[PS2.56]

Hepatitis B Virus (HBV) targets macrophage reverse cholesterol transport to infect hepatocytes

Knud Esser*[1], Georg Gasteiger[1], Xiaoming Cheng[1], Mathias Broxtermann[1], Percy Knolle[2], Ulrike Protzer[1]

[1]*Institute of Virology, Technische Universität München / Helmholtz Zentrum München, Germany, Germany,* [2]*Institute of Molecular Medicine and Experimental Immunology, Faculty of Medicine, University of Bonn, Germany, Germany*

HBV is efficiently and selectively sequestered from the circulation by the liver. Since hepatocytes bind HBV inefficiently, we hypothesized that non-parenchymal liver cells might take up HBV and mediate hepatocyte targeting by transcytosis.

To study this, we developed a model in which human liver tissue is *ex vivo* perfused via branches of the portal vein in the presence of human serum. The intact human liver microarchitecture allowed us to investigate HBV liver cell interactions occurring *in vivo*. When a fraction of fluorescence labelled viral particles (VP) was used for 45 minutes perfusion, accumulation of VP was only observed in CD68 positive Kupffer cells but not in hepatocytes. This was also the case for unlabelled VP as determined by immunofluorescence staining with anti-HBs antibodies. Ultrastructural analysis by electron microscopy showed uptake of virions into endosomes in close association to triglyceride rich lipoproteins (TRL).

To reach its host cell, however, the virus needs to be released from Kupffer cells again. When human liver tissue was perfused for further 15 hours with VP negative medium, hepatocytes became positive only when Kupffer cells became negative.

In line with HBV recycling *in vivo*, VP escaped lysosomal compartments and concentrated in FITC-transferrin positive recycling endosomes in cultured human Kupffer cells. As shown for retroendocytosis of TRL derived cholesterol, VP (coincubated with human serum) colocalized with ApoA1 and ApoE, VP containing compartments were targeted by fluorescence labelled HDL, and resecretion of fluorescence labelled as well as unlabelled VP was induced by HDL or human serum as determined by flow cytometry, quantitative RT-PCR and ELISA. Our hypothesis that HBV re-secretion out of Kupffer cells is associated with macrophage cholesterol efflux *in vivo* is emphasized by our observation that VP migrate towards and into hepatocytes along neutral lipids derived from Kupffer cells in *ex vivo* perfused human liver tissue.

Keywords: Hepatitis B Virus, Kupffer cells, macrophage reverse cholesterol transport, transcytosis