Failure To Detect Polyalbumin-Binding Sites on the Woodchuck Hepatitis Virus Surface Antigen: Implications for the Pathogenesis of Hepatitis B Virus in Humans

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Binding sites for polymerized albumin on hepatitis B virus components were reported in human hepatitis B virus chronic carriers predominantly with active viral replication (HB e antigen positive). The presence of comparable albumin-binding sites in the woodchuck hepatitis virus (WHV) model was examined on WHV components obtained from woodchucks with active viral replication (DNA polymerase positive). Binding sites for polymerized woodchuck serum albumin were not detected on the intact WHV virion, on 22-nm woodchuck hepatitis surface antigen (WHsAg), or on WHsAg polypeptides. Woodchuck albumin was not detected in purified 22-nm WHsAg, and anti-albumin antibodies were not detected in WHV chronic-carrier woodchucks. Our results in the WHV model argue against a role for viral polyalbumin-binding sites in tissue- and host-specific virus infectivity.

The woodchuck hepatitis virus (WHV) is morphologically and structurally related to the hepatitis B virus (HBV; 33). Woodchucks infected with WHV undergo disease sequelae similar to those observed in humans after HBV infection, including acute and chronic hepatitis and hepatocellular carcinoma (27). Therefore, the woodchuck and WHV are currently being investigated as a model for HBV-induced liver disease. Binding sites for polymerized human albumin have been demonstrated for the HBV virion (1, 26), the 22-nm forms of hepatitis B surface antigen (HBsAg) (10, 12, 15, 22, 25), and one or more of the constituent polypeptides (13, 21, 23, 24, 38). The polymerized albumin-binding sites (PABSs) were found to correlate with the presence of markers of active virus replication (HB e antigen [HBeAg]) and have been proposed to function in species-specific virus attachment to hepatocytes and thus mediate virus infectivity (18, 19, 23, 37). To further develop the WHV system as an experimental model for HBV-induced disease, we attempted to demonstrate PABSs on the surface antigens of WHV.

MATERIALS AND METHODS

Source of HBV and WHV antigens. Human sera from chronic HBV carriers that were HBsAg and HBeAg positive were selected from plasma units held in our laboratory. Sera positive for woodchuck hepatitis surface antigen (WHsAg) were obtained from woodchucks held at the National Institute of Allergy and Infectious Diseases animal colony (Meloy Laboratories, Inc., Gaithersburg, Md.); all woodchucks showed active virus replication as measured by DNA polymerase (DNAP) activity (14). The 22-nm forms of WHsAg and HBsAg (subtypes adw and ayw) were isolated from serum by ultracentrifugation (7). For direct-binding studies, 22-nm HBsAg and WHsAg were radioiodinated by a modification of the chloramine-T procedure (9, 30). Preparations enriched for HBV (Dane) particles were obtained by layering 10 ml of DNAP-positive serum over 2 ml of 20% sucrose followed by centrifugation at 20,000 rpm for 4.5 h in

a Beckman SW41 rotor (Beckman Instruments, Inc., Fullerton, Calif.). The pellet was resuspended in 0.5 ml of distilled water and dialyzed against phosphate-buffered saline (PBS; 0.15 M NaCl-0.01 M phosphate buffer).

RIA for surface antigens. HBsAg was assayed by the AUSRIA II radioimmunoassay (RIA; Abbott Laboratories, North Chicago, Ill.). WHsAg was assayed by site-specific solid-phase RIAs that use monoclonal antibodies (MAbs) to different epitopes of WHsAg; for the RIA, MAb 114/III was used as the immunoadsorbent and MAb 3/II or 172/III was used as a radioiodinated probe as described previously (2). For the present study, MAbs 3/II and 172/III were partially purified from ascites fluids by ammonium sulfate fractionation and were radioiodinated by the iodogen method (6).

Purification of serum albumins. Human serum albumin (HSA) (electrophoretic purity, 96%) was purchased from Behringwerke A.G., Marburg/Lahn, Federal Republic of Germany. Woodchuck serum albumin (WSA) was purified from the serum of a woodchuck that was negative for markers of WHV infection (Cocaleco Biologicals, Inc., Reamstown, Pa.). Briefly, 3 ml of woodchuck serum was diluted 1:2 in 0.1 M KCl buffer (with 0.05 M Tris hydrochloride [pH 7.0]) and then applied to an Affi-Gel Blue column (1.5 by 30 cm; Bio-Rad Laboratories, Richmond, Calif.) (3). Unbound serum proteins were eluted with 0.1 M KCl buffer, and bound albumin was recovered in 1-ml fractions by eluting with 1.5 M KCl buffer. A portion of each fraction was analyzed for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; see below). Fractions containing albumin were pooled and concentrated to 1 ml by Amicon PM 10 filtration (Amicon Corp., Lexington, Mass.). The albumin was purified further by chromatography over a Sephadex G-200 column (2.5 by 50 cm; Pharmacia, Inc., Piscataway, N.J.). Fractions of 3 ml were collected, and a portion of each was analyzed for purity by SDS-PAGE. The final preparation was concentrated to 20 mg of protein per ml; albumin concentration was measured by optical density at 280 nm (OD₂₈₀) by using an extinction coefficient of 6.7 at 10 mg/ml (4).

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Polymerization of serum albumins. HSA was polymerized by glutaraldehyde treatment as described by Hansson and Purcell (10). The same procedure was used for the polymerization of WSA. Briefly, 0.3 ml of WSA (20 mg/ml) was dialyzed against 0.1 M phosphate buffer (pH 6.8) and then polymerized by adding 35 μ l of 2.5% glutaraldehyde. The mixture was incubated for 2 h at 20°C, dialyzed against PBS (pH 7.3) for 6 h, and then quenched with an additional 0.3 ml of WSA (20 mg/ml) in 0.2 M carbonate buffer (pH 9.0). After overnight incubation at 20°C, the solution was chromatographed over a Sephadex G-200 column (2.5 by 50 cm) to obtain albumin polymers larger than 200 kilodaltons (kDa). Portions of the 200-kDa polymerized HSA (pHSA) and polymerized WSA (pWSA) were also chromatographed over a Bio-Gel A 0.5 column (Bio-Rad) to obtain polymers larger than 500 kDa. Fractions containing polyalbumins were pooled, and protein concentrations were determined by measuring OD₂₈₀ as described above.

RIA for PABs. WSA, pWSA, HSA, and pHSA were diluted to 50 μ g of protein per ml and adsorbed to microtiter plate wells (Dynatech Laboratories, Inc., Alexandria, Va.) for 18 h at 20°C. The wells were then washed with PBS and postcoated with 1% ovalbumin (Sigma Chemical Co., St. Louis, Mo.), 0.1% gelatin (Sigma), or a 1:100 dilution of normal guinea pig serum (8 h at 20°C). For the RIA, dilutions of surface antigen-positive and -negative sera (50 μ l) were incubated in microtiter wells for 18 h at 37°C. The wells were then washed several times with PBS. Bound antigen was detected with ¹²⁵I-labeled anti-HBs from the Ausab kit (Abbott) or with ¹²⁵I-labeled anti-WHs (50,000 cpm/50 μ l for 4 h at 37°C).

Assays for antibodies to albumins and polyalbumins. A MAb to HSA was derived previously (31); the MAb is species-specific for HSA and also reacts with pHSA (unpublished data). Guinea pigs were immunized intramuscularly with 100 μ g of WSA in 0.2 ml of complete Freund adjuvant and immunized again at 2 weeks with the same dose in incomplete Freund adjuvant. Animals were bled 2 weeks later to obtain anti-WSA serum. The anti-albumin antibodies were assayed in microtiter wells coated with optimal amounts of the individual albumin preparations described above; binding of guinea pig and mouse antibodies was detected, respectively, with ¹²⁵I-labeled *Staphylococcus* protein A (SPA; New England Nuclear Corp., Boston, Mass.) or ¹²⁵I-labeled goat anti-mouse immunoglobulin G (GAMIG) (see below). The GAMIG was provided as an affinity-purified preparation by D. W. Taylor, Georgetown University, and was radioiodinated by the chloramine-T procedure (9). For the RIA of anti-albumin antibodies in woodchucks, sera were incubated for 2 h in wells coated with pWSA. The presence of bound antibodies was assessed with ¹²⁵I-labeled rabbit anti-woodchuck IgG (50,000 cpm/50 μ l for 2 h), prepared in our laboratory by immunizing rabbits with several 100- μ g doses of woodchuck IgG purified by SPA chromatography. The rabbit anti-woodchuck IgG antibodies were subsequently purified by affinity chromatography over columns linked with purified woodchuck IgG and then radioiodinated by the chloramine-T procedure (9). The probe reacts with both 7S IgG and 19S IgM of woodchucks (unpublished data). All agar gel diffusion experiments were performed for 72 h at 4°C in 0.5% agarose (Sigma) containing 1% polyethylene glycol (molecular weight, 6,000).

Precipitation of HBV and WHV virions by polyalbumins. Albumin preparations were covalently linked to Sepharose-4B gels by the cyanogen bromide procedure at a ratio of 2 mg of albumin per g of gel. A gel linked with a MAb to

respiratory syncytial virus was also prepared for use as a control. Portions (100 μ l) of a 50% slurry of the gels were centrifuged in microfuge tubes, aspirated, and then suspended in 100 μ l of the test sample. Samples used for these experiments included DNAP-positive woodchuck and human sera, an HBV Dane particle preparation, and control sera that were seronegative for all markers of WHV. After 18 h at room temperature, the gels were washed three times with PBS, suspended in 35 μ l of lysis buffer (14), and then further processed for DNAP as described previously (14). DNAP bound to the gels was calculated as a P/N ratio, in which N was the activity for the corresponding negative control sample. P/N ratios for DNAP were also determined in parallel for the original samples. For these determinations, 100 μ l of each sample was centrifuged at 95,000 rpm for 20 min in an air-driven ultracentrifuge (Beckman). After centrifugation, the top 80 μ l of the sample was removed. The pellet was then resuspended in the remaining 20 μ l and adjusted to 35 μ l with PBS before the assay. The total percent precipitation of DNAP was derived by dividing P/N ratios obtained in the gel-binding experiments by those obtained for the original samples.

SDS-PAGE. SDS-PAGE was performed in slab gels under reducing or nonreducing conditions by the method of Laemmli (17), except that *N,N'*-diallyltartardiamide (Bio-Rad) was substituted (at 1:5.66 [wt/wt] *N,N'*-diallyltartardiamide/acrylamide) for bisacrylamide. Gels were stained with Coomassie brilliant blue or with silver by using the Rapid-Ag stain kit (ICN Radiochemicals, Irvine, Calif.).

Western blot analysis. After SDS-PAGE, proteins were electrophoretically transferred to nitrocellulose membranes for Western blot analysis (36) with the following modifications. The membranes were cut into strips, and nonspecific binding sites were blocked for 2 h at 20°C with 3% gelatin in isotonic washing buffer (0.15 M NaCl, 0.01 M Tris, 0.05% [vol/vol] Tween 20, 1% gelatin [pH 7.4]). The strips were then incubated individually with the different albumin preparations (10 μ g/ml for 2 h at 37°C) and then with either an HSA-specific MAb or a guinea pig anti-WSA serum (1:500 dilution for 1.5 h at 20°C). As a control, several strips were incubated with the anti-HSA and anti-WSA antibodies alone. The strips were then washed and probed with ¹²⁵I-GAMIG or with ¹²⁵I-SPA if guinea pig serum was used (150,000 cpm/ml for 1.5 h at 20°C). The blots were then washed and developed by exposure to a Kodak X-Omat AR film for 6 to 48 h.

RESULTS

Purification of WSA and preparation of polyalbumins. Albumin and polyalbumin products were compared by SDS-PAGE analysis in 9% acrylamide gels under nonreducing conditions (Fig. 1). WSA had a slightly greater *M_r* when compared with commercially available HSA (lanes 1 and 7), and polymerized albumin did not enter the gel (lanes 2 and 6). Analysis of woodchuck serum diluted 1:350 revealed that the most prominent component in the serum had the same mobility as the protein we purified by affinity chromatography (lanes 5 and 7). Purified HSA and WSA were further characterized by Western blot analysis after reduction and SDS-PAGE in 15% acrylamide gels. The anti-HSA MAb used throughout these studies was species specific for HSA (Fig. 2B), whereas the guinea pig anti-WSA serum cross-reacted with both HSA and WSA (Fig. 2A). The difference in *M_r* between reduced HSA and WSA was greater in the 15% gel than in the 9% gels (Fig. 1 versus Fig. 2). This difference may have been due to the reduction process.

Glutaraldehyde-treated WSA and HSA were fractionated over a Sephadex G-200 column and resolved into two peaks (Fig. 3). In each case, the first peak consisted largely of polymerized albumin (pWSA or pHSA, respectively) (Fig. 1, lanes 2 and 6); the second peak consisted of the monomeric WSA or HSA added to quench the polymerization reaction (Fig. 1, lanes 3 and 4). Glutaraldehyde treatment of these proteins resulted, therefore, in the comparable formation of polymers greater than 200 kDa, based on exclusion chromatography and based on the failure of the proteins to migrate into a 9% polyacrylamide gel. Chromatography of the high-molecular-mass polymers over a Bio-Gel A 0.5 column (exclusion limit, 500 kDa) revealed approximately 75% of pHSA and 40% of pWSA in the form of polymers exceeding 500 kDa in molecular mass (Fig. 3).

RIA for PABS. Some of the experiments described below were performed initially with polyalbumins isolated by Sephadex G-200 chromatography (molecular mass, >200 kDa); the results were also confirmed where indicated with larger polyalbumins isolated by Bio-Gel A 0.5 chromatography (molecular mass, >500 kDa). Portions of the fractions obtained from Sephadex G-200 chromatography of pWSA were adsorbed in triplicate to individual microtiter plate wells and tested for binding of WHsAg by RIA. The results (Fig. 4) show that none of the WSA species in either chromatographic peak was able to bind WHsAg. Increasing the concentrations of WHsAg assayed and the amounts of adsorbed pWSA or changing the type of postcoating material did not change the results. Sera from 39 chronic WHV-carrier woodchucks with active viral replication (DNAP positive), from 10 HBV chronic carriers (HBeAg positive), and purified 22-nm surface antigens from a number of these sera were assayed for PABSs in wells coated individually with either pWSA, WSA, pHSA, HSA, or ovalbumin. Sera were also assayed in parallel in antigen-specific assays. A representative analysis for a typical set of antigen preparations is shown in Fig. 5. The 39 WHsAg-positive sera and purified WHsAg from three of the sera were negative for PABSs. In contrast, we detected species-specific PABSs in each of the HBsAg-positive human sera (e.g., Fig. 5) and also in all corresponding preparations of purified 22-nm HBsAg (data not shown). The overall results of parallel experiments demonstrate species-specific PABSs for HBsAg but not for WHsAg.

FIG. 1. SDS-PAGE of polymerized and monomeric albumins. Albumin preparations were separated by 9% SDS-PAGE, and protein bands were visualized by Coomassie blue staining. Lanes: 1, affinity-purified HSA (7 μ g); 2, pHSA (peak 1; 15 μ g); 3, monomeric HSA (mHSA; peak 2; 7 μ g); 4, monomeric WSA (mWSA; peak 2; 7 μ g); 5, normal woodchuck serum (20 μ l of a 1:350 dilution); 6, pWSA (peak 1; 15 μ g); 7, affinity-purified WSA (7 μ g). Polymerized albumin did not enter the gel (lanes 2 and 6).

FIG. 2. Western blot analysis of binding of anti-WSA serum and anti-HSA MAb to HSA and WSA. Albumin preparations were separated by 15% SDS-PAGE and transferred to nitrocellulose membranes. Strips were incubated (A) in anti-WSA serum (1:500 for 1.5 h at 20°C) and probed with 125 I-SPA or (B) in anti-HSA MAb (1:500 for 1.5 h at 20°C) and probed with 125 I-GAMiG (1.5×10^5 cpm for 1.5 h at 20°C) or 125 I-SPA (1.5×10^5 cpm for 1.5 h at 20°C) and autoradiographed. Lanes: 1, HSA (5 μ g); 2, WSA (5 μ g); M, molecular mass markers. The relative molecular masses (in kilodaltons) of the protein mass markers are indicated at the left.

To rule out effects of polymer size on antigen binding in the RIA, the above set of experiments was repeated with higher-molecular-mass polyalbumins (e.g., Fig. 3). The results of these experiments were the same as those obtained above. In additional control experiments (data not shown), we did not detect binding of WHsAg by using a second 125 I-labeled MAb (172/III) that is directed against an antigenic site different from 3/II (2). Moreover, pWSA did not block detection of WHsAg in standard RIAs for WHsAg that use these MAbs as radiolabeled probes. In other experi-

FIG. 3. Fractionation of glutaraldehyde-treated HSA and WSA by Bio-Gel A 0.5 chromatography. pHSA (1 ml; ●) or pWSA (1 ml; ○) (>200 kDa) were separated on a Bio-Gel A 0.5 column as described in Materials and Methods. Fractions (1 ml) were collected, and protein concentration was determined by OD₂₈₀.

FIG. 4. Binding of WHsAg to fractions of glutaraldehyde-treated WSA. Glutaraldehyde-treated WSA was eluted from a Sephadex G-200 column as described in Materials and Methods. The protein concentration of each fraction was determined by OD_{280} (○). Microtiter plate wells were coated with 50 μ g of protein per ml from each fraction, and WHsAg-positive (●) and -negative (▲) sera were assayed at 1:50 dilutions. Bound antigen was detected with 125 I-labeled MAb 3/II and is plotted as 125 I counts per minute.

ments, direct binding of either 125 I-HBsAg or 125 I-WHsAg to polyalbumins was not detected. PABs were not detected when labeled and unlabeled WHsAg preparations were assayed in fresh normal human or woodchuck serum (i.e., to provide a source of fresh C1q complement factor [22]). These additional experiments further indicate that species-specific PABs are absent from intact WHsAg.

Assay of woodchuck sera for antibodies to polyalbumin. Antibodies to WSA in woodchuck serum could theoretically block the interaction between pWSA and WHsAg. To rule out this possibility, sera from 20 WHV chronic carriers were assayed by agar gel diffusion against pWSA. Guinea pig anti-WSA gave a visible precipitin line with pWSA, but there was no evidence for precipitating antibodies in woodchuck sera (data not shown). Furthermore, we were unable to detect anti-pWSA activity by solid-phase RIA techniques. Therefore, antibodies to polyalbumin were not evident in these WHV chronic-carrier woodchucks.

Precipitation assay for virion-associated PABs. Since the ratio of polypeptides in 22-nm WHsAg may differ slightly from that found on the surface of the intact virion, we assayed specifically for virion-associated PABs. We used precipitation of DNAP in a gel-binding assay as an indicator that PABs were present on the intact viral particles. Precipitation of DNAP was not observed when HBV-positive serum was assayed in this system because of competition from the 22-nm HBsAg particles (data not shown). We therefore used a preparation of HBV Dane particles for the experiment.

For woodchucks, we assayed viremic serum directly because the sera were negative for polyalbumin-binding activity by RIA. Even if PABs were present only on the WHV virion, then the RIA may not have been sensitive enough to detect antigen binding (i.e., owing to the relatively low concentrations of intact virions in serum). However, PABs on the intact WHV virion should have been specifically depleted from serum by an excess of polyalbumin linked to Sepharose gels.

The pHSA gel precipitated about 60% of the total HBV DNAP activity, which was two to three times more than that precipitated by the other gels. The uniformly higher DNAP activity precipitated by the other gels was probably due to the relative lack of serum proteins, which may block non-specific binding. Despite the fact that WHV samples had higher DNAP activities than the HBV samples (data not shown), neither pHSA nor any of the other albumin gels was able to specifically precipitate DNAP from woodchuck sera. Owing to the higher background activity observed in the HBV samples, these results were not statistically significant. However, they indicate that species-specific PABs are present on the intact HBV virion but were not detectable on the intact WHV virion.

Polyalbumin binding by individual surface antigen polypeptides. Polypeptide profiles of purified HBsAg and

FIG. 5. RIA for PABs. Microtiter plates were coated with surface antigen-specific antibodies, i.e., 50 to 100 μ g of pHSA, pWSA, monomeric HSA (mHSA), monomeric WSA (mWSA), or ovalbumin per ml, as indicated. Sera from an HBV chronic carrier (○), a WHV chronic carrier (●), and a WHV marker-negative woodchuck (▲) were incubated with each of these plates at the given dilution. Binding of surface antigen to any of these preparations was detected with surface antigen-specific 125 I-labeled antibodies and expressed in bound 125 I counts per minute.

WHsAg were compared by silver staining. PABSs were identified on individual polypeptides by Western blot analysis. These studies were performed to rule out the presence of cryptic PABSs in the WHV system. The silver stain in Fig. 6 compares a representative polypeptide profile of WHsAg and those from two HBsAg preparations (subtypes adw and ayw). Both HBsAg and WHsAg contained large amounts of the S gene products in both unglycosylated (P24) and glycosylated (GP27) forms (approximately 80 to 90% of total protein). WHsAg contained higher-molecular-mass polypeptides, analogous to the pre-S-containing polypeptides of HBsAg. In WHsAg, the cluster of proteins between 33 and 42 kDa appeared to represent glycosylated and nonglycosylated forms of the pre-S translation products (5; C. J. Pohl, P. J. Cote, R. M. Faust, J. T. Nupp, E. C. Ford and J. L. Gerin, manuscript in preparation). The polypeptides in 22-nm HBsAg were distinct species at 33 and 36 kDa (Fig. 6).

To identify polyalbumin-binding polypeptides, blots of surface antigen polypeptides were incubated with pWSA or pHSA under isotonic conditions, and then with antibodies to the respective albumins, and finally with 125 I-labeled probes. In the control experiment, in which polyalbumin was omitted, neither the anti-HSA MAb nor the anti-WSA antiserum reacted with surface antigen polypeptides under 50 kDa. When blots were reacted first with polyalbumin, the anti-HSA MAb detected the interaction of pHSA with two HBsAg polypeptides, GP33 and GP36 (Fig. 7A, lanes 1 and 2). The pHSA did not bind to WHsAg polypeptides (lane 3), and no interactions were detected between pWSA and the polypeptides of either antigen (Fig. 7B).

Faint bands were detected in these blots among higher-molecular-weight components migrating between 60 and 70 kDa; the most prominent were detected only for HBsAg (Fig. 7A and B, lanes 1 and 2). The above observations could be explained by (i) PABSs on multimeric structures of HBsAg polypeptides or (ii) the HSA associated with the

FIG. 6. SDS-PAGE of HBsAg and WHsAg polypeptides. Purified 22-nm forms of HBsAg and WHsAg were separated by 15% SDS-PAGE. Protein bands were visualized by silver staining. Lanes: left, HBsAg subtype ad (1 μ g); middle, HBsAg subtype ay (1 μ g); right, WHsAg (1 μ g). The relative molecular mass in kilodaltons of the glycosylated (GP) or nonglycosylated (P) polypeptides of HBsAg are indicated on the right (11).

FIG. 7. Western blot analysis of HBsAg and WHsAg polypeptides for PABSs. Purified HBsAg and WHsAg were separated by 15% SDS-PAGE and transferred to nitrocellulose membranes. (A) Strips were preincubated with pHSA (10 μ g/ml for 3 h at 37°C), incubated with anti-HSA MAb (1:500 for 1.5 h at 20°C), and probed with 125 I-GAMIGG (1.5 $\times$ 10⁵ cpm for 1.5 h at 20°C). (B) Strips were preincubated with pWSA (10 μ g/ml for 3 h at 37°C), incubated with anti-WSA (1:500 for 1.5 h at 20°C), probed with 125 I-SPA (1.5 $\times$ 10⁵ cpm for 1.5 h at 20°C), and autoradiographed. Lanes: 1, HBsAg subtype ad; 2, HBsAg subtype ay; 3, WHsAg; M, molecular mass markers. Relative molecular mass in kilodaltons is indicated at the left. Polymerized albumin-binding polypeptides and their relative molecular masses in kilodaltons are designated P33 and P36.

22-nm antigen particles (18, 19, 31). When the same experiment was repeated with high-salt incubation and washing buffers (0.5 M NaCl, 0.01 M Tris hydrochloride [pH 7.4]), the interactions between polyalbumins and surface antigen polypeptides were abolished (data not shown) (22). The only bands present were those in HBsAg at 68 kDa that were stained directly with anti-albumin antibodies; no bands were detected in WHsAg at 68 kDa. Apparently, the reaction of anti-albumin antibodies and the 68-kDa protein was less dependent on ionic strength than was polymerized albumin binding. If the 68-kDa protein represents a virus-encoded protein(s) that cross-reacts with host albumin, then it is only found in HBV and not WHV. More than likely, the 68-kDa species detected here actually represents host albumin present as a minor component in HBsAg (31) but not in WHsAg.

The overall results of experiments conducted at physiological pH and osmolarity demonstrate the presence of a species-specific PABS on the HBV virion, on the 22-nm HBsAg particles, and on two of the constituent polypeptides of HBsAg (GP33 and GP36). In contrast, under similar conditions, we failed to detect binding sites for pWSA on the WHV virion, on the 22-nm WHsAg particles, or on the individual WHsAg polypeptides. Moreover, antigens representing host albumin were more prominent in HBsAg than in WHsAg.

DISCUSSION

Species-specific PABSs have been demonstrated for HBV (1, 10, 12, 13, 15, 21–26, 38), but their putative role in pathogenesis and disease is controversial (19, 20, 23). The PABSs are specific for HSA polymerized by glutaraldehyde but not by other means (e.g., aging, heat treatment, or carbodiimide crosslinking) (19, 38). One current hypothesis is that HBV infectivity is mediated by viral polyalbumin receptors and albumin exposed on the hepatocyte surface

(12, 19, 34, 37). Although receptors for polyalbumins have been demonstrated on the surface of normal and HBV-infected liver cells (8, 20, 34, 35, 37), an *in vivo* component resembling glutaraldehyde-treated albumin has not been clearly demonstrated. In terms of immunopathogenesis, viral polyalbumin receptors could trigger an autoimmune antibody response against the corresponding liver cell receptors (34, 35). Similarly, albuminlike viral structures (28, 31) or host albumin incorporated into HBsAg (18, 19, 31) could induce antibodies that cross-react with host albumin and thus lead to liver cell injury (18, 19).

In the present study, we did not detect PABs in the WHV model by using several approaches that detect the corresponding sites of HBV (10, 12, 15, 21, 38). The detection of PABs in the HBV system correlated with active viral replication (i.e., HBeAg positive [21]). Since an antigen corresponding to the HBeAg has not yet been demonstrated in the woodchuck, DNAP activity was used as a marker for viral replication. Our survey by RIA in large numbers of WHV carrier woodchucks with active viral replication suggested that PABs are absent from WHV.

Western blot experiments supported the observation that species-specific PABs of HBsAg are located on products of the pre-S2 region of the HBV surface gene (11, 21, 23, 38). PABs were not consistently observed on the high-molecular-mass surface polypeptides of HBsAg (P39/GP42) (38); the latter could arise from masking by upstream amino acid residues. Cryptic binding sites for pWSA were not detected by Western blot analysis of the analogous WHsAg polypeptides. Although we cannot exclude the possibility that PABs of these polypeptides bind to the nitrocellulose and are therefore not detectable, this finding is probably due to the wide divergence in pre-S2 amino acid sequences between HBsAg and WHsAg (16, 29).

Polyalbumin-binding activity was not observed for the major polypeptides of HBsAg or WHsAg (P24/GP27). This has been reported for hepatoma-derived HBsAg, which is composed only of P24 and GP27 (13, 15). Particle binding could therefore be explained by sites formed from multimers of these polypeptides. The faint detection of polyalbumin binding by HBsAg components in the lower 60-kDa molecular-mass range could arise from this type of binding (e.g., Fig. 7A, lanes 1 and 2). Some of the reported interactions could also arise owing to varying physical conditions. For example, species specificity of the interaction was lost at low ionic strength (0.01 M NaCl) and no binding was detected at high ionic strength (0.5 M NaCl) (22); the latter was also shown in the present study.

The loss of polyalbumin binding in high-salt buffers revealed the presence of a 68-kDa surface antigen component with albuminlike determinants (Fig. 7). The protein was prominent in HBsAg but not in WHsAg. Presuming that hepadnavirus surface antigens contain virus-encoded proteins of less than 50-kDa molecular mass (11, 16, 32) and that reducing conditions (2.5% β -mercaptoethanol at 100°C for 10 min) led to nearly complete disruption of HBsAg particles, we suggest that the 68-kDa protein corresponded to host albumin. Although the presence of protein in this region was suggested by silver staining in all gels (Fig. 6), only the 68-kDa protein(s) from HBsAg was consistently detected with anti-albumin antibodies. Under favorable physical conditions it is possible that albumin in the infected hepatocyte becomes incorporated in HBsAg via the polyalbumin receptor. The incorporation of albumin may raise the question of what role this might play in the natural course of the HBV infection and will require further study.

In summary, observations with glutaraldehyde-polymerized albumin in the WHV model contrasted with those for HBV in humans. (i) PABs were not detected on the intact WHV virion, on 22-nm WHsAg, and on WHsAg polypeptides; (ii) WSA was not detected in 22-nm WHsAg; and (iii) anti-albumin antibodies were not detected in WHV chronic-carrier woodchucks. While these observations do not exclude binding sites to albumin polymerized by means other than glutaraldehyde, no comparable system has been detected in HBV infection of humans (38). Further studies are required to determine whether these observations are correlated with minor differences in the histopathology of HBV- and WHV-induced disease (27). In conclusion, our failure to detect PABs in the WHV model argues against a role for PABs as viral receptors mediating tissue- and host-specific virus infectivity.

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