The preS1 domain of hepatitis B virus and IgA cross-react in their binding to the hepatocyte surface

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Using a solid-phase assay we have demonstrated specific competition between the preS1 sequence of hepatitis B virus and human IgA in their binding to isolated normal human liver plasma membranes, suggesting molecular mimicry. Monoclonal and polyclonal antibodies raised against virus and IgA epitopes were used to detect and map immunological cross-reactivity to the virus sequence involved in liver cell binding. These findings suggest the existence of a common receptor or of closely related receptors for the attachment of HBV and IgA to human liver cells.

Introduction

Hepatitis B virus (HBV) infects in vivo its target cell, the hepatocyte, with very high efficiency. Infection of cultured hepatocytes or hepatoma cells is, however, very inefficient. This difficulty has prevented the unequivocal identification of the cellular receptors for infectious HBV. However, attachment of purified hepatitis B surface (HBs) particles to cultured hepatoma cells (Neurath et al., 1985) or isolated plasma membranes from fresh human liver (Pontisso et al., 1989a) has been described and attributed to amino acids 21 to 47 in the preS1 domain of the large HBs protein (Neurath et al., 1986; Pontisso et al., 1989b). The existence of a limited sequence similarity between this attachment site and the C region of the human IgA α-1 chain (Neurath et al., 1986, Neurath et al., 1990) has raised the hypothesis that HBV may attach to receptors on human hepatocytes which are also used by IgA. To explore this issue further, we have investigated immunological and functional cross-reactivity of the sequence involved in the binding of preS1 and IgA to isolated human liver plasma membranes.

Methods

Isolation of human liver plasma membranes. Plasma membranes were obtained from normal human liver by the procedure of Hubbard et al. (1983), as described previously (Pontisso et al., 1989a). Briefly, the liver tissue was minced and homogenized in STM (0.25 M-sucrose, 5 mM-Tris-HCl pH 8, 0.5 mM-MgCl2) in ice and centrifuged at 280 g for 5 min. The supernatant was collected and centrifuged at 1500 g for 10 min. The pellet was resuspended in STM and adjusted to a sucrose density of 1.18 g/ml. This solution was overlaid with STM and ultracentrifuged at 78000 g for 1 h using an SW40Ti rotor (Beckman). All the centrifugation procedures were carried out at 4 °C. The plasma membrane fraction was then collected from the interface and stored at −70 °C until use. Plasma membrane fractions were characterized by measuring the enzymatic activities of 5′-nucleotidase (5′NT) and glucose-6-phosphatase (6GP) (Pontisso et al., 1989a). Protein concentration was determined by the Bio-Rad Protein Assay and BSA was used as the control. 5′NT activity was enriched 6-5-fold over that of the starting homogenate and the 5′NT/6GP ratio was 2.35, indicating a satisfactory degree of purification in the plasma membrane fraction, as found by Hubbard et al. (1983). Rat liver plasma membranes (control) were prepared by using the same technique and showed a comparable grade of purification, as indicated by a very similar degree of marker enzyme enrichment.

Viral particles and reagents. A preparation enriched in HBV particles was obtained from the serum of an HBsAg/HBV DNA-positive carrier, as previously described (Alberti et al., 1984). Briefly, 10 ml of serum was ultracentrifuged through a discontinuous sucrose density gradient (10% to 20% to 30% in 0.01 M-Tris–HCl pH 8, 1% BSA, 0.15 M-NaCl) for 4 h at 36000 r.p.m. using an SW40Ti rotor. The pellet was resuspended in PBS and the presence of complete HBV particles was ascertained by its strong reactivity for HBsAg (AUSRIA, Abbott Diagnostics) and for HBV–DNA (dot blot hybridization) (Alberti et al., 1986). The preparation was proved to contain preS1 reactivity (P/N ratio 42) by radiolmmunoassay (RIA), based on the use of an anti-preS1 monoclonal antibody (MAb) (MA18/7) on the solid phase (10 μg/ml) and 125I-labelled anti-HBs (Abbott) as the revealing tracer. Different synthetic peptides corresponding to preS1 (12–22), preS1 (32–47), preS2 (120–130) and preS2 (130–138) protein sequences of HBV, subtype ad2, were obtained from the American Peptide Company and purified by HPLC. Human plasma IgA (dimeric) with an Mr of about 400000 and purity higher than 98% of the total protein content was purchased from Protogen. These characteristics were confirmed in our laboratory by Western blot analysis after denaturation using, in parallel, Coomassie blue staining and peroxidase-linked anti-human IgA staining. The IgA was free of anti-HBs AUSAB Abbott Diagnostics) and of anti-preS1, as detected by solid-phase RIA with synthetic peptides (Alberti et al., 1990). Human IgG was prepared from normal human serum by ion-exchange chromatography using a DE-52 resin (Whatman). Essentially globulin-free human serum albumin
(HSA) was purchased from Sigma. Asialoglycoprotein (ASOR) was prepared by desialylation of orosomucoid (Sigma) according to Daniels et al. (1989). The sialic acid content before and after treatment was 489 µg/ml and 71-6 µg/ml, respectively. Ligands were labelled with carrier-free Na2125I (Sorin Biomedica) using the lactoperoxidase method for proteins and the chloramine-T procedure for synthetic peptide (21-32).

Specific activities (µCi/µg) were 1395 for synthetic peptide (21-32), 18.8 for IgA and 21.2 for HBV particles. Rabbit polyclonal anti-human IgA (α chain) and rabbit polyclonal anti-human IgG (γ chain) were purchased from Behringwerke. Mouse MAb C20/02 was directed against the S domain of HBsAg (W.H. Gerlich, unpublished results) and MAb MA18/7 recognized the 29 to 36 preSI sequence, as previously described (Deepen et al., 1990). MAb T0606 to the 95 to 107 preSI sequence (Takai et al., 1986) was obtained from Sorin Biomedica.

**Binding and inhibition experiments**

(i) **Binding of different plasma proteins, virus particles and synthetic peptides to liver plasma membranes and cross-inhibition experiments.** Polystyrene microplates (Linbro, Flow Laboratories) were coated with human liver plasma membranes (20 µg/ml in PBS). After an overnight incubation, wells were saturated with 10% BSA for 4 h at room temperature and further incubated with labelled ligands (about 100000 c.p.m. per well) for 18 h. Plates were washed four times with PBS containing 1% BSA-0.05% Tween 20 and each well was cut out and counted in a gamma counter. Inhibition experiments with unlabelled reagents were carried out by the following procedure: 25 µl of PBS or unlabelled proteins (10 µg/ml) was mixed with the 125I conjugate just before incubation with plasma membranes. The radioactivity of the membrane-coated wells was then evaluated after 18 h, as described above. The specific binding was calculated by subtracting from the total bound c.p.m., the c.p.m. of the non-specific binding, determined as the difference between total c.p.m. and c.p.m. obtained after incubation of the labelled ligand with a 100-fold excess of unlabelled ligand. Percent inhibition was calculated as follows:

\[
100 - \frac{(\text{ligand} + \text{inhibitor}) \text{ c.p.m.}}{(\text{ligand} + \text{PBS}) \text{ c.p.m.}} \times 100.
\]

A reduction of 50% or more of the initial binding was considered significant.

(ii) **Binding of anti-preSI MAb to HBV particles and inhibition by plasma proteins.** Polystyrene microplate wells (Nunc) were incubated overnight with 50 µg/ml of serum-derived HBV particles. The plates were then washed with PBS containing 1% Tween 20 and incubated with 10% BSA in PBS for 5 h. Fifty µl of anti-preSI MAb (MA18/7) (1 µg/ml) was mixed with different amounts (0 to 50 µg) of IgA in a final volume of 50 µl, and added to coated wells for 18 h at 4 °C. Similar experiments were performed where MA18/7 was mixed with different amounts of HSA or with PBS. Binding to the solid phase was revealed by the addition of peroxidase-linked anti-mouse IgG (Amersham) for 3 h at 37 °C, followed by H2O2 and H2SO4, and the absorbance was measured at 490 nm in each well was evaluated using a Titertek Multiskan. Percent inhibition was calculated as described above.

(iii) **Immunological cross-reactivity between preSI and IgA.** The solid phase was coated with 100 µl of IgA, containing 5 µg protein, for 18 h, washed with PBS containing 1% BSA and post-coated with 10% BSA for 2 hours at 37 °C. Anti-preSI MAb (MA18/7) (0.0001 to 1 µg) in a final volume of 100 µl was subsequently added for 2 h at 37 °C. Binding of antibody was detected by the addition of peroxidase-linked anti-mouse IgG, as described above. Similarly, the microplate was coated with 100 µg/ml rabbit anti-human IgA antibody and post-coated by the addition of a 10% BSA solution. After an overnight incubation, serial dilutions of HBV particles (total proteins ranging between 0-1 and 10 µg) were added and incubated for 2 h at 37 °C, followed by washing with PBS-Tween 20 and incubation with anti-HBs mouse MAb (C20/02) for 2 h at 37 °C. Binding was then revealed by adding anti-mouse IgG conjugated with peroxidase.

**Results**

As reported previously, 125I-labelled hepatitis B virions bound efficiently to purified human liver plasma membranes absorbed to microplate wells (Table 1). Conversely, only trivial binding (130 c.p.m.) was detectable using rat liver plasma membranes coated onto the solid phase. Binding of native HBV particles to liver plasma membranes was inhibited by preSI peptide (21-32) and by a MAb (MA18/7) directed against the (29-36) preSI region. Low level inhibition (33%) was observed with preSI peptide (32-47) which was considered non-specific. Indeed, this peptide was observed to exert similar low level inhibition (28%) on the binding of peptide (21-32) to human liver plasma membranes (Table 2). Human IgA and rabbit anti-human IgA caused significant inhibition of HBV binding to human liver plasma membranes, whereas human albumin and IgG did not. Anti-human IgG caused low level inhibition

<table>
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<tr>
<th>Viral particles plus</th>
<th>Specific c.p.m.*</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>9634</td>
<td>0</td>
</tr>
<tr>
<td>Peptide preSI (21-32) (10 µg/ml)</td>
<td>2304</td>
<td>76</td>
</tr>
<tr>
<td>Peptide preSI (32-47) (10 µg/ml)</td>
<td>6454</td>
<td>33</td>
</tr>
<tr>
<td>MAb MA18/7 (0-1 µg/ml)</td>
<td>795</td>
<td>92</td>
</tr>
<tr>
<td>MAb T0606 (0-1 µg/ml)</td>
<td>10124</td>
<td>0</td>
</tr>
<tr>
<td>Human IgA (10 µg/ml)</td>
<td>1834</td>
<td>81</td>
</tr>
<tr>
<td>Human IgG (10 µg/ml)</td>
<td>9894</td>
<td>90</td>
</tr>
<tr>
<td>Human albumin (10 µg/ml)</td>
<td>9464</td>
<td>2</td>
</tr>
<tr>
<td>ASOR (10 µg/ml)</td>
<td>3556</td>
<td>63</td>
</tr>
<tr>
<td>Anti-human IgA (1:10^-1)</td>
<td>184</td>
<td>98</td>
</tr>
<tr>
<td>Anti-human IgG (1:10^-2)</td>
<td>6307</td>
<td>35</td>
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</table>

* Specific c.p.m. was calculated, as described in Methods, by subtracting the c.p.m. of non-specific binding, which was in this case 1465, from total bound c.p.m.

<table>
<thead>
<tr>
<th>Synthetic peptide plus</th>
<th>Specific c.p.m.*</th>
<th>Inhibition (%)</th>
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</thead>
<tbody>
<tr>
<td>PBS</td>
<td>4580</td>
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</tr>
<tr>
<td>Peptide preSI (32-47) (10 µg/ml)</td>
<td>3298</td>
<td>28</td>
</tr>
<tr>
<td>Peptide preS2 (120-139) (10 µg/ml)</td>
<td>4732</td>
<td>0</td>
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<tr>
<td>Peptide preS2 (130-138) (10 µg/ml)</td>
<td>3664</td>
<td>20</td>
</tr>
<tr>
<td>Human IgA (10 µg/ml)</td>
<td>64</td>
<td>99</td>
</tr>
</tbody>
</table>

* Specific c.p.m. was calculated, as described in Methods, by subtracting the non-specific c.p.m., which was in this case 1820, from total bound c.p.m.
IgA and preS1 cross-reactivity

Fig. 1. Binding of $^{125}$I-labelled human IgA to human liver plasma membranes in the presence of increasing amounts of ligands: HSA (○), peptide (21–32) (□), HBV particles (●), peptide (32–47) (⋆), ASOR (■), IgA (●). Results are expressed as net c.p.m., calculated by subtracting from total bound c.p.m. the background c.p.m. (360), obtained by incubating labelled IgA in microplate wells without human plasma membranes, saturated with 10% BSA.

(35%) which was considered non-specific and could have been due to interaction with IgG bound in vivo to native HBV particles. The involvement of the (21–32) preS1 sequence was further confirmed by using a $^{125}$I-labelled preS1 (21–32) peptide which bound to liver plasma membranes (Table 2) and was strongly competed by human IgA, but not by IgG, albumin or peptides from other regions of the preS1 and preS2 domains.

We next examined whether purified IgA bound to liver plasma membranes and the competition exerted by entire HBV and by preS1. Indeed, IgA bound to liver plasma membranes; this interaction was blocked by preS1-containing HBV particles as efficiently as by unlabelled IgA (Fig. 1). PreS1 peptide (32–47) showed greater inhibition than did preS1 peptide (21–32). These results suggest molecular mimicry of IgA and HBV on the liver cell membrane. This may be the consequence of sequence similarity (Neurath et al., 1986, 1990). Cross-reactivity between the α-1 chain and the preS1 (29–36) domain was indeed demonstrated in the following experiments. Anti-preS1 MAb MA18/7, raised against purified HBV, bound IgA in a solid-phase immune assay (Fig. 2a) and anti-α chain antibody bound purified HBV particles at concentrations up to 1 µg/ml (Fig. 2b). Furthermore, IgA specifically competed with MAb MA18/7 in binding purified HBV particles (Fig. 3). To explore the nature of the IgA binding site on liver plasma membrane, the possible role of the ASOR receptor was examined. Binding of IgA to liver plasma membranes was inhibited by an excess (100-fold) of a desialylated glycoprotein such as ASOR. However, a similar effect was seen also using the preS1 peptides which have no carbohydrate side-chains. ASOR also inhibited the

Fig. 2. (a) Binding of increasing amounts of anti-preS1 MAb MA18/7 to solid-phase-coated IgA. (b) Binding of increasing amounts of HBV particles to solid-phase-coated anti-human IgA. The dotted lines represent the cutoff.
binding of HBV to liver plasma membranes (Table 1). These findings would suggest that the ASOR receptor could be contiguous with but not identical to the preS1/IgA binding site. This would appear in agreement with the findings of Neurath et al. (1990) who, in addition, excluded involvement of the receptor for secretory IgA. This is expected as this receptor is thought not to be expressed on human hepatocytes (Delacroix et al., 1983; Nagura et al., 1981).

**Discussion**

Viruses often use cell receptors for physiological ligands as sites of attachment and entry. Infection of hepatocytes by HBV could not be investigated in our system and, consequently, identification of a true receptor function for HBV was beyond the purpose of this study. Antibodies against preS1 sequence (21–47) have been shown to neutralize infectivity of HBV in chimpanzees (Neurath et al., 1989) and peptide (21–47) is able to induce protective immunity against HBV (Thornton et al., 1989). These data are consistent with an essential role of this amino acid sequence in *in vivo* attachment of HBV to hepatocytes. Here, we have demonstrated that the preS1 (21–32) sequence contains binding sites for the hepatocyte membranes which express functional and immunological cross-reactivity with binding sites on human IgA. This molecular mimicry may be the consequence of sequence similarity, the amino acid composition of preS1 (21–32) being PLGFEPDHGLDP and that of IgA chain (28–39) being VQGFPQQLPSV (identical amino acids are underlined). This does not necessarily mean that IgA and preS1 in fact share the same receptor. One way in which IgA binds to human hepatocytes is through the receptor for ASORs (Stockert et al., 1982; Tomana et al., 1988; Daniels et al., 1989). In our experiments ASOR inhibited binding of both IgA and HBV to liver plasma membranes. These findings could mean that the observed binding indeed involved terminal galactose of N-linked glycan, but may also merely reflect steric hindrance by the bulky inhibitor used. The inhibition also exerted by the much smaller preS1 (21–32) and (32–47) peptides, which do not contain terminal galactose residues, supports the latter possibility. Thus, the ASOR receptor might be contiguous with the binding site of preS1, but not identical to it. Involvement of another IgA receptor, the secretory component (SC) is also highly unlikely in our system as the SC is not expressed on human hepatocytes (Nagura et al., 1981; Delacroix et al., 1983) also justifying the negative results recently obtained by Neurath et al. (1990).

The nature of the cellular receptor which is used *in vivo* by HBV to enter hepatocytes and other cellular compon-

**References**


IgA and preS1 cross-reactivity


(Received 7 February 1992; Accepted 27 April 1992)