STUDIES OF THE INTERACTION OF HEPATITIS B VIRUS WITH HUMAN HAEMOPOIETIC CELLS

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CONTENTS

ABSTRACT

This thesis studied aspects of the interaction of hepatitis B virus (HBV) with haemopoietic cells and cell lines, to address the reported tropism of HBV for haemopoietic tissues. Emphasis was directed at demonstrating specific attachment of HBV to defined sub-populations of peripheral blood leucocytes (PBL) and bone marrow cells (BM), and the distribution of receptors for HBV on well-defined haemopoietic cell lines. Biochemical characterisation of the virus-cell interaction was also performed, and the question of infectivity of haemopoietic cell lines was addressed.

Firstly, a quantitative assay of HBV binding to liver plasma membranes (PM) was adapted to show that isolated PBL PM bound serum-derived HBV particles to a similar degree, based on their protein content. Using synthetic peptides representative of various amino acid sequences of the preS1 and preS2 regions of L HBsAg to inhibit HBV binding to the PM, it was found that peptide preS1(12-32) inhibited binding to PBL PM by 60-80% and peptide preS1(21-47) inhibited by 0-30% (depending on the source of PM), while peptides preS1(32-49) and preS2(120-145) did not inhibit binding. This contrasts with results obtained using liver PM, where peptide preS1(12-32) did not inhibit binding, while peptide preS1(21-47) inhibited by 70%, and preS1(32-49) inhibited by approximately 12%. Peptide preS2(120-145) had no effect on binding. Thus, different regions of the L surface protein appear to mediate attachment to PBL and hepatocytes.

HBV particles isolated from serum are complexed with serum proteins including IgG. To test the involvement of receptors for IgG and complement fragments (opsonins) in the HBV-PM interaction, a panel of ligand-blocking monoclonal antibodies (MAbs) to opsonin receptors was used, and it was shown that the three classes of receptors for IgG (FcγRI, FcγRII and FcγRIII) and CR3, are not major receptors for HBV on PBL or hepatocytes, as MAbs to these did not inhibit HBV binding. It was also shown that HBV does not utilise the receptor for IgA, FcαR, for attachment to PBL, despite reported sequence homology between the large envelope protein of HBV and the Fc portion of human IgA. In contrast to a published report

that IL-6 mediates binding of HBV to hepatocytes, IL-6 was shown not to mediate attachment to either liver or PBL PM, by virtue of pre-incubation with a blocking polyclonal anti-serum to IL-6.

Glycosaminoglycans (GAGs) were found to influence HBV binding to PM: soluble heparin (HE) inhibited binding to liver PM by up to 80%, and to leucocyte PM by up to 40%; chondroitin sulphate C (CS-C) enhanced virus binding (approximately 1.5-fold) to leucocyte PM only. Chondroitin sulphate A and hyaluronate had no effect on binding to either PM, arguing that simple electrostatic properties of GAGs were not responsible for the observed effects. The incomplete inhibition by HE and enhancement by CS-C could indicate the presence of more than one class of binding site for HBV on the respective PM, and coupled with the differential pattern of inhibition in the presence of synthetic peptides, argues that receptors for HBV on PBL and hepatocytes may be either different, or altered forms of the same molecule(s).

To extend these studies, whole cell binding assays were developed in order to accurately define which subsets of PBL and BM cells could bind HBV. Using purified HBV particles as the first stage in an immunofluorescence-based detection system, followed by detection of bound HBV using anti-preS1 MAbs F35.25 or MA18/7, and a FITC-conjugated third-stage antibody, specific membrane staining of peripheral blood monocytes from 8/9 donors was observed. In addition, binding of HBV to the erythroleukaemia cell line K562 was observed, while other myeloid cell lines did not appear to bind virus. This assay was then adapted to a suspension cell assay with analysis by flow cytometry, using phycoerythrin as the detecting fluorochrome. The parameters of binding were optimised for K562 cells and these were then applied to analyse HBV binding to PBL and BM cells obtained from healthy volunteers, whose serum was free of HBV markers. Based on their light scatter characteristics, monocytes and neutrophils were the only cell types in the peripheral blood that bound HBV, while binding to lymphocytes was not observed. This was confirmed by two-colour immunofluorescence to simultaneously detect bound HBV and subset-specific leucocyte

markers. Similarly, in the BM, only monocytes bound HBV. Importantly, haemopoietic stem cells (CD34+) did not bind HBV. Binding was tested to 'activated' populations of lymphocytes (PHA-treated), monocytes (LPS-treated), and neutrophils (fMLP-treated). The pattern of HBV binding was not affected by these treatments. Monocytes cultured *in vitro*, bound significantly more virus than freshly isolated monocytes. Taken together, these results indicate that only monocytes, and to a lesser extent neutrophils, express potential receptors for HBV, and a differentiation-dependent upregulation of receptor sites for HBV is observed on monocytes.

The distribution of potential HBV receptors was determined on a number of haemopoietic cell lines, representative of various haemopoietic lineages. K562 (erythroid), and the monocyte cell line THP-1, were the only haemopoietic cell lines which bound HBV, while binding was also observed to the human hepatoma cell line HepG2. A number of other erythroid and monocyte cell lines, as well as T and B lymphoid, and a megakaryocytic line, all failed to bind HBV. A comparison of the surface immunophenotypes of all the cell lines tested excluded all known CD-classified molecules (including opsonin receptors), as candidate HBV receptors.

The biochemical characteristics of the interaction of HBV with all of these cell types were then examined. On K562 and THP-1, HBV binding was sensitive to the protease chymopapain but insensitive to trypsin, indicating that the molecule was a glycosylated protein. Pre-treatment of these cell lines with tunicamycin, to inhibit post-translational addition of *N*-linked carbohydrate to surface glycoproteins, did not influence HBV binding, indicating that these moieties are not important for virus attachment. Enzymatic removal of cell surface sialic acids with neuraminidase significantly enhanced HBV binding to K562 and THP-1 cells but did not confer binding to otherwise 'negative' cell lines. Binding of HBV to cultured monocytes and HepG2 cells was trypsin and chymopapain sensitive, and was not increased by neuraminidase pre-treatment. Cation chelation demonstrated that HBV binding of to all cell types was Ca^{2+}/Mg^{2+}-independent, and acid elution of cells showed that binding

was not mediated by peripherally-bound molecules. Binding of HBV to monocytes and to HepG2 cells was significantly reduced by pre-treatment of the cells with PI-PLC, implying that the molecule responsible for binding to these cells is GPI-linked. In this case, a comparison with HBV binding to K562 was not informative due to the resistance of the GPI linkages on these cells, and possibly also on THP-1 cells (based on CD59 cleavage), to hydrolysis by PI-PLC. Thus, cells expressing potential receptor(s) for HBV, whose characteristics do not correlate with any other proposed candidate, have been identified.

Immunoprecipitation analysis using HBV particles covalently cross-linked to the surface of ^{125}I-labelled K562 cells, and anti-S MAb coupled to goat anti-mouse IgG-Sepharose, resulted in the identification of a 50 kDa species as a putative HBV receptor.

Contrary to reports that HBV inhibits colony formation by myeloid cell lines in semi-solid media, no inhibitory effect by HBV was seen on clonal cell growth in liquid culture. K562 cells were found to be able to efficiently internalise HBV particles, which accumulated in a perinuclear compartment. In infection studies, K562 cells positive for HBsAg after 2-4 days post-infection became enlarged, and their numbers decreased steadily over an 11 day period. It is not clear whether these cells represent a transient or differentiated cell type. Similarly, it appears that the level of HBV DNA in these cells declines steadily during the infection course.