The asialoglycoprotein receptor mediates hepatic binding and uptake of natural hepatitis B virus particles derived from viraemic carriers

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As a putative mechanism of hepatitis B virus (HBV) uptake into hepatocytes the interaction between HBV and the hepatic, human-derived asialoglycoprotein receptor (ASGPR) was investigated. Sera from patients with different variations of hepatitis B surface antigen (HBsAg) positive chronic hepatitis, HBV particles isolated from HBV carriers with high-titre viraemia and commercial HBsAg served as sources of HBV. ASGPR was affinity-purified from human liver. HBV that had bound to isolated ASGPR was either detected by radioimmunoassay using solid-phase bound ASGPR or enzyme immunoassay with biotin–ASGPR bound to immobilized HBV. Furthermore, binding and uptake of purified, 125I-labelled HBV particles into human hepatoma cell lines (HepG2 and HuH7), which constitutively express functional ASGPR molecules, were compared to that of ASGPR-negative COS cells. As a result HBV was found to bind to purified human ASGPR in two different assays. Circulating virus particles from sera with high titre viraemia showed the highest attachment activity to ASGPR. HBV binding to purified ASGPR was saturable and inhibitable by an excess of D-galactose-bearing ligands, by EDTA and anti-receptor immunoglobulin. Lysis of particles by adding detergent abolished immunodetectable HBV binding to purified ASGPR. Commercial HBsAg did not adhere to solid phase-immobilized ASGPR. Monoclonal anti-preS1 antibody (MA18/7) but not anti-preS2 antibody (Q19/10) inhibited virus attachment. Purified and radiolabelled HBV particles showed binding to HepG2 and HuH7 cells but to much lesser degree to COS cells. Cellular binding of HBV was significantly inhibited by blocking ASGPR function. Both ASGPR ligands and rabbit anti-ASGPR immunoglobulin but not non-immune rabbit serum inhibited uptake of radiolabelled HBV particles into HepG2 cells or HuH7 cells, respectively. This study suggests that HBV virions may enter human hepatocytes via ASGPR molecules by attachment of viral preS1-related envelope binding sites to this receptor.

Introduction

The hepatitis B virus (HBV) is a major cause of chronic inflammatory liver diseases and liver cirrhosis associated with the development of hepatocellular carcinoma (Hoofnagle & Seef, 1982). A specific receptor for hepatic HBV infection has not yet been found. HBV particles express three related envelope glycoproteins (S, preS1 and preS2) (Heermann et al., 1984), which bear candidate attachment sites for the host cell. Hepatitis B surface antigen (HBsAg), preS1 and preS2 are distinct in size and amino acid composition, forming glycoproteins with different qualities and quantities of glycosides (Wong et al., 1985). It has been suggested that preS1 and preS2 possess binding sites for virus attachment i.e. a hepatocyte receptor on preS1 and a receptor for polymerized albumin on preS2 molecules (Pontisso et al., 1989; Petit et al., 1989). In HepG2 cells (Petit et al., 1991) and on human liver plasma membranes (Pontisso et al., 1990), the preS1 region has also been shown to be responsible for HBV binding. A glycosylated receptor of Mr 31K responsible for binding of synthetic preS1 peptide was described recently (Dash et al., 1992). The pathobiological significance of these findings remains unclear. Recent studies reported the successful in vitro infection of human hepatoma (HepG2) cells (Bchini et al., 1990) and of human hepatocytes by adding polyethylene glycol (Gripon et al., 1993), however, without defining a cellular receptor for HBV.

The cellular uptake mechanism of HBV is not understood. In particular it remains unclear whether phagocytic or endocytic pathways are dominantly involved. In T lymphocytes, the transferrin receptor has been suggested to mediate uptake of HBV particles into the cell (Franco et al., 1992), whereby the preS1 region appeared to represent the main binding site (Pontisso et al., 1991). It therefore was supposed that transferrin receptors present on hepatocytes may also be responsible...
for hepatic HBV endocytosis. In a different study, interleukin 6 (IL-6) was shown to bind HBV thereby probably serving as a mediator for HBV infection of the liver cell via the IL-6 receptor (Neurath et al., 1992).

The hepatic asialoglycoprotein receptor (ASGPR) is a transmembrane molecule specifically expressed on the sinusoidal and basolateral hepatocellular membrane mediating uptake and intracellular degradation of desialylated glycoproteins by liver cells. Its biochemical properties are well known (Harford & Ashwell, 1982; Spiess, 1990) as well as the DNA sequences of its two major subunits, H1 and H2 (Paietta et al., 1992). The assembly of these subunits is required to provide a highly active system for the endocytosis of asialoglycoproteins into hepatocytes (Shia & Lodish, 1989). However, the exact physiological role of the ASGPR has so far not been defined. ASGPR function was used to establish cell-directed drug targeting (Meijer et al., 1992) and, with respect to integration of foreign DNA such as virus DNA, successful gene transfer into hepatocytes via the ASGPR has been reported (Wu & Wu, 1988). After ASGPR-mediated endocytosis of carrier–HBV DNA constructs experimental animals demonstrated HBsAg synthesis (Wu et al., 1992).

This study now suggests that the hepatic ASGPR is able to bind natural HBV specifically by its preS1 region and that this attachment might provide a mechanism for the hepatic endocytosis of HBV.

**Methods**

**Patients and sera.** Two hundred sera from HBV carriers with acute or chronic hepatitis B were included. Sixty-one viraemic sera [HBsAg- and HBeAg-positive (n = 47) and HBsAg- and anti-HBe-positive (n = 14)] exhibited more than 10 pg HBV DNA/ml (Genetics assay; Abbott Laboratories). In addition, 139 sera [HBsAg- and HBeAg-positive (n = 44) and HBsAg- and anti-HBe-positive (n = 95)] showed low levels of HBV (less than 10 pg/ml). HBV status was assessed by commercial assays (Abbott Laboratories). Control sera were from healthy blood donors (n = 5) and from patients with HBsAg-negative chronic hepatitis either of autoimmune origin (n = 5) or HCV-related (n = 5) according to internationally agreed criteria (Fogarty Proceedings, 1976).

Rabbit anti-human ASGPR immunoglobulin was described recently and its specificity was proven by immunoblotting of liver lysate (Fig. 1b) (Stockert & Morell, 1990). 125I-dye-labelled anti-HBsAg monoclonal antibody (MAb) was purchased from Abbott Laboratories. Murine anti-HBsAg MAb was obtained from Dianova. Murine MAb against the large HBV envelope protein (anti-preS1, MA18/7) and against the glycosylated middle HBV envelope protein (anti-preS2, Q19/10) were kindly provided by Prof Dr W. H. Gerlich, Justus Liebig University, Gießen. All chemicals were from Merck, unless otherwise indicated.

Purified hepatitis B virus surface protein (HBsAg type ad) was purchased from Biogenetics. HBV particles were isolated from sera containing a high titre of HBV as described (Gerken et al., 1991). Briefly, using three sequences of differential ultracentrifugation on sucrose layers, HBV particles were separated from the fresh plasma of viraemic HBV carriers. HBV particle preparations containing 10 to 20 mg protein/ml (Lowry et al., 1951) were characterized by SDS-PAGE, immunoblotting and electron microscopy as described earlier (Gerken et al., 1989). Fig. 1(b) shows a representative result demonstrating both HBsAg, preS1 and preS2 molecules appearing as doublets indicating glycosylated and non-glycosylated proteins. Particle preparations were kept in small aliquots at −20 °C until use for binding assays or 125I-radiolabelling.

**ASGPR and plasma membrane preparations.** Human ASGPR was prepared as described earlier (Treichel et al., 1990) from fresh human liver from kidney donors whose livers were not suitable for transplantation. Briefly, this method utilizes double affinity chromatography on lactose–agarose followed by fast desalting. The galactose-binding portion of proteins in liver lysates corresponds to the ASGPR. The preparations were analysed for purity by silver staining after SDS-PAGE (Fogarty Proceeding) showing a highly purified doublet band at 46K, for protein content according to the Lowry method (Lowry et al., 1951) and for biological activity by defining the binding capacity of 125I-asialoorosomucoid (ASOR) as previously described (Stockert & Morell, 1990). The preparations used in this study showed a mean binding activity of approximately 100 µg ASOR per mg purified ASGPR. Plasma membranes from human liver were prepared according to the method of Hubbard et al. (1983).

**Cells.** The human hepatoblastoma cell line HepG2 (Schwartz et al., 1982), the hepatocarcinoma cell line HuH7 (Nakahayashi et al., 1982), both expressing functionally active ASGPR molecules, and COS cells as ASGPR-negative cells were kept under standard cell culture conditions. Culture medium [ Dulbecco’s modified Eagle’s medium DMEM; Gibco] was supplemented with 10% fetal calf serum (FCS) (Boehringer Mannheim) and penicillin/streptomycin solution from Gibco.

**Solid phase radioimmunoassay (RIA) for HBV/plasma membrane or HBV/ASGPR interaction.** Five hundred ng of plasma membranes or of highly purified human ASGPR or anti-HBsAg antibody (Dianova), anti-preS1 MA18/7 and anti-preS2 Q19/10, respectively, were diluted in 50 µl of PBS pH 7.5 and coated to solid-phase RIA strips (Dianova) overnight at 4 °C. Post-coating occurred in 1% immunoglobulin/PBS for 1 h at room temperature. After three washes in binding buffer (10 mM-HEPES pH 7.5, 150 mM-NaCl, 10 mM-CaCl2, 0.1% immunoglobulin) various dilutions of HBV particle preparations or sera diluted 1:10, were incubated for 1 h at room temperature. After three further washes, commercial 125I-labelled anti-HBsAg antibody (Abbott Laboratories) was added for 1 h at room temperature. After three final washes the radioactivity in the wells was counted using a gamma counter. Results were expressed as sample/background ratio according to the manufacturers’ guidelines (except Table 1, 2 and Fig. 2a). Here the c.p.m. are given from an experiment done 48 h after delivery of the antibody. The background without coated plasma membranes or ASGPR was always below 10% of the total counts and then subtracted. In other experiments sample/background ratios were used according to the manufacturers’ guidelines.

**Solid-phase enzyme immunoassay for HBV/ASGPR interaction.** Purified ASGPR was biotinylated using sulpho-N-hydroxysuccinimidobiotin (Pierce) according to the manufacturer’s guidelines and kept at −20 °C until use at a concentration of 0.1 mg/ml. Flat-bottom 96-well plates (Dynatech) were coated with commercial anti-HBsAg antibody diluted 1:800 in PBS. After post-coating in 0.1% immunoglobulin/PBS (1 h, room temperature) purified HBV particles diluted 1:100 in binding buffer (10 mM-HEPES pH 7.5, 150 mM-NaCl, 10 mM-CaCl2, 0.1% immunoglobulin) were added to the wells. After an incubation period of 1 h at room temperature followed by five washings in binding buffer, biotinylated ASGPR diluted 1:10 up to 1:1024 was added. After another washing, avidin–horseradish peroxidase complex
HBV binding to hepatic ASGPR receptor

Results

HBV attachment to hepatocellular membrane-specific molecules was assessed using both plasma membranes and ASGPR purified from human liver. Fig. 1(a) indicates by silver staining that ASGPR preparations used in this study did not contain major contaminants. Anti-receptor antibody reacted specifically with ASGPR in liver lysate as shown by immunoblotting. HBV particles derived from HBsAg carriers with high titre viraemia are characterized in Fig. 1(b), complete Dane particles are present as demonstrated by immunoblotting of preS1 and preS2 molecules.

A solid-phase RIA coating either plasma membranes or purified ASGPR and detecting specifically bound HBsAg/HBV using a commercial, low level labelled anti-HBsAg antibody was employed for the analysis of HBV/ASGPR interaction. Maximum and saturable binding for HBV was achieved by adding approximately 1000 ng/ml protein equivalent of HBV particles (Fig. 2a). Fig. 2(b), showing a different assay using soluble preparations was proven by silver staining of SDS–polyacrylamide gels. Immunoblotting for ASGPR protein in crude liver lysate used a rabbit anti-ASGPR antibody indicating its immunospecificity. (b) Immunoblotting of HBV particles with anti-preS1, anti-preS2 and anti-S antibodies. The presence of all three HBV envelope proteins, namely small-sized (SHBs), mid-sized (MHBs) and large HBsAg (LHBs) was demonstrated by specific anti-preS1 (MA18/7, peptide specific, lane 1), anti-preS2 (Q19/10, glycan-specific, lane 2) and anti-S (peptide specific, from Abbott Laboratories, lane 3) antigen immunoblotting.

Fig. 1. Purity of HBV particles and ASGPR preparations. (a) Silver-stained human ASGPR preparation and immunoblotting of liver lysate with polyclonal rabbit anti-ASGPR antiserum. Purity of the ASGPR (Sigma) diluted 1:1000 was added and incubated for 1 h. After final washing 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) was added as substrate (Sigma; 5 mg/ml in PBS containing 0.01% H2O2) and colour was allowed to develop for 30 min. The reaction was stopped with NaOH and Aopt values were measured (Titertek). ASGPR-specific activity was proven by adding an excess of ligand or EDTA, respectively.

ASGPR-mediated binding and uptake of HBV in cell culture. HBV particles were labelled with 125I as described (Stockert & Morell, 1990), those exhibiting 1000 c.p.m./ng protein were used for studies of cellular ASGPR as described previously in detail (Stockert & Morell, 1990). Briefly, radiolabelled HBV particles (normalized to 0·5 × 10⁶ c.p.m./well) were incubated for 1 h on ice for binding studies. After three washings with ice-cold DMEM, 125I–HBV particles bound to cells were released by rinsing in ice-cold PBS containing 10 mM-EDTA. The radioactivity was measured using a gamma counter. Total cells were harvested by cell scraping. For uptake studies, cells were incubated with 125I–HBV diluted in DMEM without FCS for 2 h at 37 °C in a humified incubator. Degradation of 125I–HBV was then assessed by analysing TCA-soluble radioactivity in the supernatant. TCA always precipitated more than 90% of non-degraded, radiolabelled HBV. In both binding and uptake assays ASGPR-specific binding was analysed either by adding 0·5 mg ASGPR ligands i.e. asialofetuin, ASOR, rabbit anti-ASGPR immunoglobulin, normal non-immune rabbit serum diluted 1:500 or 5 mM-EDTA just prior to the incubation period, respectively.
biotinylated ASGPR bound to immobilized HBV, confirmed specific ASGPR/HBV interaction. Therefore, Fig. 2 demonstrates that natural HBV particles bind to isolated ASGPR. In addition, Fig. 3 shows that the reaction was detergent (Lubrol) sensitive if Lubrol was used above the critical micelle concentration. At low concentration (0.01%) both specific and non-specific binding increased, this was probably due to better binding conditions for the integral membrane protein ASGPR in detergent.
Table 2. Inhibition of HBV attachment to human ASGPR

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<thead>
<tr>
<th>Inhibitor</th>
<th>Inhibition (%)</th>
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<tr>
<td></td>
<td>100 µg per well</td>
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<tr>
<td>Asialoorosomucoid</td>
<td>67</td>
</tr>
<tr>
<td>Orosomucoid</td>
<td>0</td>
</tr>
<tr>
<td>Asialofetuin</td>
<td>56</td>
</tr>
<tr>
<td>Fetuin</td>
<td>0</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>3.6</td>
</tr>
<tr>
<td>Human serum albumin</td>
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</tr>
<tr>
<td>Bovine haemoglobin</td>
<td>0</td>
</tr>
<tr>
<td>Human immunoglobulin*</td>
<td>0</td>
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<tr>
<td>EDTA (10 mM)</td>
<td>98</td>
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</tbody>
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* Inhibitory activity of human immunoglobulin on HBV attachment to ASGPR was determined by using haemoglobin as protein reagent in both post-coating and incubation buffer.

Fig. 4. Inhibition of binding activity of HBV particles to isolated ASGPR. Binding of purified HBV particles to solid-phase bound ASGPR was assessed by coincubating anti-preS1 and anti-preS2 MAbs in decreasing titre from 1:200 to 1:10000, respectively. The results are expressed as sample radioactivity/background.

Table 3. Binding of HBV particles and HBsAg to anti-HBsAg, anti-preS1, anti-preS2 and ASGPR

<table>
<thead>
<tr>
<th>Target</th>
<th>Bound radiolabelled ligand (c.p.m.)</th>
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<tr>
<td></td>
<td>Anti-HBsAg</td>
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<tr>
<td></td>
<td>(1:500)</td>
</tr>
<tr>
<td>HBV (1 µg)</td>
<td>2943 ± 189</td>
</tr>
<tr>
<td>HBsAg (1 µg)</td>
<td>2603 ± 98</td>
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MA18/7, completely inhibited HBV binding to ASGPR in a dose-dependent manner. In contrast, similar dilutions of anti-preS2 antibody Q19/10 had no effect. In a different approach, as shown in Table 3, binding of HBV to purified ASGPR was shown to be restricted to the preS1 or preS2 but not HBsAg envelope proteins. Whereas HBV particles derived from viraemic carriers bound to both commercial anti-HBsAg or preS1/preS2 antibodies as well as to ASGPR, purified HBsAg, which only contained small amounts of impurities, did not bind to the receptor. Thus, the preS1 region of the HBV envelope detected by the MA18/7 MAb is related to the attachment site of HBV to the ASGPR.

Fig. 5 demonstrates that circulating HBV particles from HBsAg-positive patients were also found to bind to the ASGPR. This finding provides evidence that circulating factors do not interfere with HBV binding to ASGPR. Sera from patients with high-titre viraemia (as defined by HBV DNA concentrations of more than 10 pg/ml) demonstrated significantly more HBV bound to the ASGPR than sera with low-titre viraemia (P < 0.05 by Student's t-test of unpaired variables).
Fig. 6. ASGPR-mediated uptake of 125I-HBV particles into hepatoma cells. (a) Purity of 125I-HBV particles. Radiolabelled HBV particles (10000 c.p.m./lane) were separated by SDS–PAGE (10% polyacrylamide). After separation and gel drying autoradiography was performed for 24 h. The arrows indicate a broad band corresponding to the small-sized envelope protein and fine bands for the mid-sized and large HBV envelope proteins, respectively. (b) HepG2 cells, HuH7 cells and COS cells were incubated with 2 x 10^6 c.p.m. of 125I-labelled HBV particles per well in the absence (□) or presence of 500 μg/ml asialofetuin (□) or polyvalent rabbit anti-ASGPR immunoglobulin (■) or non-immune rabbit serum (■) for 1 h at 37 °C. TCA soluble supernatant radioactivity was measured in a gamma counter. Results are given as c.p.m. per 0.5 x 10^6 cells.

Table 4. 125I–HBV particle binding to HepG2, HuH7 and COS cells in vitro

<table>
<thead>
<tr>
<th>Modifier</th>
<th>Cell line</th>
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<tr>
<td></td>
<td>HepG2</td>
<td>HuH7</td>
<td>COS</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3063±1501</td>
<td>9260±1861</td>
<td>613±65</td>
<td></td>
</tr>
<tr>
<td>Asialofetuin†</td>
<td>1605±414</td>
<td>5160±3058</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HSA†</td>
<td>4280±1456</td>
<td>6526±1786</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anti-ASGPR‡</td>
<td>733±565</td>
<td>1860±441</td>
<td>0</td>
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</table>

* Results are given as EDTA-released radioactivity (c.p.m.)±s.d. after a 1 h binding incubation on ice.
† Asialofetuin and HSA (human serum albumin) were used at a concentration of 0.5 mg/ml.
‡ Anti-ASGPR was used at a dilution of 1:500.

Fig. 6(a) gives a representative autoradiograph of radiolabelled, purified HBV particles separated by SDS–PAGE indicating the bands that correspond to the three envelope proteins of HBV (arrows): HBsAg at approximately 24K, preS2 at 34K and preS1 at 42K. Therefore, 125I-labelled HBV surface proteins were apparently obtained by the labelling procedure. In Table 4, 125I–HBV binding to different cell types (HepG2, HuH7 and COS cells) is shown. Evidently, human hepatoma cell lines are susceptible to HBV particle binding that is strikingly blocked by anti-ASGPR antibody. HBV binding to ASGPR-negative COS cells is at a nearly 10-fold lower level. Therefore, HBV attachment to hepatocyte-derived cell lines appear to be ASGPR-restricted. Fig. 6(b) clearly demonstrates that HepG2 cells and HuH7 cells but not COS cells can endocytose 125I-labelled HBV particles as indicated by their intracellular degradation to non-precipitable peptides. The uptake of highly purified virus protein was inhibitable using both excess of ligand and polyclonal anti-ASGPR immunoglobulin but not by normal rabbit
serum. These results suggest that more than 50% of the HBV endocytosis into hepatocytes is mediated by the ASGPR.

Discussion

The ASGPR was the first mammalian lectin described (Morell et al., 1968), mediating the uptake of a variety of ligands. Endogenous ligands for the ASGPR are highly organ-specific (Treichel et al., 1989) and differ in affinity, which is dependent upon glycosylation status (Harford & Ashwell, 1982). The presumed recognition of glycosides related to the preS1 region of the HBV envelope appears to be restricted to a specific binding site on the virus as indicated by blocking experiments with both ASGPR ligands, anti-ASGPR antibody and HBV surface-directed antibodies. Earlier studies have shown that recombinant-derived HBV binds to human-derived liver plasma membranes and HepG2 cells at a much reduced level compared with purified HBV particles (Alberti et al., 1990; Kaiser et al., 1986). It could be postulated that these findings are explained by inadequate glycosylation of recombinant virus particles.

Our data defining the interaction of HBV particles with the ASGPR as classical C-type lectin-linked lead to the assumption that ϑ-galactose-containing residues are present on the virus. Other glycosides recognized by the ASGPR provide much weaker binding avidity, which would be inconsistent with binding of HBV virion to the ASGPR in presence of potential ligands in circulation (Fig. 5). Moreover, a similar glycan-mediated infection was recently demonstrated for the murine coronavirus that causes a viral hepatitis in mice (Williams et al., 1991). The receptor was identified as a member of the receptors for the carcinoembryonic antigen family, a glycoprotein which was also shown to represent a ligand for the ASGPR (Harford & Ashwell, 1982). In addition, it must be considered that desialylated serum factors attached to the HBV may serve as mediators of interaction with the receptors' binding site. HBV particles attached to an asialoglycoprotein may contribute a carrier-mediated mechanism for HBV binding on liver cells.

The effect of anti-preS1 MAb remains unclear concerning a candidate attachment site on the HBV. The MAb MA18/7 is directed against the preS1 polypeptide. A steric inhibition effect could be responsible for the interference with a ϑ-galactose-bearing glycoside on the virus. The possible role of the large surface protein on HBV expressing the likely binding site for the receptor is supported by the experiment shown in Table 3. Binding of HBV to ASGPR is clearly dependent on the presence of preS1 or preS2 but cannot be mediated by the glycosylated HBsAg. However, the glycosylation of the preS1 region is a controversial issue. PreS1 exhibits 10 potential sites for N-linked glycosylation. Using a yeast expression system in vitro, both O- and N-linked glycosylation of the preS1 polypeptide have been described (Kuroda et al., 1992). In contrast, despite being highly glycosylated, evidently preS2 molecules do not contain galactose residues on their N-linked glycans (Yu Ip et al., 1992). Therefore, the extent and quality of preS1 glycosides remains unclear and has to be defined more clearly in the future.

The recognition of HBV via preS1-related glycosides may have potential significance for disease progression. Attachment of HBV to the ASGPR was clinically associated with a high titre viraemia carrier status, however, with a large margin of variation in titre. It could be concluded that each patient carries a distinct amount of HBV that is capable of binding to the ASGPR or presents with different amounts of ASGPR inhibitors in blood circulation. Nonetheless, Fig. 5 clearly indicates the rule that the higher the amount of HBV the more HBV binds to the ASGPR. This observation could be explained by a higher amount of particles expressing binding sites for attachment to hepatocytes. This appears to be of major importance since a close relationship between preS1/HBs antigens to HBV carriers with high titre viraemia has been described (Petit et al., 1990). The role of preS1 molecules in a humoral immune response is presumably essential for the course of HBV-related disorders since preS1 persistence is correlated to chronicity (Gerken et al., 1987).

An open question is the relative organotropism of HBV infection. A recently published report postulates a receptor on liver cells of Mf, 31K (Dash et al., 1992). The authors, however, used synthetic peptide for their crosslinking studies and did not find binding to peripheral blood lymphocytes, a cell type often infected by HBV. Another publication had demonstrated that endoxenin II binds to small HBsAg (Hertogs et al., 1993). Our data lead to the hypothesis that, at least in part, hepatocyte-specific ASGPR-mediated HBV uptake is responsible for the organotropism. In vitro, hepatoma cells like HepG2 and HuH7 cells are much more capable of binding and endocytosis of HBV particles than ASGPR-negative COS cells. It might therefore be likely that an HBV virion is rapidly cleared by the hepatic ASGPR after injection, similar to its physiological ligands. In addition, HBV infection of non-hepatic cells via similar mechanisms could be explained by ASGPR-related molecules: Kupffer cells possess a distinct asialoglycoprotein binding system which uses C-reactive protein as carrier (Kolb-Bachofen, 1991). ASGPR-related protein has also been described in testis (Abdullah & Kierszenbaum, 1989) and in the developing intestine (Hu et al., 1991). However, most ϑ-galactose-containing
glycoproteins are, after injection, almost exclusively cleared by the liver within the first passage which could explain the organotropic infection of the liver by α-galactose-carrying HBV.

Our findings suggest that the ASGPR is a candidate receptor for HBV attachment on hepatocytes. After ASGPR-mediated endocytosis and degradation, virus DNA could be enabled to integrate into the genome of the host leading to chronic viraemia and liver inflammation. Further studies have to define potential glycoconjugates on the virus envelope and to prove the ASGPR-mediated route as a major pathobiological event of HBV infection.

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Methods


