53 THE EFFECT OF LIVER MACROPHAGE SOURCE ON VIRUS-MACROPHAGE INTERACTIONS IN A MODEL OF MURINE VIRAL HEPATITIS.

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Most experimental models of viral hepatitis in mice extrapolate the role of Kupffer cell-virus interaction from studies using non-hepatic sources of macrophages (Mos). This study was designed to compare the viral interaction of liver macrophages (Kupffer cells) with Mos from peritoneal exudate (PE), and peripheral blood (monocytes) using a Phlebovirus of the Bunyaviridiae, Punta Toro Virus (PRV). PRV causes an age-dependent lethal hepatic necrosis in 3 week old C57BL/6 mice 3-4 days after s.c. inoculation, but 8 week old mice survive with minimal hepatic necrosis.

Methods: Mos were derived from 3 week old (susceptible) and 8 week old (resistant) C57BL/6 mice and isolated from liver by collagenase perfusion and centrifugal elutriation, from PE by lavage after thioglycolate, and from blood by Percoll gradient centrifugation. Mo Monolayers were infected with PRV (MOI 0.05) after 24 hours of culture in the presence of anti-interferon. PRV titers in supernatant were measured as plaque-forming units in Vero Kidney cells.

Results: PRV could replicate to a variable extent in Mos from both age groups and all sources, however, only Kupffer cells expressed an age-related susceptibility to the virus in vitro ($P<0.05$).

Conclusions: Inherent age-related differences in PTV-liver macrophage interactions are uniquely expressed in Kupffer cells vs Mos from other sources. The results suggest that the nature of Kupffer cell-virus interactions cannot be presumed from studies using other macrophage populations.

54 RETINOIDS AFFECT PHENOTYPE OF FAT-STORING CELLS

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Fat-storing cells (FSCs) are the main storage site of vitamin A in the mammalian body. During hepatic fibrogenesis, FSCs loose their vitamin A rich fat-droplets, transform into myofibroblast-like cells and deposit extracellular matrix components. In this study, we have analyzed these relationships on FSCs in culture.

FSCs were isolated from adult Wistar rats. The morphology of the cells was studied by phase contrast and electron microscopy. Protein synthesis was measured by ^{3}H-labeled proline incorporation. Collagenous protein was measured by a collagenase assay.

FSCs spontaneously transformed into cells with the ultrastructural characteristics of myofibroblasts : the cells spread on the culture dishes and became very flat. The heterochromatin in the nuclei transformed almost completely into less dense euchromatin. The cisternae of the rough endoplasmic reticulum became dilated. Bundles of microfilaments with typical, smooth muscle-like condensations appeared. The number and size of the vitamin A-rich fat droplets decreased. Simultaneously, a twofold increase in the incorporation of ^{3}H-proline into secretory proteins and an eleven-fold increase into secreted collagenous proteins occurred.

The influence of retinol, retinyl acetate and retinoic acid on transformed FSCs was examined at concentrations ranging from 1 to 50 µM. The retinoids counteracted the spreading of the cells. At high retinol concentration, cells with rounded perikaryon, long branched processes and large cytoplasmic fat-droplets were observed. At concentrations above 10 µM, the three retinoids had an inhibitory effect on the protein synthesis of transformed fat-storing cells.

At present, we are evaluating whether these substances have a similar regulatory effect in vivo. If so, these observations may have interesting clinical implications in preventing hepatic fibrosis.

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55 EFFECTS OF HEPATIC STIMULATOR SUBSTANCE (HSS) ON $[Ca^{2+}]_i$ IN HTC HEPATOMA CELLS.

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Hepatic Stimulator Substance (HSS) is a 12-18000 M.W. peptide which stimulates growth of normal and malignant hepatocytes. HSS induces a rapid uptake of Na^+ by HTC hepatoma cells via the Na^+/H^+ antiport which is essential, but not sufficient, to stimulate DNA synthesis.

Changes in intracellular free calcium concentrations $[Ca^{2+}]_i$ are thought to play a role in growth regulation, with Ca^{2+} acting as a second messenger. The present experiments examined the effects of HSS on the regulation of $[Ca^{2+}]_i$ in individual cultured HTC hepatoma cells, monitored by quantifying the fluorescence of intracellularly trapped Fura-2, using a digital microscopic image analysis system. The average basal $[Ca^{2+}]_i$ in these cells was 90 nM.

Addition of 50 µg/ml HSS to the hepatic cells, resulted in a gradual increase in $[Ca^{2+}]_i$ to nearly 500 nM. The $[Ca^{2+}]_i$ in response to HSS stimulation was uniformly distributed in the cell. A cell-to-cell variation was observed in the response to HSS and not all cells responded to HSS with identical kinetics. Thus, in some cells, $[Ca^{2+}]_i$ increased to 210 nM, while in others it rose to 450 nM. $[Ca^{2+}]_i$ reached a maximum between 4-6 sec.

Addition of 200 µg/ml HSS, a concentration known to promote maximum cell proliferation, produced a significantly higher $[Ca^{2+}]_i$ compared to 50 µg/ml HSS (540 ± 50 nM v.s. 750 ± 60 nM). The $[Ca^{2+}]_i$ in response to 200 µg/ml reached a peak in 3-4 seconds, twice as fast as the response to 50 µg/ml. Preliminary studies suggest that the increase in $[Ca^{2+}]_i$ is due to Ca^{2+} release from intracellular stores and is not dependent on the availability of extracellular Ca^{2+}.

Thus HSS appears to affect two key components of the growth regulation cascade; Na^+/H^+ exchange and $[Ca^{2+}]_i$.

56 PARTIAL PURIFICATION AND CHARACTERIZATION OF AN INHIBITOR (KcHI) PRODUCED BY KUPFFER CELLS IN CULTURE WHICH INHIBITS HEPATOCYTE PROLIFERATION IN VITRO

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The liver is an organ composed of different cytotypes. The most abundant cells are hepatocytes, Kupffer cells and endothelial cells. In the past, studies on liver regeneration have neglected possible interactions between the other cell types and hepatocytes. Herein, we report the partial purification and characterization of an inhibitor from Kupffer cell conditioned medium which is active on hepatocytes in primary culture. Kupffer cells from Fisher rats were purified by centrifugal elutriation and kept in serum-free Dulbecco's medium (DEM) for 8-72 hours. The purity of Kupffer cell preparation was confirmed by the method of peroxidase activity, latex bead endocytosis and E.M. The activity of the Kupffer cell Hepatic Inhibitor (KcHI) was maximum when Kupffer cells were cultured for 48 hours. To prepare the KcHI, media collected from Kupffer cell primary cultures were dialyzed, lyophylized and then stored at -70°C. Before use, the lyophylized material was resuspended in the medium at a concentration of 5µg of protein/ml. At this concentration, KcHI inhibited hepatocyte proliferation in primary cultures in the presence of Insulin (I) and epidermal growth factor (EGF) as determined by DNA synthesis and labeling index. The inhibition was not due to proteolytic activity but it was specific for hepatocytes and was dependent on the time point of the addition during the incubation. The KcHI, added to the incubation medium (serum-free DEM + I + EGF) at the beginning of the incubation and kept for 48 hours, produced at 53+6% inhibition of hepatocyte proliferation. This inhibition disappeared when KcHI was added after 12 hours incubation. The initial characterization of KcHI demonstrated that the inhibitor was retained on PM-50 Amicon ultrafiltration membranes. The PM-50 fraction was active at 2µg/ml. KcHI seems to be a heat-sensitive protein with a MW > 50 Kd, not inactivated by trypsin, chymotrypsin and acid treatment. It is precipitated by ammonium sulfate at 80% concentration. The identification of KcHI will represent an important step in the understanding of the mechanism(s) of normal and pathological hepatocyte regeneration.