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Hepatitis B Virus (HBV) Binding Factor in Human Serum: Candidate for a Soluble Form of Hepatocyte HBV Receptor

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A hepatitis B virus (HBV) binding factor (HBV-BF) was identified in normal human serum interacting with the pre-S1 and pre-S2 epitopes of the viral envelope located within the protein domains involved in recognition of hepatocyte receptor(s). This molecule was characterized as a 50-kDa glycoprotein showing an isoelectric point of 7.13 with a biological activity depending on its native molecular conformation and on intact sulfhydryl bonds. Monoclonal antibodies to HBV-BF recognized a membrane component of the normal human liver whereas they were unreactive with hepatocyte membranes of other species and with those of the HepG2 cell line. These results suggest that the HBV-BF represents a soluble fragment of the membrane component and can be related to the HBV receptor mediating attachment of HBV to human liver cells.

In analogy with other viral diseases, hepatitis B virus (HBV) infection is likely initiated by specific binding of the virions to cell membrane structures via one—or potentially several—viral envelope protein(s). HBV has not been propagated in established cell lines, and only humans and higher apes are susceptible to infection. HBV replication and cellular injury are largely confined to the liver, and the hepatocyte is considered the primary target cell for infection, whereas the significance of extrahepatic replication of HBV is not yet well understood (27).

The HBV envelope consists of three distinct coterminal proteins which are encoded by a single *env* gene. The domains of these proteins encoded by the pre-S region of the viral genome represent potential attachment sites of HBV to the hepatocyte, since pre-S1 and pre-S2 antibodies neutralize infectivity in vitro (20) as well as in experimental models (11, 22) and inhibit binding of virions to isolated hepatocyte membranes (30, 31). Neurath et al. (21) demonstrated that both pre-S1- and pre-S2-encoded domains of the HBV envelope proteins are involved in recognition of hepatocyte receptors. For these authors, the crucial HBV binding site is located within the amino acid sequence 21 to 47 of the pre-S1 domain and the auxiliary binding site is within the pre-S2 amino acid sequence 120 to 145.

Although the viral structures involved in attachment to the target cell have been identified, the cellular receptor(s) for HBV has not yet been determined and the biochemical events leading to infection remain unknown. Besides hepatocytes, many cells of nonhepatic origin, including hematopoietic cells, of the B lymphocyte lineage, peripheral blood lymphocytes, and even some nonhuman, simian virus 40-transformed cell lines have receptors for the pre-S1 amino acid 21-to-47 region (25). Since the pre-S1 domain of the large envelope protein has a partial sequence homology with the Fc moiety of the α chain of immunoglobulin A (IgA) (23), a common receptor for the attachment of HBV and IgA to human liver cells has been proposed (32). On the other hand, interleukin 6, containing recognition sites for the pre-S1 domain, could mediate HBV-cell interaction (24), and the transferrin receptor might play a role in the binding of HBV to hepatocytes via the pre-S2 protein sequence, as this domain is involved in the binding of hepatitis B surface antigen (HBsAg) particles with T cells (6).

The present report describes the isolation and characterization of a serum glycoprotein which interacts with pre-S epitopes of the viral envelope and inhibits binding of pre-S1 (Ma18/7)- and pre-S2 (F124)-specific monoclonal antibodies (MAbs) to viral and recombinant HBV particles. The localization of the molecule, antigenically related to HBV binding factor (HBV-BF) in the liver cell membrane, suggests that this circulating glycoprotein could represent a soluble form, or a fragment, of the hepatocyte receptor mediating attachment of HBV to liver cells.

MATERIALS AND METHODS

Detection of the pre-S-binding protein by inhibition assays. Solid-phase inhibition enzyme-linked immunosorbent assay (ELISA) has been previously used for detection of antibodies directed against pre-S1 and pre-S2 region-encoded epitopes in patients' sera (1). These assays were based on inhibition of the binding of horseradish peroxidase (HRPO)-labeled MAbs to the pre-S epitopes present on HBV or on recombinant HBsAg particles with pre-S2 epitopes (m-HBsAg [18]) preadsorbed onto microwells. HBV-BF was detected by both these assays in some human sera collected from healthy subjects without any HBV markers as well as from hepatitis patients.

Inhibition of the binding of pre-S1-specific MAb Ma18/7 (10) to pre-S1 epitopes was carried out with viral particles. This MAb recognizing the amino acid sequence 29 to 36 of the pre-S1 region was kindly provided by W. H. Gerlich. Serum-derived viral particles rich in pre-S1 were captured from HBV concentrates to the wells of microtiter plates (Nunc, Roskilde, Denmark) by means of anti-HBs MAb 39-10 produced in our laboratory. The plates were overcoated with 1% bovine serum albumin–0.05% Tween 20 in phosphate-buffered saline (PBS). Samples examined for HBV-BF activity were incubated for 2 h at 37°C on HBV-coated plates and, after washing, the wells were reacted with

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HRPO-labeled anti-pre-S1 Ma18/7 MAb and then with *o*-phenylenediamine as a substrate.

HBV-BF was also detected by inhibition of pre-S2-specific MAb F124 (2) recognizing an epitope localized in the N-terminal part of the pre-S2 domain, close to the pre-S2 glycosylation site. Recombinant m-HBsAg particles (1 μg/ml) containing S and pre-S2 determinants (18) were directly applied as a coating to the plate and overcoated with bovine serum albumin-Tween 20 in PBS. After washing, samples tested for the pre-S binding activity were incubated on the plate for 2 h at 37°C. After washing, the wells were reacted with HRPO-labeled MAb F124 and *o*-phenylenediamine.

Inhibition assays of anti-HBs MAbs were carried out under similar conditions with m-HBsAg particles applied as a coating to the solid-phase and HRPO-labeled anti-HBs MAbs 144 and 112 (Sanofi Diagnostics Pasteur).

The results of inhibition assays were expressed as percent inhibition calculated from the A_{492} values (A) substituted in the following formula:

$$\text{percent inhibition} = \frac{A_{\text{negative control}} - A_{\text{test sample}}}{A_{\text{negative control}} - A_{\text{positive control}}} \times 100$$

The samples were considered positive when providing more than 30% inhibition.

Purification of the pre-S-binding protein from human serum. Globulins were precipitated from normal human serum with 50% ammonium sulfate and dialyzed against 10 mM phosphate buffer, pH 7.4. The sample was then applied to a column of DEAE-cellulose (DE-52, Whatman) equilibrated with this buffer. After recovery of the unbound fraction, molecules retained on the column were eluted stepwise with 20, 30, 50, and 300 mM of the same buffer. Fractions positive for HBV-BF activity were subjected to gel permeation with a series of three Sephacryl S200 columns (1.8 by 90 cm) in PBS containing sodium azide. The final preparation was absorbed with insolubilized anti-IgG (anti-Fc and anti-Fab) to remove contaminating IgG and its fragments.

HPLC. The apparent molecular mass of the HBV-BF was estimated by gel permeation. High-performance liquid chromatography (HPLC) was carried out with a Superose 12 column (Pharmacia, Uppsala, Sweden) at a flow rate of 12 ml/h. Fractions were collected every 2.5 min. A_{280} was detected with an HPLC spectrophotometer (Gilson, Villiers le Bel, France) connected to a CR3A integrator (Shimadzu, Kyoto, Japan). Molecular mass of the HBV-BF was established by comparison with standards of human IgG and chymotrypsin and of IgM, secretory IgA, $F(ab')_2$, and Fab fragments prepared in our laboratory.

Electrofocusing. Preparative electrofocusing was carried out in liquid phase, with a Rotor cell (Bio-Rad, Richmond, Calif.). The sample was dialyzed versus 1% (wt/vol) glycine containing 1% (vol/vol) glycerol. Fractions were collected in separate tubes, according to the prescriptions of the manufacturer, dialyzed against PBS after measurement of the pH gradient, and analyzed for HBV-BF activity.

Absorption of HBV-BF activity with lectins. Concanavalin A, wheat germ agglutinin, and Helix Pomatia lectin bound to Sepharose 6MB were from Pharmacia. Peanut agglutinin bound to Ultrogel beads was from IBF (Villeneuve-la-Garenne, France). Absorption procedures were carried out according to the manufacturer's prescriptions.

HBV and HBsAg particles. Natural HBV particles were prepared from the plasma of a virus carrier by pelleting of viral particles for 6 h at 220,000 × *g* followed by three washings with cold PBS and isopycnic centrifugation in a 10 to 60% sucrose gradient for 26 h at 280,000 × *g*. Fractions rich in HBV DNA as determined by the Abbott hepatitis B DNA kit, banding at 38 to 43.5% sucrose, were pooled and concentrated. This preparation consisted mainly of complete virions (Dane particles) and tubules and contained only small amounts of 22-nm forms, as evidenced by electron microscopy. Recombinant m-HBsAg particles, containing the middle and the major HBV proteins (18), were kindly provided by R. Vinas (Pasteur Vaccin). These particles were purified by the producer.

Control experiments for proving that HBV-BF does not degrade pre-S epitopes. The inhibition assays were carried out in the presence of inhibitors of proteolytic enzymes. For this purpose, recombinant HBsAg–pre-S2 particles were incubated with purified HBV-BF in PBS containing 0.002 M phenylmethanesulfonyl fluoride and/or 2000 K.I.U of aprotinin per ml (Trasylol). The activities of HBV-BF with and without inhibitors were compared.

In the second series of experiments, HBV particles, purified from serum, were incubated with HBV-BF until pre-S epitopes were no longer detectable by ELISA with pre-S-specific MAbs. The treated sample and a control sample were then denatured and subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and immunoblotting.

Western immunoblot. Specimens were solubilized in a sample buffer containing 2% SDS, 5% 2-mercaptoethanol, 0.01% bromophenol blue, and 20% glycerol for 2 min at 100°C. The proteins were separated on 5 to 15% gels and electroblotted to nitrocellulose. The membrane strips were postcoated overnight at 4°C with 5% skim milk and washed and reacted for 1 h at room temperature with anti-pre-S1 5a-19 MAb diluted in 1% skim milk. This MAb, produced in our laboratory, recognizes pre-S1 amino acid sequence 35 to 53 of the ay antigenic subtype. HRPO-labeled anti-mouse Ig (heavy plus light chains) (Dako) served as a second antibody. After final rinses, the blots were visualized with an enhanced chemiluminescent detection system (Amersham, Little Chalfont, United Kingdom).

Preparation of MAbs to the pre-S-binding protein. Antibodies to the pre-S-binding protein were prepared by immunization of BALB/c mice with HBV-BF purified from human serum. The purification procedure involved precipitation with ammonium sulfate and DEAE-cellulose chromatography followed by either HPLC or electrofocusing. Mice were immunized subcutaneously with 0.5-ml samples emulsified in complete Freund's adjuvant; immunization was followed by two intramuscular injections with incomplete Freund's adjuvant, given at 10-day intervals. A final injection was given 3 days before fusion with NSO myeloma cells. Hybridomas were screened for the presence of antibodies against HBV-BF by ELISA on purified antigen-coated plates with HRPO-conjugated anti-mouse Igs (heavy plus light chains) (Dako).

Ascites were produced by intraperitoneal injections of hybridoma cells into mice pretreated with 0.5 ml of pristane.

Dot blot analysis. Fractions eluted from the HPLC column were tested for their reactivity with anti-HBV-BF MAb by dot blot. Aliquots (10 μl) of the samples were deposited on a nitrocellulose membrane and air dried. The membranes were postcoated overnight with 5% skim milk, washed, and incubated with anti-HBV-BF MAb diluted in skim milk. The antibody bound to the strips was reacted with HRPO-labeled anti-mouse Ig (heavy plus light chains) (Dako) and revealed by the enhanced chemiluminescent detection system (Amersham).

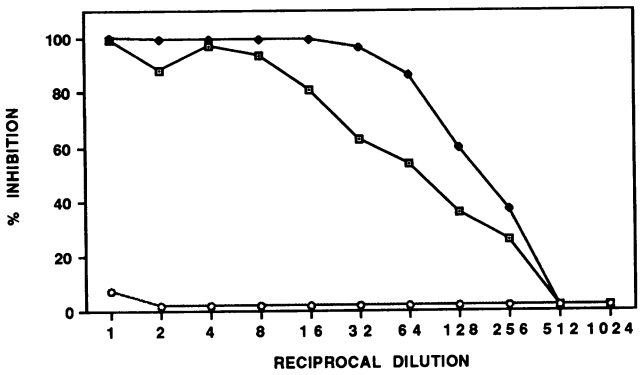

FIG. 1. Inhibition of the binding of HRPO-labeled pre-S1-specific Ma18/7 MAb (⊡) and of pre-S2-specific F124 MAb (◆) to HBV or to recombinant m-HBsAg particles by HBV-BF from human serum. No inhibition was observed for the mixture of anti-HBs MAbs 144 and 112 (○).

Immunohistochemistry. Tissue localization of antigens reacting with MAbs was investigated with frozen liver sections. Samples of normal human liver were from patients who underwent surgical resection for a benign tumor. Liver samples of Pekin duck, chicken, and woodchuck were kindly provided by L. Cova (Institut National de la Santé et de la Recherche Médicale U 271, Lyon, France). Liver tissue was embedded in ornithine carbamyl transferase, quickly frozen in liquid nitrogen, and stored at −80°C until use. Serial 5-μm-thick sections were cut, fixed for 5 min with cold acetone, and air dried. Immunohistochemical staining was carried out according to the avidin-biotin peroxidase complex method with the Vectastain ABC Elite kit (Vector Laboratories, Burlingame, Calif.) according to the instructions of the manufacturer. In order to block unspecific background, sections were preincubated in 3% normal sheep serum diluted in PBS. Sections were then incubated with the primary antibody at a 1:200 dilution in PBS for 30 min at room temperature. Biotinylated anti-mouse IgG (Vector) was used as a second step. Detection was carried out with 3-amino-9-ethyl carbazole as a chromogenic substrate. Controls included sections in which the primary antibody was omitted or replaced by irrelevant antibodies or PBS.

Immunohistochemical staining was also carried out on cultured hepatoma cells. HepG2 cells were grown under standard conditions in Dulbecco essential medium supplemented with 10% fetal calf serum. The cells were plated on slides (22 by 22 mm) for 48 h. After being washed with PBS, the cells were fixed and stained according to the procedure described above.

RESULTS

Identification of HBV-BF in human serum. HBV-BF was detected in some normal human sera by its ability to inhibit the binding of pre-S1-specific Ma18/7 MAb and pre-S2-specific F124 MAb to corresponding epitopes in an inhibition ELISA (Fig. 1). In contrast, there was no inhibition of the binding of MAbs to the major HBV S protein. Preadsorption of the HBV-BF-containing sera with 2 μg of recombinant m-HBsAg particles, containing pre-S2 and S epitopes, did not influence the inhibitory activity of the factor against anti-pre-S1 MAb. Therefore, inhibition involved the epitopes located in both pre-S1 and pre-S2 domains. The pre-S blocking activity could not be removed by affinity chromatography by using either anti-IgG, IgM, IgA, or anti-Fab immunosorbents (data not shown). Therefore, the pre-S blocking activity in these sera was due not to anti-pre-S antibodies or to their fragments but to an unknown factor which we termed HBV-BF.

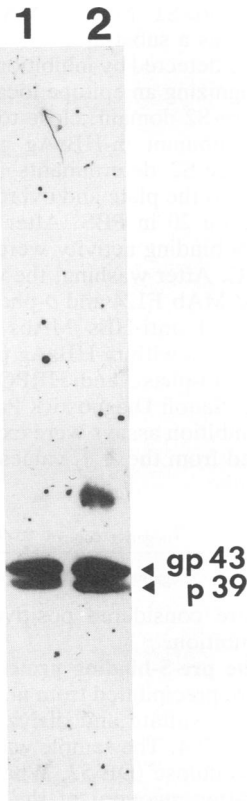

FIG. 2. Reactivity of anti-pre-S1 MAb 5a-19 with HBV preincubated with HBV-BF (lane 1) and with a control HBV preparation (lane 2). Protein bands were visualized by an enhanced chemiluminescent detection system after immunoblotting with HRPO-labeled anti-mouse IgG.

The activity of HBV-BF against pre-S epitopes was not influenced by inhibitors of proteolytic enzymes such as phenylmethanesulfonyl fluoride and aprotinin. Furthermore, the large HBV protein bands—containing pre-S1 and pre-S2 region-encoded sequences—had unchanged molecular masses (39 and 43 kDa) after treatment with HBV-BF (Fig. 2).

Characterization of HBV-BF from human serum. The HBV-BF was recovered in the pellet after precipitation of normal human serum with ammonium sulfate. The pre-S binding fraction was eluted from the anion exchanger with 10 and 30 mM phosphate buffers. The active fractions were then isolated by gel filtration and electrofocusing as outlined in Materials and Methods. At all steps of the purification procedure, the inhibitory activity of HBV-BF concerned always both pre-S1 and pre-S2 MAbs; the inhibition of MAb F124 was used in all further studies.

The purified factor exhibited an apparent molecular mass of 50 kDa as determined by HPLC (Fig. 3A). Its isoelectric point was neutral (pH 7.13), as judged by electrofocusing (Fig. 3B). Digestion with subtilisin abolished HBV-BF activity, indicating that HBV-BF was a protein (Fig. 4). Complete inactivation of its pre-S binding activity occurred

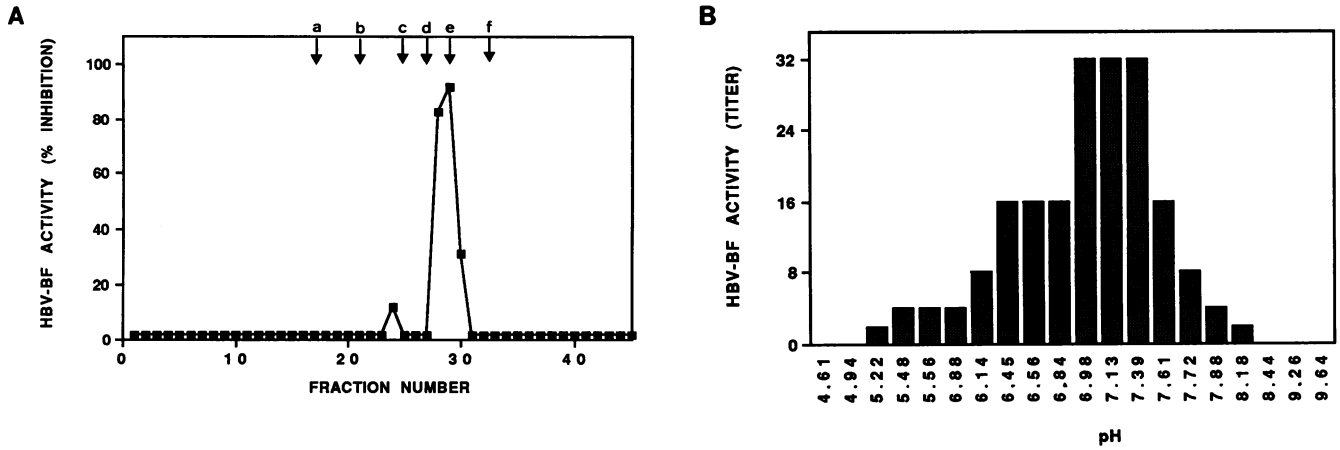

FIG. 3. (A) Molecular mass of HBV-BF determined by HPLC. Molecular mass markers: a, IgM (900 kDa); b, secretory IgA (410 kDa); c, IgG (150 kDa); d, F(ab')$_2$ (100 kDa); e, Fab (50 kDa); f, chymotrypsin (25 kDa). HBV-BF activity is expressed as percent inhibition of anti-pre-S2 MAb F124. (B) Isoelectric point of HBV-BF determined by electrofocusing. Titration of HBV-BF activity (inhibition of MAb F124 as described above) was performed on each fraction.

at 60°C, as well as after treatment with SDS or 2-mercaptoethanol or at acidic pH; therefore, native conformation or an intact disulfide bond(s) is essential for HBV-BF biological activity.

To determine whether carbohydrates were present in HBV-BF, the purified preparations were absorbed with various insolubilized lectins. As illustrated in Fig. 5, incubation of HBV-BF with lectins from wheat germ and Helix Pomatia (specifically binding to *N*-acetyl-β-D-glucosamine and to *N*-acetyl-galactosamine residues, respectively) reduced HBV-BF activity by more than fourfold, indicating that most of the HBV-BF remained bound to these lectins. Peanut agglutinin—specific for lactose residues—had only little effect on HBV-BF, and concanavalin A—reactive with α-methyl-glucose and methyl mannose—did not influence its activity.

Selection and characterization of MAbs against HBV-BF. Several hybridoma supernatants were found positive by direct ELISA with HBV-BF used as a coating on the solid phase and were subcloned. To determine whether MAbs were reactive specifically with HBV-BF, the purified preparation of HBV-BF was preabsorbed with these antibodies prior to the reaction with pre-S-containing HBsAg particles. This treatment completely abolished the pre-S binding activity of HBV-BF (data not shown). Furthermore, the reactivity of MAbs with HBV-BF was confirmed by dot blot analysis of HPLC fractions. MAb 9H11B3, representative of the group of antibodies tested, recognized the fractions with HBV-BF activity eluted from the HPLC column (Fig. 6).

Reactivity of MAbs with hepatocyte membranes. In human liver, a strong and specific staining was observed on the cytoplasmic membrane of hepatocytes (Fig. 7A). The staining pattern was thin and sustained all along the cell membrane, giving the parenchyma a honeycomb pattern. No staining was seen on liver sections when PBS or irrelevant antibodies were used. For livers of other species (rabbit, rat, chicken, Pekin duck, mouse, and woodchuck), no staining was observed on cell membranes (e.g., Fig. 7B). Furthermore, the membranes of HepG2 cells, fixed and stained under the same conditions, remained unlabeled (Fig. 7C).

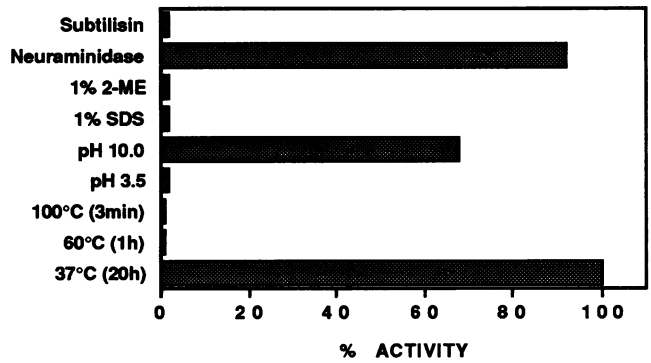

FIG. 4. Effect of the treatment of HBV-BF with enzymes and various physicochemical agents. Results of the treatment are presented as percent pre-S binding activity (inhibition of F124 MAb) in comparison with a control untreated sample. 2-ME, 2-mercaptoethanol.

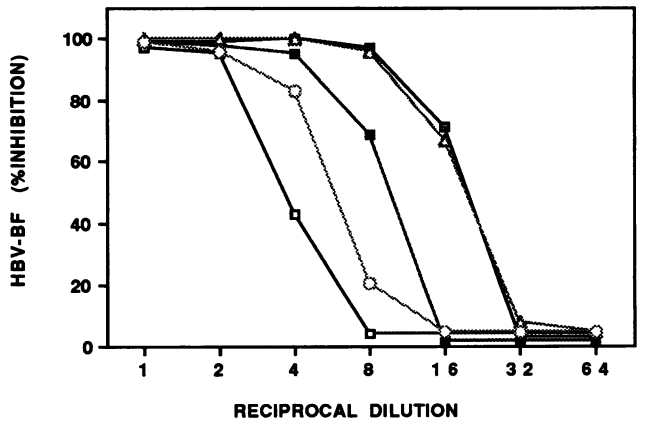

FIG. 5. Absorption of HBV-BF activity with lectins. Shown are nonabsorbed control (⊡) and samples absorbed with wheat germ agglutinin (□), peanut lectin (■), concanavalin A (△), and Helix Pomatia lectin (○). Results are expressed as residual activity after absorption.

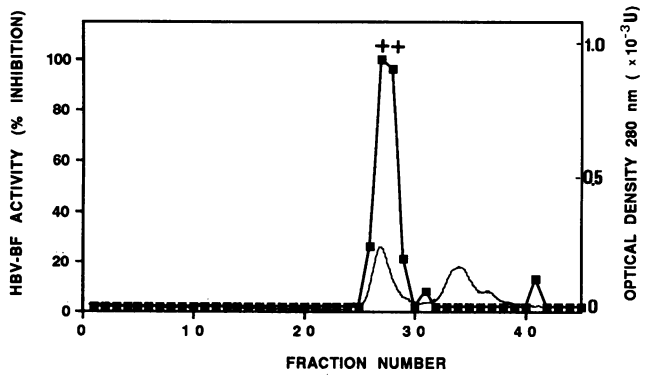

FIG. 6. Immunoreactivity of MAb with HBV-BF purified from serum. The fractions eluted from the HPLC column were tested for HBV-BF activity by an inhibition assay with F124 MAb (■) and for their reactivity with 9H11B3 MAb by dot blot (+). Optical density at 280 nm (——) is shown.

DISCUSSION

In this study, we identified a serum glycoprotein that interacts with the epitopes localized within the pre-S1 and pre-S2 domains of the viral envelope and might be related to the membrane receptor mediating the attachment of HBV to the liver cell.

Solid-phase enzyme immunoassays have been previously used for the detection of anti-pre-S1 and anti-pre-S2 antibodies in sera (1). A series of control experiments performed on normal and pathological sera ascertained the specificity of the detection of these antibodies (1). However, using these assays, we evidenced in some normal human sera a factor different from circulating antibodies, blocking binding of the pre-S1 and pre-S2 MAbs with the corresponding viral epitopes. This factor did not interfere with the reactivity of two different anti-S domain-specific MAbs. Therefore, the inhibitory activity was due not to an eventual lack of specificity of these assays but apparently to the presence in human serum of a definite molecule with affinity for the pre-S protein domains. Masking of pre-S1 and pre-S2 region-encoded epitopes by circulating host factors has also been observed by others (9, 19). In these studies, the blocking activity in human serum was apparently not associated with the presence of specific antibodies, since it was not removed by protein A (19). This molecule was considered to be monomeric albumin since it eluted in the same fractions by 0.5 M Bio-Gel chromatography (9).

A mixture of proteolytic enzyme inhibitors failed to impair the blocking activity of HBV-BF against pre-S epitopes. Furthermore, the presence of the large envelope protein, containing both pre-S1 and pre-S2 region-encoded epitopes, with an unchanged molecular weight was evidenced by Western blot with HBV-BF-treated viral particles. Thus, HBV-BF is not a protease degrading pre-S determinants but rather a molecule specifically masking these epitopes.

Analysis of the apparent molecular weight of the inhibiting factor by size exclusion HPLC revealed in our study a molecule of 50 kDa. A complete inactivation of HBV-BF was observed after treatment with either SDS or 2-mercaptoethanol. This finding demonstrates the absolute requirement of intact disulfide bonds—likely interchain bridges—and of the presence of hydrophobic bonds for binding to pre-S domains. Inactivation of HBV-BF occurred also at high temperature and at acidic pH, showing that additional noncovalent links are essential for maintaining the conformation necessary for recognition of HBV. Other physicochemical characteristics evidenced that HBV-BF is a glycoprotein, containing *N*-acetyl-galactosamine and *N*-acetyl-glucosamine residues.

The interaction of the HBV-BF with pre-S epitopes is of special interest. The pre-S region-encoded domains of HBV envelope proteins are candidates for the viral components mediating the attachment of virions to susceptible cells (21, 30, 31). The amino acid sequence 21 to 47 of the pre-S1

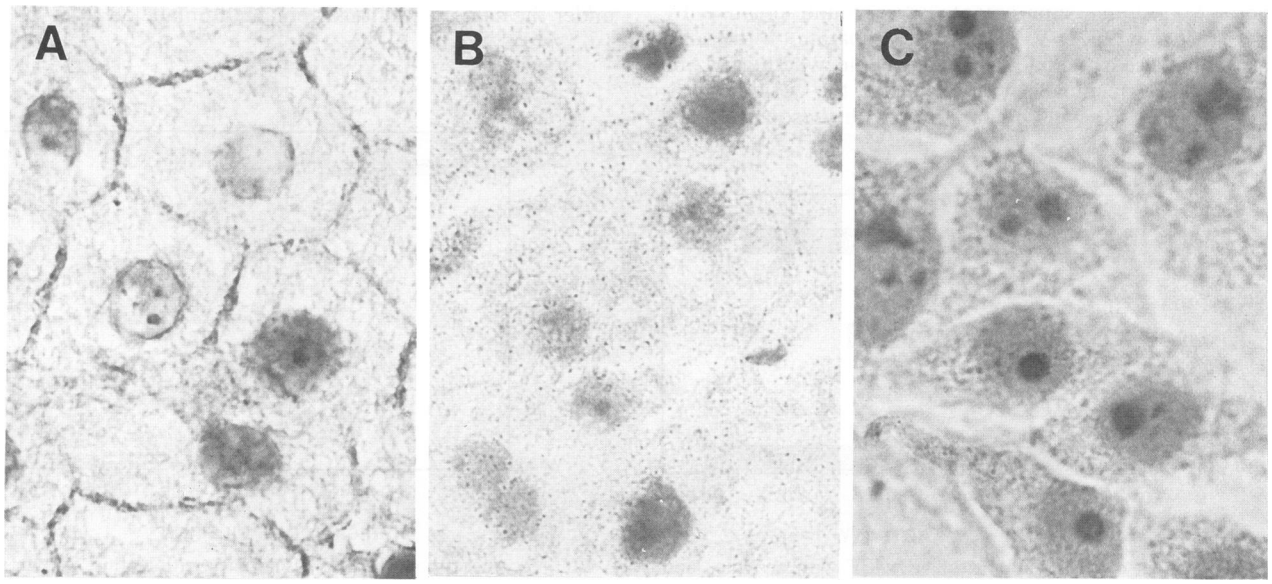

FIG. 7. Immunohistochemical staining of human liver (A), woodchuck liver (B), and HepG2 (C) cells with MAb against HBV-BF. Note immunostaining of human hepatocyte membranes (A) and lack of membrane staining of woodchuck hepatocytes (B) and of HepG2 cells (C). Counterstaining was with Mayer's hemalun. Magnification, ×250.

region has been shown to be crucial for viral binding (21, 30, 31). The auxiliary binding site has been localized within amino acid sequence 120 to 145 of the pre-S2 domain (21, 30). The involvement of these two sites in virus attachment is compatible with neutralization effects of both anti-pre-S1 and anti-pre-S2 antibodies in vivo (11, 22) and with the inhibitory effect of MAbs directed to these two domains in vitro (30, 31). As demonstrated in this study, HBV-BF interacts with both protein domains of the viral envelope representing putative HBV attachment proteins. Indeed, inhibition of Ma18/7 MAb locates the binding of HBV-BF to the pre-S1 sequence between amino acids 28 and 36, within the main HBV cell-receptor binding site determined by Neurath et al. (21). Inhibition of MAb F124 determines the second interaction point of HBV-BF in the N-terminal part of the pre-S2 domain, close to the glycosylation site, located in the auxiliary HBV binding site.

Finally, in immunocytochemical studies, these MAbs recognized the plasma membrane component of normal human hepatocytes, but not that of hepatocytes of other species. Therefore, the serum 50-kDa glycoprotein is immunologically related to a membrane component of normal human hepatocytes, and absent from—or present as undetectable amounts in—hepatocyte membranes of species nonsusceptible to infection.

Since (i) HBV-BF binds to viral envelope proteins involved in the attachment and since (ii) MAb anti-HBV-BF detects a membrane component present only on the surface of hepatocytes capable of replicating HBV, it seems likely that serum HBV-BF can be related to the cellular structures involved in HBV infection of normal hepatocytes.

Different groups have previously identified HepG2 proteins showing molecular masses of 66 kDa (25) and 31 kDa (3) or multiple 35- to 50-kDa bands (29) as HBV receptor components. Indeed, these cells have a capacity to effectively adsorb HBV (3, 21, 29), as well as many other cultured cell lines (25). However, to date HBV has not been effectively propagated in any continuous cell line. HepG2 hepatoma cells are able to replicate HBV after transfection with viral DNA but apparently are nonsusceptible to infection under conditions which allow the propagation of HBV in normal adult human hepatocytes (8). In this study, the 50-kDa glycoprotein, or at least the epitope recognized by MAb 9H11B3, was not detected in the HepG2 cell membrane. Therefore, it can be hypothesized that the HBV-BF-related membrane component is specific for the cells susceptible to infection, whereas HepG2 cells may express different receptors or a truncated protein(s) lacking the structure recognized by our MAb.

A number of receptors for several hormones, cytokines, growth factors, and other membrane molecules, such as major histocompatibility complex class I, CD4, and CD8, exist under soluble forms in the biological fluids of both animals and humans (5, 7, 13–16, 26, 28). Widespread distribution of these molecules suggests that the soluble receptors in biological fluids can act as carriers for these mediators or can be part of a general regulatory mechanism for their activity. Moreover, soluble receptors for poliovirus (12), rhinovirus (17), and human immunodeficiency virus (4, 28) have been recently described and may have potential interest as antiviral agents in vivo. The binding properties and chemical composition of HBV-BF as well as membranous localization of the HBV-BF-related antigen suggest that the serum HBV-BF represents a soluble form, or a fragment, of the cellular HBV receptor. This molecule might be generated, like other secreted forms of receptors, by proteolytic cleavage of the cellular receptors, or by alternative splicing of the receptor transcript, deleting sequences necessary for membrane anchoring (5). HBV-BF, undetectable by inhibition assays in most tested normal serum samples, may exist under a complexed form with a still-undetermined physiological ligand(s). MAbs will be helpful for identifying its origin and its specific ligand.

The membrane component recognized by MAbs is at present under characterization, and other MAbs against the HBV-binding protein have been prepared in our laboratory. The use of these reagents promises to play an important role in the final identification of the HBV receptor and to facilitate isolation of this molecule for future studies and eventual clinical applications.

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