THE EFFECT OF LIVER MACROPHAGE SOURCE ON VIRUS-
MACROPHAGE INTERACTIONS IN A MODEL OF MURINE
VIRAL HEPATITIS. P.S. Latham and S.B. Seipel, Deps. of
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Most experimental models of viral hepatitis in mice
extrapolate the role of Kupffer cell-virus interaction from
studies using non-hepatitic sources of macrophages (Mos).
This study was designed to compare the virus interactions
of liver macrophages (Kupffer cells) with Mos from peritoneal
exudate (PE), and peripheral blood (monocytes) using a
Phanovirus of the Bunyaviridae, Punta Toro Virus (PTV).
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,
and from PE by lavage after thiglycolate, and from blood by
Percol gradient centrifugation. Mos 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.

EFFECTS OF HEPATIC STIMULATOR SUBSTANCE (HSS) ON [Ca\(^{2+}\)],
IN HTC HEPATOMA CELLS: J.M. Lachat, N.V. Sharma and R.C. Bhalla, Dept. of
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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\(^+\) antport which is
essential, but not sufficient, to stimulate DNA
synthesis.

Changes in intracellular free calcium concentrations
([Ca\(^{2+}\)]) 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\(^{2+}\)] in individual cultured HTC hepatoma cells,
monitored by quantifying the fluorescence of
intracellularly trapped Flura-2, using a digital
microscopic image analysis system. The average basal
([Ca\(^{2+}\)]) in these cells was 90 nM.

Addition of 50 μg/ml HSS to the Hepatic cells,
resulted in a gradual increase in [Ca\(^{2+}\)], to nearly 500
nM. The [Ca\(^{2+}\)] 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\(^{2+}\)] increased to 210 nM, while in others
it rose to 450 nM. [Ca\(^{2+}\)] reached a maximum between 4-6
sec.

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

Thus HSS appears to effect two key components of
the growth regulation cascade; Na\(^+\)/H\(^+\) exchange and [Ca\(^{2+}\)].

RETIROIDS 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 lose their
vitamin A rich fat-droplets, transform into myohepatoblast-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.
Collagenous protein was measured by a collagenase assay.

FSCs spontaneously transformed into cells with the ultrastruc-
structural characteristics of myohepatoblasts: the cells spread on the cul-
ture dishes and became very flat. The heterochromatin in the nuclei
transformed almost completely into less dense euchromatin. The
cisema of the rough endoplasmic reticulum became dilated. Bun-
dies of microfilaments with smooth muscle-like condensa-
tions appeared. The number of some cells, [Ca\(^{2+}\)] in response to 50
M, [Ca\(^{2+}\)], the availability of extracellular Ca\(^{2+}\).

The influence of retinol, retinyl acetate and retinoic acid on
transformed FSCs was measured at concentrations ranging from 1 to
50 μM. The retinoids counteracted the spreading of the cells. At high
retinol concentration, cells with rounded perikarya, long
branched processes and large cytoplasmatic 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.

PARTIAL PURIFICATION AND CHARACTERIZATION OF AN INHIBITOR
(KH1) PRODUCED BY KUPFER CELLS IN CULTURE WHICH INHIBITS
HEPATOCHINE PROLIFERATION. A. Francavilla, L. Polimeni, M. Barone, L.
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The liver is an organ composed of different cytotypos. The most abundant
cells are hepatocytes, Kupffer cells and cholangiobells. In the past, regen-
eration have neglected possible interactions between
the other cell types and Kupffer cells. We report the
partial purification and characterization of an inhibitor
from Kupffer cell conditioned medium which is active on
hepatocytes in primary cultures. Kupffer cells from Fisher rats were purified
by centrifugal elutriation and kept in serum-free Dulbeco's medium (RIM)
for 8-72 hours. The purity of Kupffer cell preparation was confirmed by
the method of peroxidase activity, latex bead endocytosis and
EM. The activity of the Kupffer cell Hepatic Inhibitor
(KH1) was maximum when Kupffer cells were cultured for 48
hours. To prepare the KH1, media collected from Kupffer
cells primary cultures were dialyzed, lyophilized and then
stored at -70°C. Before use, the lyophylized material was
desuspended in the medium at a concentration of 3mg of
protein/ml. At this concentration, KH1 inhibited the
hepatocyte proliferation in primary cultures in the presence of Insu-
lin (I) and epidermal growth factor (EGF) as determined by
DNA synthesis and labeling index. The inhibition was not
due to prohibitive activity but it was specific for hepato-
cytes and was dependent on the time point of the addition
during the incubation. The KH1, added to the incubation
medium (serum-free Dulbeco's I + EGF) for 2 hours,
reduced the incorporation of [3H]-thymidine in the
incubation and kept for 48 hours, produced at 50-68
inhibition of hepatocyte proliferation. This inhibition dis-
appeared when added after the 24 hours of incubation.

The initial characterization of KH1 demonstrated that the
inhibitor was retained on PM-50, Amicon ultrafiltration
membranes. The PM-50 fraction was active at 0.5 μg/ml. KH1
seems to be a heat-sensitive protein with a Nm>50 Kd, not
inactivated by trypsin, chymotrypsin and acid treatment.

It is precipitated by ammonium sulfate at 80% concentration.
The identification of KH1 will represent an important step in
understanding(s) of normal and pathological hepaticocyte regeneration.