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Surface Antigenic Determinants of Mammalian "Hepadnaviruses" Defined by Group- and Class-Specific Monoclonal Antibodies

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The hepatitis B-like viruses (human hepatitis B virus, woodchuck hepatitis virus, ground squirrel hepatitis virus, and duck hepatitis B virus) are hepatotropic DNA viruses which have been referred to collectively as "hepadnaviruses." Using a murine monoclonal antibody (101-2) to the surface antigen of woodchuck hepatitis virus, we have shown that the surface antigens of mammalian hepadnaviruses (HBsAg, WHsAg, and GSHsAg) are antigenically related via a common determinant (HV/101). Furthermore, analysis with other monoclonal antibodies to WHsAg revealed that WHsAg and GHsAg are antigenically distinct, although the antigens had more determinants in common with each other than with HBsAg. The hepadnavirus group-specific antibody (101-2) reacted with HBsAg subtypic variants in a group-specific rather than subtype-specific manner. In conjunction with observations with an HBsAg-specific, group-reactive monoclonal antibody (BX259), the present data suggest that there are at least two group-reactive epitopes of HBsAg: one which is virus specific (HBV/259) and one which is common to two other mammalian hepadnaviruses (HV/101).

Hepatitis B-like viruses have been discovered in woodchucks (31), Beechy ground squirrels (18), and Pekin ducks (20). These viruses comprise a unique family of DNA viruses which are morphologically and structurally similar and which are capable of establishing hepatotropic persistent infections in their respective hosts (for review, see reference 30). In humans, chronic hepatitis B virus (HBV) infection has been associated with an increased risk of active hepatitis and liver cirrhosis and with the development of primary hepatocellular carcinoma (19, 23). Primary hepatocellular carcinoma associated with chronic viral hepatitis has also been observed in the woodchuck (*Marmota monax*) hepatitis virus system (6, 21, 28, 29), but has not yet been described in ground squirrels or ducks (30). The viral infections are characterized by an accumulation in the blood of excess viral surface antigen (20- to 25-nm-diameter lipoprotein particles and filaments) and lesser amounts of the virion (40- to 50-nm diameter) (30, 32). The virus consists of an outer envelope bearing surface antigenic determinants and an inner 27-nm-diameter protein capsid (core antigen) which surrounds a partially single-stranded, circular DNA genome and the endogenous DNA polymerase (12, 13, 32). Studies involving reciprocal antigen-antiserum reactions have shown that there is some degree of cross-reactivity among the surface antigen-antibody systems of woodchuck (WHsAg-anti-WHs), human (HBsAg-anti-HBs), and ground squirrel (GSHsAg-anti-GSHs) viruses; however, that of the duck virus appears to be antigenically distinct (3, 6, 9, 20, 33). These previous observations establish, but do not define, the antigenic relationship between the viruses. The definition of antigenic interrelationships among the HBV-like viruses can more readily be accomplished through the use of virus-specific and cross-reacting monoclonal antibodies. In the present study, we used hybridoma techniques (14, 16) to generate monoclonal antibodies to WHsAg. Of 11 antibodies, 3 were found to be specific for WHsAg, and 8 cross-reacted with the surface antigen of ground squirrel hepatitis virus. One of these eight antibodies also cross-reacted with HBsAg.

MATERIALS AND METHODS

Isolation of WHsAg. WHsAg was isolated from plasma of a chronically infected, captive woodchuck by isopycnic ultracentrifugation procedures developed previously for a two-step isolation of 22-nm HBsAg (7). Cesium chloride gradient fractions were assayed for WHsAg by the commercial Ausria II assay (Abbott

Laboratories, North Chicago, Ill.), which takes advantage of limited cross-reactions in the human and woodchuck surface antigen-antibody systems (6, 33).

Animal immunizations. Female BALB/c mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass., 6 to 8 weeks old) were immunized by the intraperitoneal route with 10 to 15 μg of WHsAg emulsified in Freund complete adjuvant (0.2 ml). Individual mice were periodically test bled after 5 weeks by the orbital sinus technique, and diluted sera (1:10) were tested for anti-WHs (see assay below). After 10 weeks, boosters of 2 to 4 μg of WHsAg in Freund incomplete adjuvant were administered subcutaneously at least 1 month before hybridization experiments. Otherwise, aqueous boosters were administered by the intraperitoneal route in conjunction with hybridization protocols.

Somatic cell hybridizations and the isolation of anti-WHs-secreting clones. On days −9, −7, and −5 relative to hybridization experiments, immunized mice were boosted repeatedly with 2 to 4 μg of WHsAg in 0.4 ml of phosphate-buffered saline (0.85% NaCl, 0.01 M phosphate buffer, pH 7.3). This was followed by a final intravenous booster on day −3, and individual sera and spleens were harvested on day 0. Somatic cell hybridizations were mediated by polyethylene glycol (1,000 daltons; 42% [wt/vol]; Sigma Chemical Co., St. Louis, Mo.) by using standard techniques (4, 14) and the NS-1 mouse myeloma cell line (15). Hybridoma cultures and clones were plated, cloned, and expanded in culture and as ascites tumors in syngeneic mice as described previously (2a, 24). Hybridoma culture fluids were screened for anti-WHs activity by double-antibody radioimmune precipitation (daRIP) procedures (10, 25; see below).

daRIP assay. WHsAg was radioiodinated by the chloromine-T procedure, freed of unbound ^{125}I by gel filtration, and further purified by sucrose gradient centrifugation at 40,000 rpm for 4 h (2). For screening tests, 5,000 cpm of ^{125}I-WHsAg per 5 μl of 1% bovine serum albumin–phosphate-buffered saline and 5 μl of test sample were incubated (2 h, 25°C) in wells of V-bottom microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.). Precipitation of antigen-antibody complexes was accomplished by the addition of 100 μl of goat anti-mouse immunoglobulin G (Cappel Laboratories, Inc., Cochranville, Pa.; diluted 1:25 [vol/vol] in phosphate-buffered saline). Plates were incubated overnight (4°C) and then centrifuged (2,800 rpm, 25 min, 25°C). The resulting supernatants and pellets were counted and the percent ^{125}I-WHsAg precipitated (%PPT) was calculated as %PPT = counts per minute of the pellet/(counts per minute of the pellet + supernatant) × 100. The %PPT by blank serum or culture fluids (8 to 12%) was subtracted from that of the test sample. For titration analysis, dilutions of ascites fluids containing monoclonal antibody were prepared in 1:20 normal mouse serum (Dutchland Laboratories, Denver, Pa.) and incubated with ^{125}I-WHsAg; an additional 5 μl of 1:40 normal mouse serum was then added as a blank competitor (see below).

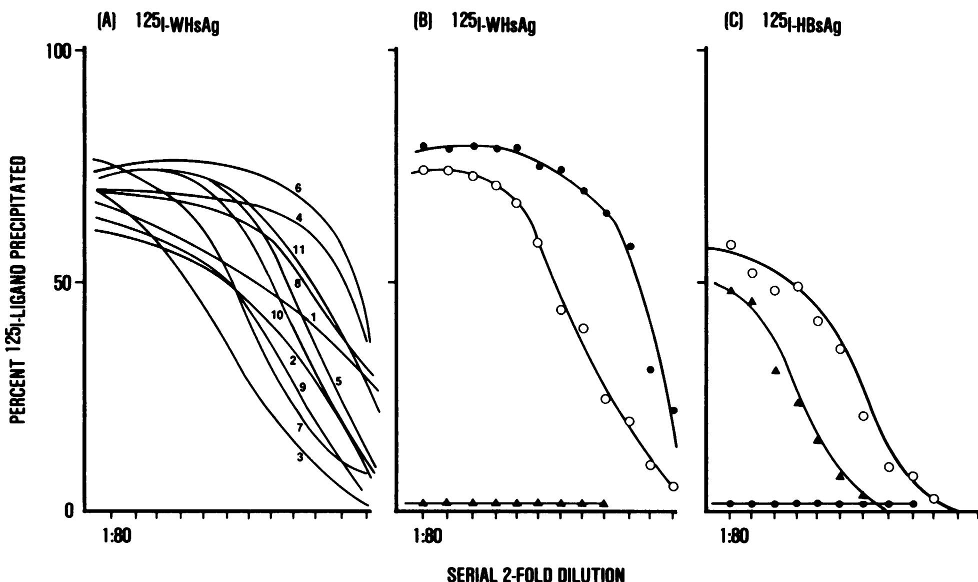

FIG. 1. Titration analysis of monoclonal antibodies and heteroantisera by daRIP. A, Assay by daRIP of serial twofold dilutions of ascites fluids from hybridoma clones with ^{125}I-WHsAg ligand; numbered titration curves (1 through 11) correspond, respectively, to monoclonal antibodies from hybridoma clones designated WCN 3-2, 14-1, 19-4, 31-1, 41-1, 71-3, 101-2, 114-1, 151-1, 172-1, and 181-1. See the text for other details of the daRIP assay. B, Titration analysis comparison of daRIP with ^{125}I-WHsAg ligand and either mouse anti-WHs (●), mouse anti-HBs (▲), or WHsAg-defined monoclonal antibody 101-2 (○; curve no. 7 in A). C, Titration analysis comparison by daRIP with ^{125}I-HBsAg ligand (standard Ausab reagent) and either mouse anti-WHs (●), mouse anti-HBs (▲), or WHsAG-defined monoclonal antibody 101-2 (○).

TABLE 1. Testing of murine anti-WHs, anti-HBs, and monoclonal antibodies to WHsAg by Ausab radioimmunoassay[a]

Sample	Sample dilution		
	1:20	1:200	1:2,000
NMS[b]	1.0		
M/anti-HBs[c]	208.6	131.7	13.9
M/anti-WHs (no. 3)[d]	0.8		
M/anti-WHs (no. 5)[d]	0.7		
WCN 101-2[e]	200.9	33.5	1.9

[a] Values are positive/negative (P/N) ratios determined by the Ausab test for anti-HBs (Abbott Labs); background counts (N) for anti-HBs negative human serum controls were less than or equal to 100 cpm. P/N values greater than 2.1 were considered positive results.

[b] NMS, Normal mouse serum (Dutchland Labs). P/N values for undiluted samples were less than or equal to 1.4.

[c] Mouse anti-HBs; week 5 pooled serum from four mice immunized with HBsAg/*ad* vaccine (National Institute of Allergy and Infectious Diseases lot A-7; alum adsorbed; 8 μg/0.1 ml per mouse, intramuscular route).

[d] Mouse anti-WHs; individual samples from two mice (no. 3 and 5) hyperimmunized with WHsAg; mice were eventually used in fusion experiments (see text).

[e] Monoclonal antibody predefined by immunization of mice with WHsAg; antibody WCN 101-2 was derived from mouse no. 3; all other monoclonal antibodies derived in the present study were negative in the Ausab test (P/N ≤ 2.1).

Competition radioimmune precipitation (cRIP) assay. Combinations of ascites fluid and ^{125}I-WHsAg that yielded a net 30 to 50% of maximum ligand precipitation were prepared in replicate numbers for each antibody. Dilution series were also prepared of either purified WHsAg (in 1:40 normal mouse serum) or WHsAg-positive woodchuck serum (in 1:5 normal woodchuck serum). For each antibody-^{125}I-WHsAg series, 5 μl of unlabeled antigen was added to effect increasing competition. After 3 h of incubation (25°C), the antigen-antibody complexes were precipitated as described above for daRIP. The percent ^{125}I-WHsAg was calculated and expressed relative to that where either 1:40 normal mouse serum or 1:5 normal woodchuck serum was added as a blank competitor. Thus, the percent inhibition of precipitation by the unlabeled test antigen is equal to 100 × (1.00 − ^{125}I-WHsAg %PPT in the presence of competing antigen/^{125}I-WHsAg %PPT in the presence of blank competitor).

Source and use of other reagents. Monoclonal antibodies to WHsAg were tested for cross-reactivity with HBsAg by the commercial Ausab assay (Abbott Laboratories). The ^{125}I-HBsAg from the Ausab kit was also adapted for use in daRIP and cRIP assays by adjusting the reagent to 2% normal mouse serum; 6,000 to 8,000 cpm/10 μl was used in place of 5 μl of ^{125}I-WHsAg. Isolates of four-step-purified HBsAg subtypes (*adw2* and *ayw3*) were obtained from repository stocks held in our laboratory (1, 12). Nine HBsAg-positive human sera representing the major subtypes of HBsAg were obtained from the Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health; samples were used in cRIP tests. A GSHsAg-positive serum sample used in cRIP tests was kindly supplied by W. S. Robinson (3, 18).

RESULTS

Hybridoma colonies secreting anti-WHs were identified by the ability of culture fluids to precipitate 60 to 75% of the ^{125}I-WHsAg ligand in daRIP. Eleven positive colonies were cloned twice by limiting dilution procedures, and representative progeny clones were selected for propagation in culture and as ascites tumors in syngeneic mice. All of the cell lines produced monoclonal antibody with γ1 subtype heavy chains and κ light chains as determined by micro-Ouchterlony immunodiffusion against subtype-specific antisera (2a). Titration analysis of ascites fluids from each clone suggested that the monoclonal antibodies exhibited a variety of avidities for WHsAg when compared with the mouse anti-WHs serum (Fig. 1A and B). All antibodies were tested in daRIP tests with ^{125}I-HBsAg ligand in place of ^{125}I-WHsAg. Only monoclonal antibody 101-2 precipitated both ligands (Fig. 1B and C). The isolation of such a clone was of interest in view of the observation that mouse anti-WHs serum did not precipitate ^{125}I-HBsAg (Fig. 1B versus Fig. 1C). We have also observed that several of our mouse anti-HBs sera and anti-HBs monoclonal antibodies (24) do not cross-react with WHsAg (Fig. 1C versus Fig. 1B; Shih et al., submitted for publication).

The reactivity of WHsAg-defined antibody 101-2 with HBsAg was further confirmed by Ausab assay, which is specific for anti-HBs. Although antibody 101-2 was more reactive with HBsAg in daRIP when compared with mouse anti-HBs serum (Fig. 1C), it was comparatively less reactive than the anti-HBs serum in Ausab (Table 1). The antibody however, was consistently detected by Ausab assay at dilutions of ascites fluids between 1:200 and 1:1,000 and also in tests using the undiluted culture fluids (positive/negative ratio, 50). Clone 101-2 was further subcloned, and 30 of 30 individual progeny clones tested reacted with both WHsAg and HBsAg. Mouse anti-WHs sera from two mice used in hybridization experiments were not reactive in the Ausab assay (Table 1).

All monoclonal antibodies were used in cRIP assays involving the homologous antigen system (^{125}I-WHsAg ligand and competing unlabeled WHsAg from the donor woodchuck) (Fig. 2A). Competition to effect 50% inhibition of ligand precipitation ($cRIP_{50}$) varied depending on the

monoclonal antibody; corresponding $cRIP_{50}$ values ranged from less than 0.3 μg/ml (antibody 101-2) to greater than 80 μg/ml (antibody 19-4). The differences obtained are most likely related to differences in antibody avidity for WHsAg. Competing WHsAg was consistently detected with all antibodies at 15 to 20% inhibition of ligand precipitation. cRIP assays to detect native WHsAg in serum from the chronically infected donor woodchuck were performed with three of the monoclonal antibodies; these are compared with mouse anti-WHs serum in Fig. 2B. Monoclonal antibodies detected native WHsAg in serum at differing sensitivity levels when compared with the mouse anti-WHs. The relative ordering of $cRIP_{50}$ values for the monoclonal antibodies in the detection of native WHsAg in donor serum was similar to that obtained when the two-step-purified WHsAg was used as the competing antigen (i.e., 101-2 $<$ 172-1 $\leq$ 114-1; Fig. 2B versus Fig. 2A). Based on the monoclonal antibody data, the concentration of WHsAg in the serum sample was estimated to be 190 to 250 μg/ml.

Monoclonal antibody 101-2 was used in reciprocal cRIP tests involving WHsAg and HBsAg ligand-competitor systems (Fig. 3). The near-parallel slopes of the competition curves indicated that the effect of antigen concentration was similar in all systems; thus, $cRIP_{50}$ points reflect comparable measurements. Using either ^{125}I-WHsAg or ^{125}I-HBsAg ligand, it required relatively greater concentrations of HBsAg than WHsAg to effect 50% inhibition of ligand precipitation. This observation suggests that antibody 101-2 has a greater relative avidity for WHsAg than for HBsAg. One *ad* and three *ay* HBsAg isolates were effective as competing antigens when either ^{125}I-ligand was used (Fig. 3). This observation demonstrated HBsAg detection by the WHsAg-defined antibody in the cRIP assays and also suggested that the 101-2 antibody is directed against an HBsAg group-specific rather than subtype-specific determinant. The reac-

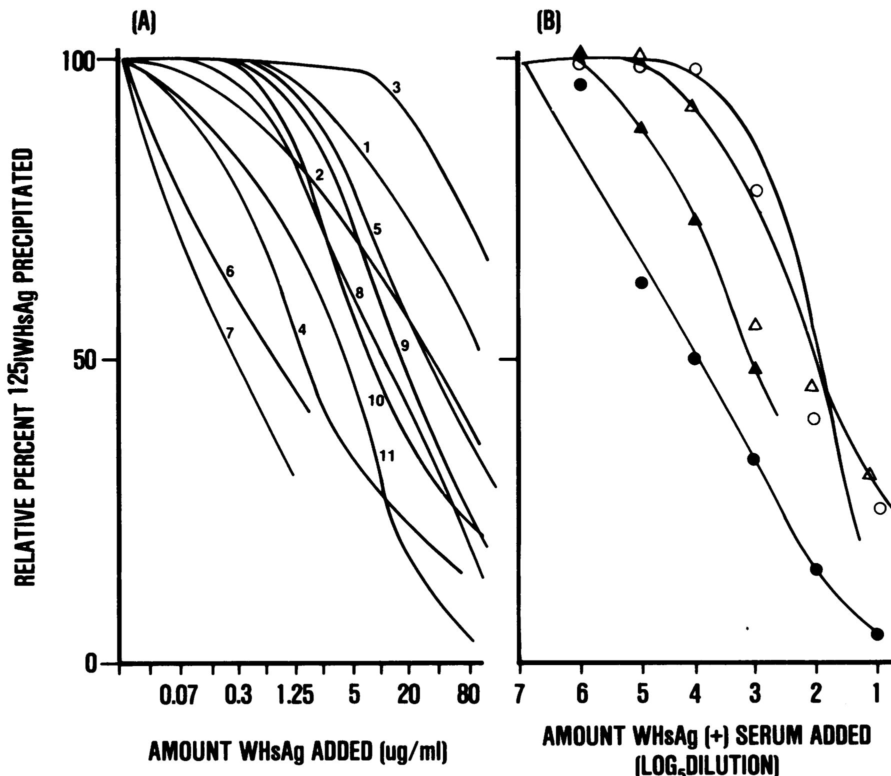

FIG. 2. Use of monoclonal antibodies in cRIP assays to detect WHsAg. A, cRIP assay with monoclonal antibodies and ^{125}I-WHsAg to detect purified WHsAg; numbered curves (1 through 11) correspond, respectively, to monoclonal antibodies WCN 3-2, 14-1, 19-4, 31-1, 41-1, 71-3, 101-2, 114-1, 151-1, 172-1, and 181-1. B, cRIP assay to detect native WHsAg in serum from the infected donor woodchuck with mouse antiserum (▲) and monoclonal antibodies 101-2 (●), 114-1 (○), and 172-1 (△). WHsAg-positive serum was diluted in 1:5 normal woodchuck serum; normal woodchuck serum was used as a blank competitor in this instance.

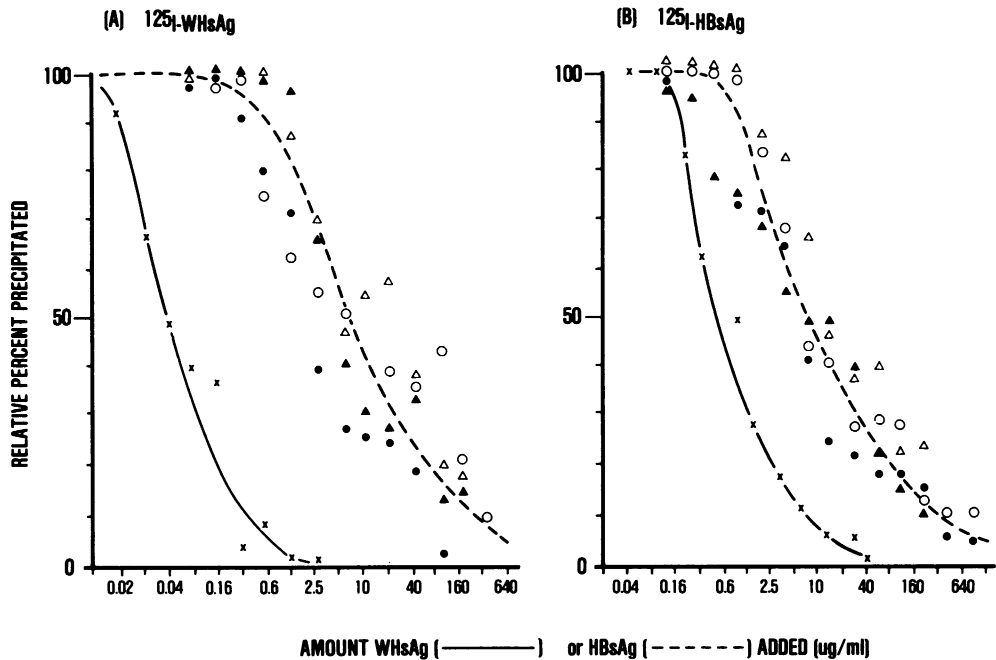

FIG. 3. cRIP analysis with WHsAg-defined monoclonal antibody (101-2) in various ^{125}I-ligand-competitor systems. A, Antibody 101-2 and ^{125}I-WHsAg ligand system; competition with WHsAg (×) and with purified HBsAg (----). These isolates were subtyped as *adw2* (●) and *ayw3* (▲, ○, △). Monoclonal antibody 101-2 from ascites fluid was used in a 1:5,120 dilution which precipitated 34% of the ^{125}I-WHsAg ligand in daRIP. The cRIP$_{50}$ points for competing WHsAg and HBsAg in this test were approximately 0.04 and 7 μg/ml, respectively. B, Antibody 101-2 and ^{125}I-HBsAg ligand system; competition with WHsAg (×) and with purified HBsAg (-----). These isolates are the same as those used in A. Monoclonal antibody 101-2 was used in this instance at a 1:160 dilution which precipitated 45% of the ^{125}I-HBsAg ligand in daRIP. The cRIP$_{50}$ points for competing WHsAg and HBsAg in this test were approximately 0.6 and 7 μg/ml, respectively.

tivity of antibody 101-2 with nine major HBsAg subtypes was assessed by the cRIP assay (Table 2). All HBsAg subtypes competed by using either ^{125}I-ligand, with the exception that the *adw4* test sample produced an enhancement of ligand precipitation over that in the absence of competing antigen. The reactive samples produced inhibition of precipitation at 1:10 and, for the most part, at 1:50 dilutions; the majority were nonreactive at dilutions greater than 1:250. These data indicate that WHsAg-defined monoclonal antibody 101-2 specifies for a group determinant of HBsAg.

The reactivity of mouse anti-WHs and WHsAg-defined monoclonal antibodies with GSHsAg was determined by using ^{125}I-WHsAg ligand versus GSHsAg-positive serum in cRIP assays. GSHsAg-positive serum diluted 1:10 produced an 80% inhibition of ^{125}I-WHsAg precipitation by anti-WHs, which compared well with control samples where WHsAg-positive serum was used (92% inhibition). Thus, mouse anti-WHs serum contains antibodies which cross-react with GSHsAg. Together with other data (Tables 1 and 2), the monoclonal antibodies could be classified into three groups representing at least three different surface antigenic determinants (Table 3). The group I determinant represented by antibody 101-2 is ubiquitous among mammalian hepadnavirus surface antigens. Group II determinants are present on WHsAg and GSHsAg, but not HBsAg. Note that two of the group II antibodies (19-4 and 172-1), although predefined by WHsAg, appeared to have a comparatively greater avidity for GSHsAg than for WHsAg. The group III determinants are WHsAg specific.

DISCUSSION

Human HBV and the HBV-like animal viruses have been referred to collectively as "hepadnaviruses" to denote their respective hepatotropism and genomic composition (22). Cross-reactions among the surface antigens of mammalian hepadnaviruses detected with homologous and heterologous antisera probably reflect the conservation of one or more hepadnavirus-group epitopes which are related to, or are part of, the classically defined group *a* determinant(s) of HBsAg (1, 12, 17). Apparently, antibodies in

TABLE 2. Reactivity of WHsAg-defined monoclonal antibody (101-2) with the major HBsAg subtypes

Competing HBsAg subtype[a]	cRIP ^{125}I-ligand[b]	
	WHsAg	HBsAg
ayw1	50	44
ayw2	43	34
ayw3	69	75
ayw4	22	28
ayr	41	66
adw2	15	26
adw4	(138)	(145)
adr	60	43
adyw	57	52

[a] See reference 34.

[b] Values obtained by cRIP assay are the percent ^{125}I-ligand precipitated relative to that in the absence of competing antigen (1:2 normal human serum). The percent inhibition of precipitation is equal to 100% minus the value in the table; values less than 80% precipitated (greater than 20% inhibition) are considered positive reactions. Values in parentheses represent an apparent enhancement of ligand precipitation above the 100% point. Values are the averages for duplicate and triplicate tests using WHsAg and HBsAg ligands, respectively, and test sample at a 1:2 dilution.

immune mouse sera which are capable of recognizing hepadnavirus-group determinants represent only a minute fraction of the total antibody response to the antigen particle. For example, anti-WHs and anti-HBs sera from BALB/c mice do not cross-react with the heterologous antigens (Fig. 1B and C; Table 1), and others (3, 9) have shown that mouse anti-GHs cross-reacted only minimally or not at all with HBsAg. The observations may reflect an immunodominance effect in the murine system among determinants of the animal (ground squirrel hepatitis virus, woodchuck hepatitis virus) and human (HBV) viral surface antigens, such that the in vivo antibody response to hepadnavirus group-specific determinants is diminished below detectable levels. This is in contrast, for example, to guinea pig anti-HBs used in commercial assays and various woodchuck anti-WHs sera, which are able to recognize determinants common to all three antigens (3; Shih et al., submitted for publication). Thus, relative antigenic characterizations within virus families by using reciprocal antigen-antisera reactions may reflect species or host factors (or both) which govern the antibody response to specific determinants, rather than the actual presence or absence of the determinants. Using hybridoma techniques, we were able to isolate and amplify an antibody-binding function that could not be detected in the antisera of donor mice. The results demonstrate the detection of an HBsAg-group determinant by using a cross-reacting murine monoclonal antibody predefined by the surface antigen of a related hepadnavirus (woodchuck hepatitis virus). Subsequently, it was shown that the determinant is specific for the mammalian hepadnavirus group. For routine hybridoma purposes, it may be possible to increase the number of murine clones producing hepadnavirus group-specific antibodies via alternate primary immunization with HBsAg and subsequent booster with WHsAg or GSHsAg. Such an approach has proven successful for obtaining increased frequencies of cross-reacting monoclonal antibodies to the hemagglutinin protein of different influenza virus strains (5).

Monoclonal antibodies were used to develop liquid-phase cRIP assays for native WHsAg (Fig. 2). Results using these assays to screen captive woodchucks for WHsAg (data not shown) were qualitatively similar to those obtained in a newly developed solid-phase competition assay system which uses hyperimmune woodchuck anti-WHs serum and ^{125}I-WHsAg (33a). Presently, we used monoclonal antibodies of differing relative avidities for WHsAg to demonstrate the antigenic similarity of native and purified WHsAg (Fig. 2A versus Fig. 2B). The 22-nm HBsAg forms are multivalent for monoclonal antibody-defined determinants in that they are composed of numerous copies of closely related, antigenically similar polypeptide

TABLE 3. Surface antigenic determinants of mammalian hepadnaviruses defined by monoclonal antibodies to WHsAg

WHsAg-defined monoclonal antibody group		Relative inhibition of ^{125}I-WHsAg precipitation by:		
		WHsAg[a]	GSHsAg[a]	HBsAg[b]
I	(101-2)	+++[c]	+++	++
II	(3-2)	+++	++	−
	(14-1)	+++	++	−
	(31-1)	++	+	−
	(71-3)	++	+	−
	(114-1)	++	++	−
	(19-4)	+	++	−
	(172-1)	++	+++	−
III	(41-1)	+	−	−
	(151-1)	++	−	−
	(181-1)	++	−	−

[a] An antigen-positive serum sample was diluted 1:10 in normal woodchuck serum (1:5 in phosphate-buffered saline), and tested in cRIP assay versus ^{125}I-WHsAg.

[b] Observations were deduced from the data presented in Table 2 and elsewhere in this manuscript.

[c] The relative values indicate a range for precipitation inhibition by the test sample; +++, 70 to 100% inhibition; ++, 35 to 69% inhibition; +, 20 to 34% inhibition; −, 0 to 19% inhibition.

chains (26, 27); a similar situation can also be assumed for WHsAg. Therefore, apparent differences in relative avidities of monoclonal antibodies for WHsAg may reflect a combination of factors such as the number, distribution, or location of a particular determinant, which may in turn influence the effective binding strength of antibody to the WHsAg particle (i.e., affinity). Detailed comparisons of the cross-reactive 101-2 determinant suggested that its antigenicity was similar among four purified HBsAg preparations (1 *adw2*, 3 *ayw3*), but differed in comparisons between HBV and woodchuck hepatitis virus surface antigen particles (see $cRIP_{50}$ values in Fig. 3). In this instance, the observations could reflect decreased binding affinity to HBsAg brought about by microheterogeneity within or immediately around the antibody-binding sites or a decreased number of 101-2 binding sites on HBsAg relative to WHsAg (or both). More basic studies are required to elucidate the nature of monoclonal antibody binding to single determinants of individual soluble proteins or polypeptides versus their binding to discrete multivalent particles formed by the aggregation of these subunits.

Using monoclonal antibody 101-2 in cRIP assay systems, we detected HBsAg in eight of nine human sera, each representing a different subtypic variant of HBsAg (Table 2). Present data considered, there is no a priori reason to conclude that the 101-2 epitope is absent from the *adw4* subtype of HBsAg. The enhancement of ligand precipitation by this sample may be related to the presence of some nonspecific factor(s) in the serum which may enhance immune complex or antigen agglutination in the liquid-phase system (11; no endogenous or contaminating anti-HBs-like activity was detected in the sample). cRIP assay systems were also employed to detect GSHsAg in an antigen-positive serum sample. In this case, the 101-2 antibody and seven other monoclonal antibodies not reactive with HBsAg were found to cross-react with GSHsAg; another three of the antibodies were WHsAg specific (Table 3). Thus, at least three major groups of surface antigenic determinants are represented by the present selection of WHsAg-defined monoclonal antibodies. Feitelson et al. (3) and Gerlich et al. (9) have used a GSHsAg-defined monoclonal antibody which is GSHsAg specific and thus have defined a fourth group of hepadnavirus surface antigenic determinants. Furthermore, we have previously described HBsAg-defined monoclonal antibodies which exhibited group and subtype specificities (8, 24), none of which cross-reacted with WHsAg (Shih et al., submitted for publication). The HBV-specific group and subtype determinants would thus comprise additional monoclonal antibody-defined categories. The present observations with the 101-2 antibody observations and with a group-reactive, HBsAg-specific monoclonal antibody (8, 24) suggest the existence of at least two group epitopes of HBsAg: one which is virus specific, and one (HV/101) which is common to two other mammalian hepadnaviruses. The latter epitopes have been defined by using monoclonal antibodies produced in the murine system and, by definition, may be considered as HBsAg-group determinants. However, the exact relationship of these epitopes to the classically defined group (*a*) determinant(s) recognized by human convalescent antisera (1, 17) has not been established.

The observations provide direct proof of the antigenic relationship among mammalian hepadnaviruses and demonstrate that different viral surface antigens possess unique determinants. Recent evidence suggests that polypeptides of GSHsAg and WHsAg are more closely related structurally when compared with those of HBV (3). Our observations and those of others (3, 9) in the murine system lend strong support to the concept of a closer antigenic relationship between the two animal viruses relative to HBV. For example, mouse anti-WHs and anti-GSHs sera recognize GSHsAg and WHsAg, respectively, but not HBsAg, and mouse anti-HBs serum does not recognize WHsAg (Fig. 1; Shih et al., submitted for publication). Second, the 101-2 antibody derived in the present study exhibited a similar relative avidity for WHsAg and GSHsAg (Table 3), but its avidity for HBsAg was relatively less (Table 3, Fig. 3). In addition, 7 of 11 WHsAg-defined clones derived from three independent fusion experiments were found to react with both WHsAg and GSHsAg (group II, Table 3). The possibility that all of the group II antibodies are directed against the same determinant cannot be ruled out at present; however, WHsAg-defined antibodies demonstrating a greater relative avidity for GSHsAg than for the homologous immunogen may specify for a determinant(s) distinct from others in that group which have a greater relative avidity for the homologous immunogen (e.g., 19-4 and 172-1 versus 14-1 and 31-1; Table 3). Although very closely related, GSHsAg and WHsAg are evidently distinct antigenically as exemplified by the present selection of group III monoclonal antibodies (Table 3) and a GSHsAg-specific monoclonal antibody used by Robinson and coworkers (3, 9). In contrast to these virus-specific and animal virus-specific determinants (group II), the HV/101 epitope appears to be the more highly conserved evolutionarily. Thus, the 101-2 antibody is of potential value in the detection of HBV-like agents in the serum of other animal species.

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LITERATURE CITED

1. **Bancroft, W. H., F. K. Mundon, and P. K. Russell.** 1972. Detection of additional antigenic determinants of hepatitis B antigen by immunoprecipitation. J. Immunol. **109**:842–848.
2. **Chisari, F. V., J. L. Gerin, and T. S. Edgington.** 1974. Immunochemistry of the hepatitis B virus: ^{125}I-HGsAg ligand. J. Immunol. 113:543–553.

2a. **Cote, P. J., Jr., B. F. Fernie, E. C. Ford, J. W.-K. Shih, and J. L. Gerin.** 1981. Monoclonal antibodies to respiratory syncytial virus: detection of virus neutralization and other antigen-antibody systems using infected human and murine cells. J. Virol. Methods 3:137–147.

3. **Feitelson, M. A., P. L. Marion, and W. S. Robinson.** 1981. Antigenic and structural relationships of the surface antigens of hepatitis B virus, ground squirrel hepatitis virus, and woodchuck hepatitis virus. J. Virol 39:447–454.
4. **Gefter, M. L., D. H. Margulies, and M. D. Scharff.** 1977. A simple method for polyethylene glycol promoted hybridization of mouse myeloma cells. Somat. Cell Genet. 2:231–236.
5. **Gerhard, W., C. M. Croce, D. Lopes, and H. Koprowski.** 1978. Repertoire of antiviral hybrids. Proc. Natl. Acad. Sci. U.S.A. **75**:1510–1514.
6. **Gerin, J. L.** 1979. The eastern woodchuck (*Marmota monax*): a potential animal model for virus induced disease in man, p. 171–175. *In* E. Bartoni, L. Chiandusi, and S. Sherlock (ed.), Systemic effects of HBsAg immune complexes. Piccin Medical Books, Padova, Italy.
7. **Gerin, J. L., R. M. Faust, and P. V. Holland.** 1975. Biophysical characterization of the *adr* subtype of hepatitis B antigen and preparation of anti-*r* sera in rabbits. J. Immunol. **115**:100–105.
8. **Gerin, J. L., J. W.-K. Shih, and B. H. Hoyer.** 1981. Biology and characterization of hepatitis B virus, chapter 5. *In* Proceedings of the International Symposium on Viral Hepatitis. Franklin Institute Press, Philadelphia.
9. **Gerlich, W. H., M. A. Feitelson, P. L. Marion, and W. S. Robinson.** 1980. Structural relationships between the surface antigens of ground squirrel hepatitis virus and human hepatitis B virus. J. Virol. **36**:787–795.
10. **Gerloff, R. K., B. H. Hoyer, and L. C. McLaren.** 1962. Precipitation of radiolabeled poliovirus with specific antibody and antiglobulin. J. Immunol. **89**:559–570.
11. **Hardin, J. A.** 1981. Cryoprecipitogogue from normal serum: mechanism for cryoprecipitation of immune complexes. Proc. Natl. Acad. Sci. U.S.A. **78**:4562–4565.
12. **Hess, G., J. W.-K. Shih, P. M. Kaplan, and J. L. Gerin.** 1977. The demonstration of subtype (*d* or *y*)-specific determinants on the surface of the presumed hepatitis B virus. J. Immunol. **119**:1542–1544.
13. **Kaplan, P. M., R. L. Greenman, J. L. Gerin, R. H. Purcell, and W. S. Robinson.** 1973. DNA polymerase associated with human hepatitis B antigen. J. Virol. **12**:995–1005.
14. **Kennet, R. H., T. J. McKearn, and K. B. Bechtol (ed).** 1980. Monoclonal antibodies. Hybridomas: a new dimension in biological analysis. Plenum Publishing Corp., New York.
15. **Kohler, G., S. C. Howe, and C. Milstein.** 1976. Fusion between immunoglobulin-secreting and non-secreting myeloma cell lines. Eur. J. Immunol. **6**:292–295.
16. **Kohler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
17. **LeBouvier, G. L.** 1971. The heterogeneity of Australia antigen. J. Infect. Dis. **123**:671–675.
18. **Marion, P. L., L. S. Oshiro, D. C. Regnery, G. H. Scullard, and W. S. Robinson.** 1980. A virus of Beechy ground squirrels that is related to hepatitis B virus of humans. Proc. Natl. Acad. Sci. U.S.A. **77**:2941–2945.
19. **Marion, P. L., and W. S. Robinson.** 1980. Hepatitis B virus and hepatocellular carcinoma, p. 423–434. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Mason, W. S., G. Seal, and J. Summers.** 1980. Virus of Pekin ducks with structural and biological relatedness to human hepatitis B virus. J. Virol. **36**:829–836.
21. **Popper, H., J. W.-K. Shih, J. L. Gerin, D. C. Wong, B. H. Hoyer, W. T. London, D. L. Sly, and R. H. Purcell.** 1981. Woodchuck hepatitis and hepatocellular carcinoma: correlation of histologic with virologic observations. Hepatology 1:91–98.
22. **Robinson, W. S.** 1980. Genetic variation among hepatitis B and related viruses. Ann. N.Y. Acad. Sci. **354**:371–378.
23. **Shafritz, D. A., and M. C. Kew.** 1981. Identification of integrated hepatitis B virus DNA sequences in human hepatocellular carcinomas. Hepatology 1:1–8.
24. **Shih, J. W.-K., P. J. Cote, Jr., G. M. Dapolito, and J. L. Gerin.** 1980. Production of monoclonal antibodies against hepatitis B surface antigen (HBsAg) by somatic cell hybrids. J. Virol. Methods 1:257–273.
25. **Shih, J. W.-K., and J. L. Gerin.** 1975. Immunochemistry of hepatitis B surface antigen (HBsAg): preparation and characterization of antibodies to the constituent polypeptides. J. Immunol. 115:634–639.
26. **Shih, J. W.-K., and J. L. Gerin.** 1977. Proteins of hepatitis B surface antigen: amino acid composition of the major polypeptides. J. Virol. **21**:1219–1222.
27. **Shih, J. W.-K., P. L. Tan, and J. L. Gerin.** 1978. Antigenicity of the major polypeptides of hepatitis B surface antigen. J. Immunol. **120**:520–525.
28. **Snyder, R. L., and H. L. Ratcliffe.** 1969. *Marmota monax*: a model for studies of cardiovascular, cerebrovascular, and neoplastic disease. Acta Zool. Pathol. Antverp. **48**:265–273.
29. **Snyder, R. L., and J. Summers.** 1980. Woodchuck hepatitis virus and hepatocellular carcinoma, p. 447–457. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Summers, J.** 1981. Three recently described animal virus models for human hepatitis B virus. Hepatology 1:179–183.
31. **Summers, J., J. M. Smolec, and R. L. Snyder.** 1978. A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proc. Natl. Acad. Sci. U.S.A. **75**:4533–4537.
32. **Summers, J., J. M. Smolec, B. G. Werner, T. J. Kelly, Jr., G. V. Tyler, and R. L. Snyder.** 1980. Hepatitis B virus and woodchuck hepatitis virus are members of a novel class of DNA viruses, p. 459–470. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Werner, B. G., J. M. Smolec, R. L. Snyder, and J. Summers.** 1979. Serological relationship of woodchuck hepatitis virus to human hepatitis B virus. J. Virol. **32**:314–322.

33a. **Wong, D. C., J.-W. Shih, R. H. Purcell, J. L. Gerin, and W. T. London.** 1982. Natural and experimental infection of woodchucks with woodchuck hepatitis virus, as measured by new, specific assays for woodchuck surface antigen and antibody. J. Clin. Microbiol. **15**:484–490.

34. **World Health Organization Expert Committee on Viral Hepatitis.** 1977 Terminology of hepatitis viruses and antigens. Morbid. Mortal. Weekly Rep. **26**:10.