Tissues, Pathology, and Diagnostic Microscopy

LS.2.P101
Visualization of early Hepatitis B Virus (HBV) infection in *ex vivo* perfused human liver tissue using fluorescence labelled viral particles

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HBV is a hepatotropic pathogen leading to a chronic inflammation of human liver in about 10% of infections. It is a main cause for the development of liver cirrhosis and hepatocellular carcinoma and represents a serious health problem worldwide. Despite many efforts, the early steps in HBV infection are still poorly understood. In humans, HBV is efficiently and selectively separated from the circulation by the liver. However, hepatocytes, the HBV host cells, are shielded from the blood by a fenestrated layer of sinusoidal non-parenchymal cells and bind HBV only inefficiently *in vitro*, especially in the presence of human serum. Thus, non-parenchymal liver cells might take up HBV *in vivo* and mediate host-cell targeting by transcytosis.

To test this hypothesis, we developed a model in which human liver tissue is *ex vivo* perfused via branches of the portal vein under cell culture conditions 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 sinusoidal CD68 positive liver macrophages -named Kupffer cells- but not in hepatocytes. To exclude artefacts due to the labelling or purification process, supernatant of HBV producing HepG2.215 cells was used for liver perfusion and immunofluorescence analysis confirmed former results. Ultrastructural analysis by electron microscopy showed uptake of virions into endosomes where they exclusively located membrane associated, a geometric constraint essential for component recycling. In line with a recycling within Kupffer cells, VP concentrated in FITC-transferrin positive recycling endosomes in cultured liver macrophages. 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 human serum containing but VP negative medium, hepatocytes became positive only when Kupffer cells became negative. Taken together, our data suggest that HBV is removed from the circulation by Kupffer cells *in vivo*. Within these cells, HBV connects to a recycling pathway along that HBV targets and finally trans-infects its host cell.