells were derived from cadaveric organ donors. Pieces of human liver 5 to 50 grams were minced, washed, and incubated in collagenase at 37 degrees C. After washing, the cell suspension was plated into culture vessels that had been briefly pre-treated with an extract derived from human liver. A mixed population of liver cells, including endothelial cells, hepatocytes, and Kupffer cells, attached within hours. At the end of 2 to 3 weeks there developed clusters of densely packed cells of two types. The most numerous cells were initially fusiform but grew as a monolayer even when densely packed. As density increased they assumed a polygonal form; cells with this morphological appearance stained immunocytochemically for von Willebrand factor antigen. They were relatively small and resembled cells derived from human umbilical vein except that the cytoplasm was more filmy in appearance. The second prominent cell type was significantly larger and likewise replicated to form clusters. These large cells sometimes contained multiple nuclei, exhibited a relatively low nuclear to cytoplasmic ratio, and immunocytochemically stained for human fibrinogen. A more distinct nuclear membrane and prominent nucleoli were characteristics of hepatocytes that were useful light microscopically in distinguishing these cells from sinusoidal endothelial cells. Ultrastructurally, endothelial cells were characterized by small size, holes in and among the cells that probably were the in vitro analogue of fenestrae, and numerous Weibel-Palade bodies in the cytoplasm, which otherwise was relatively bland. Hepatocytes, by contrast, had an active appearing cytoplasm containing more organelles. Canaliculi and typical tight junctions formed between adjacent hepatocytes. Levels of vWF and fibrinogen increased in a time dependent manner in media overlying this mixed population of cells. Human factor VIII has not yet been detected in the media overlying these mixed cells derived from human liver, and factor VIII antigen has not yet been demonstrable immunocytochemically in either cell type.

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PERMSELECTIVITY OF CULTURED ENDOTHELIAL MONOLAYERS: EFFECT OF SIZE AND CHARGE OF THE TRANSPORTED MOLECULES. R. Bizios (1), F.A. Blumenstock (2), P.J. Del Vecchio (3), and A.B. Malik (2). Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, U.S.A. (1) Department of Physiology (2), and Department of Opthalmology (3), Albany Medical College, Albany, NY 12208, U.S.A.

Known molecular size neutral dextrans (molecular weight (MW) range ~6,000-~500,000), anionic (dextran sulfate, ~500,000 MW), and cationic (DEAE-dextran, ~500,000 MW) were used to determine the permselectivity characteristics of bovine pulmonary arterial endothelial monolayers. The experimental system consisted of two compartments separated by a gelatinized polycarbonate membrane (0.8 μm pore size) on one side of which endothelial monolayers were grown to confluence. Dextran solutions (1 gram %) were prepared in phosphate buffered saline buffer (containing 0.5 gram % serum albumin) and placed on the luminal side. Transendothelial dextran transport at 37°C was studied as a function of time. Dextran concentrations were determined spectrophotometrically using the anthrone method. The results (mean ± S.E.) of the dextran concentrations in the abluminal compartment at 45 minutes are given in the table.

Approximate Dextran Molecular Weight	Dextran Concentration (μg/ml) Transported Across the Endothelium: Membrane Control	EC Monolayer
6,000	87.5 (n=2)	53.25 ± 3.83 (n=5)
10,000	40.90 ± 4.98 (n=4)	11.20 ± 1.10 (n=8)
20,000	33.40 (n=2)	10.94 ± 1.34 (n=6)
40,000	43.10 ± 6.97 (n=4)	4.73 ± 0.58 (n=7)
70,000	30.5 (n=2)	3.96 ± 0.26 (n=6)
170,000	32.2 (n=2)	3.32 ± 0.31 (n=5)
500,000 (Neutral)	36.67 ± 2.41 (n=6)	3.10 ± 0.32 (n=6
500,000 (DEAE)	15.9 ± 4.15 (n=4)	1.81 ± 0.29 (n=5)
500,000 (Sulfate)	44.06 ± 6.05 (n=4)	9.44 ± 1.21 (n=9)

The endothelial monolayers markedly restricted dextran transport compared to transport across gelatinized, polycarbonate membranes alone (controls). Furthermore, the results indicate that size and charge of the transported molecules determine the sieving mechanism responsible for their passage across the cultured endothelial monolayer. (Supported by HL32418.)

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THE EFFECTS OF HEMORRHAGIC FEVER VIRUS INFECTION OF ENDOTHELIAL CELLS. R.M. Lewis (1), P.B. Jahrling (2), B.P. Griffin (1) and T.M. Cosgriff (1). Medical Division (1) and Disease Assessment Division (2), United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD 21701-5011, U.S.A.

Pichindé viral infection of strain 13 guinea pigs is a model for Lassa fever virus in humans. Infected animals show impaired platelet function and altered coagulation parameters. Human endothelial cells and the human endothelial-like cell line, EA926, were infected with Pichindé virus. Following infection, cultures were monitored by phase contract microscopy for cytopathic effect (CPE). Assays of supernatant were used to document viral growth and to measure those endothelial-produced components that might affect hemostasis. In addition, the cells were stimulated with phorbol ester (PMA), which stimulates the production of prostacyclin. Infection showed no noticeable effect on the endothelial cells or EA926 cells which were untreated with PMA. PHA-treated EA926 cells were subject to CPE. Factor VIII antigen was not significantly affected by viral infection, PMA treatment, or endotoxin exposure. The production of PGF1, measured as an estimate of prostacyclin synthesis, was dependent on the concentration of stimulating PMA. Infected cultures showed decreased responsiveness to PMA stimulation when infected by increasing concentrations of Pichindé. The most noticeable effect was noted when cultures were infected with a multiplicity of infection of 0.1 and 100 ng/ml PMA. Thromboxane B_2, an estimate of thromboxane A_2, showed no significant change. No detectable leukotriene C_4^2 was produced and no significant change in leukotriene B_4 was measured. The decreased prostacyclin production by the infected endothelial cells may indicate a role for the endothelium in the hemorrhagic syndrome that accompanies some viral diseases.

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FACTOR VIII IN VIRUS INFECTED CELLS: A LIGHT MICROSCOPIC OBSERVATION. C.A. Bruggeman (1), W.H.M. Debie (1) and M.C.E. van Dam-Mieras (2). Department of Medical Microbiology (1) and Department of Biochemistry (2), University of Limburg, Maastricht, The Netherlands

Viruses may be important in atherosclerosis as inciters of arterial injury or as modulators of the metabolism of the infected cells. Cytomegalovirus (CMV) is one of the members of the herpesvirus family that infect human beings. Virus was detected in arterial walls of patients with atherosclerosis (1). The effect of CMV on endothelial cells and on the metabolism of these cells was subject of this study.
Human endothelial cells monolayers were established from cells obtained by collagenase treatment of human umbilical cord veins and arteries. The cells were grown in medium 199 containing growth factor and 20% fetal calf serum. All monolayers were near confluency at the time of viral infection. The cells were purified by FACS cell sorting using dilacLDL as marker (2). Infection of endothelial cells was performed using CMV laboratory strains AD169 and Kerr and wild strains isolated from immunosuppressed patients (obtained by Dr. H.T. Weiland, Dept. Virology, University of Leiden, The Netherlands). Intracytoplasmic and intranuclear viral antigens were detected in 10-20% of the endothelial cells by fluorescence microscopy using TRITC-labeled monoclonal antibodies against CMV. Centrifugation of the cell monolayer during virus incubation resulted in an increase in the percentage of virus positive cells. The effect of virus infection on presence of factor VIII in the cells was studied using fluorescence microscopic examination of cell monolayers using a double staining technique. The presence of factor VIII in the cytoplasm of the cells was demonstrated using FITC-labeled antibodies and viral antigens were demonstrated by TRITC-labeled antibodies. The results of these experiments showed that HCMV-infection of endothelial cells resulted in the disappearance of factor VIII from the cytoplasm of the endothelial cells at 48-72 hrs post infection without affecting the viability of the infected cells. The importance of this finding in relation to the function of the endothelial cells will be discussed.
References:
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