INFECTION AND IMMUNITY, Sept. 1983, p. 950–958
0019-9567/83/090950-09$02.00/0

Vol. 41, No. 3

Complexes of Hepatitis B Surface Antigen and Immunoglobulin M in the Sera of Patients with Hepatitis B Virus Infection

MARIO PALLA,[1] ROBERTO RIZZI,[2] MARIO TOTI,[3] PAOLO ALMI,[3] MARIO RIZZETTO,[2]* FERRUCCIO BONINO,[2] AND ROBERT PURCELL[4]

Clinical Immunochemistry Unit, SORIN Biomedica, and Clinic Fisiology Institute, National Council of Research, Saluggia and Pisa, Italy[1]; Department of Gastroenterology, S. Giovanni Hospital, Turin, Italy[2]; Department of Infectious Diseases, S. Maria della Scala Hospital, Siena, Italy[3]; and Hepatitis Viruses Section, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205[4]

Received 18 October 1982/Accepted 17 March 1983

Hepatitis B surface antigen (HBsAg) bound to immunoglobulin M (IgM) was detected in sera of HBsAg carriers by a radioimmunoassay based on selective absorption of the immunoglobulin on a solid phase coated with antiserum to human IgM. Isopycnic banding and rate-zonal sedimentation have shown that the reaction is related to particulate forms of the HBsAg complexed with IgM. The binding of IgM possibly occurred because of a selective affinity of these molecules to the surface of HBsAg particles. HBsAg/IgM was found transiently in 24 of 25 (96%) patients with acute self-limited hepatitis B and persistently in 6 of 25 patients whose acute hepatitis B progressed to chronicity. It was also found in 20 of 39 (51%) chronic HBsAg carriers with inactive and asymptomatic infection. The HBsAg/IgM phenomenon is not dependent on replication of hepatitis B virions; its persistence in patients with acute hepatitis B may provide complementary evidence of transition of the infection to chronicity.

The finding of antibodies of the immunoglobulin M (IgM) class is of clinical relevance in the diagnosis of hepatitis A, rubella, and other viral diseases, because an IgM immune response occurs very early during the acute phase of these infections (4, 5, 10, 11, 14). IgM anti-hepatitis B core (anti-HBc) and anti-hepatitis B surface (anti-HBs) antibodies have been detected in acute hepatitis B virus (HBV), suggesting that synthesis of this class of antibodies occurs in this disease also (10, 19, 20).

While studying the IgM antibody response to HBs antigen (HBsAg), we observed that the immunological removal of IgM from sera of patients with acute HBV resulted in the removal of most of the HBsAg. These observations suggested the presence of circulating complexes containing HBsAg and IgM antibodies; the analysis of this phenomenon and its occurrence and clinical significance in individuals with HBs antigenemia are reported in this study.

MATERIALS AND METHODS

Reagents. Serum specimens were obtained from humans and chimpanzees with HBV infection, as follows. (i) Forty patients with clinical and biochemical signs of acute hepatitis were studied. Twenty-five had hepatitis B; 10, hepatitis A; and 5, hepatitis non-A, non-B. Serial blood samples were collected at admission to hospital and weekly thereafter. (ii) One hundred twenty chronic HBsAg carriers were subjected to evaluation of hepatic histology. Their histological diagnoses were as follows: minimal histological changes in 17, persistent hepatitis in 17, chronic active hepatitis in 63, chronic active hepatitis with cirrhosis in 13, and inactive cirrhosis in 10. (iii) Twelve asymptomatic HBsAg carriers with persistently normal liver function tests were included in the study. (iv) Two experimentally infected chimpanzees (no. 33 and 36) with type B hepatitis were studied; serum specimens and liver biopsies were obtained before inoculation and weekly thereafter.

Blood samples were obtained by venipuncture, allowed to clot at room temperature in glass tubes for about 2 h, and separated by centrifugation at 2,000 rpm for 5 min. Samples were stored at −30°C for a period ranging from a few months to 3 years before testing.

The following sera were used as controls: (i) 47 sera from normal healthy blood donors; (ii) 20 sera which contained autoantibodies, 10 with antimitochondrial antibody from primary biliary cirrhosis, 5 with anti-smooth muscle antibodies from alcoholic liver diseases, and 2 with antinuclear antibodies from autoimmune hepatitis; (iii) 15 HBsAg-negative sera from individuals positive for anti-HBs; (iv) 13 sera positive for rheumatoid factor; (v) 3 sera containing immune complexes obtained from patients with acute non-A, non-B hepatitis (kindly provided by H. Alter, National Institutes of Health).

Assay. HBsAg, anti-HBs, hepatitis B antigen (HBeAg), anti-HBe, anti-HBc, and anti-hepatitis A

virus IgM were measured by commercial radioimmunoassays (Ausria II, Ausab, kit HBe Abbott, Corab, and Havab; Abbott Laboratories, N. Chicago, Ill.); HBcAg, anti-HBc IgM, and anti-δ were tested by solid-phase radioimmunoassays (20, 31, 33), and rheumatoid factor was measured with commercial reagents (Behring Latex RF, RGA 15). HBsAg, HBcAg, and HBV-associated δ antigen were detected by immunofluorescence (IFL) in the liver biopsies as previously described (32).

Detection of HBsAg/IgM. The assay for detection of HBsAg/IgM was based on the method first described by Duermeyer et al. (11): polystyrene beads (0.25 in [0.64 cm]; Spherotech AG, Zurich, Switzerland) were coated with 30 μg of rabbit IgG anti-human IgM specific for "μ" chain (code 10091; Dako, Copenhagen, Denmark) in 0.05 M phosphate buffer, pH 7.4. After 24 h, the beads were washed and incubated overnight at 4°C with 1% bovine serum albumin in 0.85% NaCl plus 0.01 M phosphate buffer, pH 7.4 (PBS).

Duplicate beads were incubated for 4 h at 37°C with 200 μl of test or control sera diluted 1/1,000 in PBS plus 1% bovine serum albumin; after washing, 200 μl of ^{125}I-labeled sheep IgG anti-HBs (Sorin, Saluggia VC, Italy) was added, and incubation was for 2 h at 37°C. The beads were washed and counted for residual radioactivity in an LKB gamma counter. Sheep IgG anti-HBs was labeled by the Chloramine T procedure (11) at a specific activity of 45 μCi/μg.

Each specimen was also tested after incubation with purified HBsAg between the first and second steps of the standard procedure. After incubation with the test serum, duplicate beads were washed and incubated overnight at room temperature with 200 μl of a solution (200 μg/ml) of purified HBsAg (*ad* and *ay* subtypes; Centre National de Transfusion Sanguine, Paris, France) in 0.05 M phosphate buffer, pH 7.4, containing 20% human serum, negative for HBV-associated markers; radiolabeled anti-HBs was then added.

RESULTS

Incubation of some HBsAg-positive sera with beads coated with anti-human IgM, followed by addition of ^{125}I-labeled anti-HBs, resulted in a significantly higher binding of the radiolabel (>2.1) than that obtained with HBsAg-negative controls, with sera containing autoantibodies and cryoglobulins or with anti-hepatitis A virus of IgG and IgM types and sera obtained from patients with acute non-A, non-B hepatitis.

The addition of purified HBsAg after incubation of the positive sera did not modify the reaction significantly; a non-reproducible increase in the number of counts per minute was sometimes observed in wells with additional purified HBsAg. Of the anti-HBs-positive sera, only one from a patient convalescent from acute hepatitis was positive for free anti-HBs IgM after addition of purified HBsAg.

To assess the specificity of the IgM binding, a positive serum sample (100 μl) was incubated with aliquots of 100 μl of PBS plus 1% bovine serum albumin containing various amounts (0.1 to 6 mg/ml) of purified human IgG, IgM (40), and IgG anti-HBc (purified from an HBsAg, HBeAg-positive serum) (31). Addition of IgM inhibited the reaction progressively, starting from a concentration of 1 mg/ml, whereas IgG and anti-HBc IgG did not inhibit at any concentration (Fig. 1).

To rule out interference due to rheumatoid factor, beads coated with anti-IgM were incubated with sera containing various titers of rheumatoid factor and then with ^{125}I-labeled anti-HBs: no significant binding of the radiolabel was observed.

Negative results were also obtained when serial dilutions (200 μl in PBS) of cryoglobulins with IgG and IgM components (purified from sera of patients with primary biliary cirrhosis) were tested as such or after overnight incubation with purified HBsAg, with or without fresh human serum as source of complement. No reactivity was obtained when anti-human IgG or IgA (anti-human IgG-Fc or anti-human IgA (11); 9477SA, 9465SA BRL; Bethesda Research Laboratories, Inc., Gaithersburg, Md.) and anti-

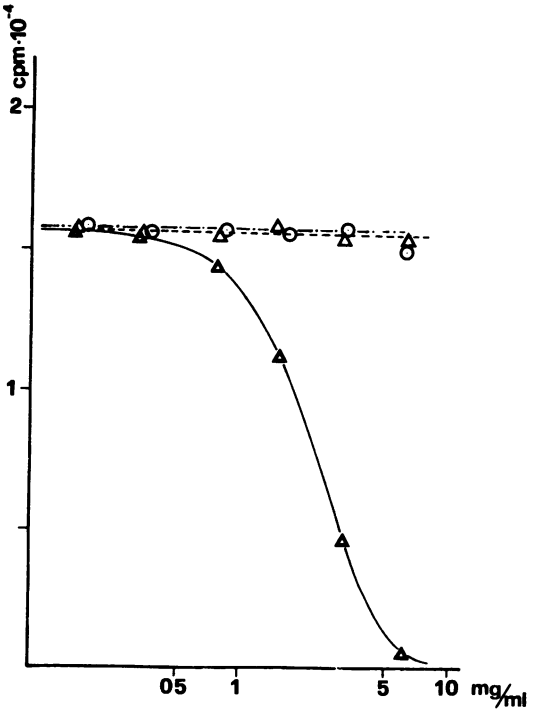

FIG. 1. Aliquots (200 μl) of PBS plus 1% bovine serum albumin containing increasing amounts of IgG, IgG anti-HBc, or IgM (0.1 to 6 mg/ml) were added to an HBsAg/IgM-positive serum. Increasing amounts of IgM (△—△) progressively inhibited the HBsAg/IgM reactivity; addition of IgG (△----△) or IgG anti-HBc (○—·—○) did not affect the reaction.

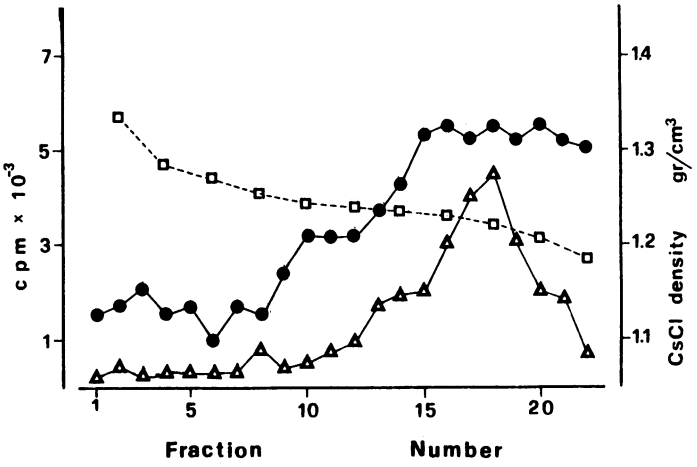

FIG. 2. Equilibrium sedimentation of HBsAg/IgM in CsCl. An aliquot of P1 pellet (0.250 ml) was layered on a 11.5-ml discontinuous gradient (1.2 to 1.5 g/cm^3) of CsCl in PBS and centrifuged for 24 h at 108,000 × *g* and 4°C. Fractions were collected by bottom puncture and assayed for HBsAg (1/200 in PBS; ●) and HBsAg/IgM (diluted 1/100; △). CsCl density was determined by refractometry (□).

HBc or anti-δ (31, 33) were substituted for the anti-human IgM coating on the solid phase.

Characterization of the HBsAg/IgM reaction. A 5-ml portion of an HBsAg/IgM-positive serum from a patient with chronic hepatitis was pelleted through a cushion of 20% (wt/wt) sucrose in PBS and centrifuged for 5 h at 193,000 × *g* and 4°C. A total of 95% of the reactivity was recovered in the pellet, which was resuspended to a final volume of 0.5 ml in PBS and divided into two 250-μl aliquots. An aliquot was submitted to equilibrium sedimentation in a discontinuous CsCl gradient (1.2 to 1.5 g/cm^3 for 24 h at 108,000 × *g* in a Beckman SW41 rotor); HBsAg/IgM and free HBsAg were measured in the 22 fractions obtained from the gradient (Fig. 2). The other aliquot was mixed with 250 μl of purified HBsAg labeled with ^{125}I (3) and subjected to rate-zonal sedimentation in sucrose (Fig. 3). HBsAg/IgM was at equilibrium in CsCl, with the bulk of free HBsAg, at a density between 1.20 and 1.24 g/cm^3. In sucrose, the front of HBsAg/IgM activity sedimented faster than purified ^{125}I-labeled HBsAg (22 nm) (Fig. 3).

A positive serum sample (100 μl; diluted 1/1,000) was incubated with 100-μl aliquots of

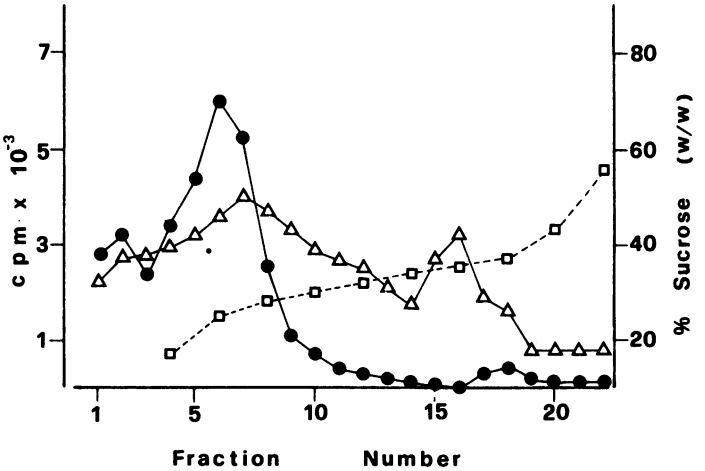

FIG. 3. Rate-zonal sedimentation of HBsAg/IgM in sucrose. An aliquot of P1 pellet (0.250 ml) was mixed with 0.200 ml of purified ^{125}I-labeled HBsAg layered on a 11.0-ml linear (10 to 30% [wt/wt]) gradient of sucrose in PBS with a 65% (wt/wt) sucrose cushion at the bottom and centrifuged for 2.5 h at 101,000 × *g* and 4°C. Fractions were collected by top aspiration, counted for ^{125}I-labeled HBsAg (diluted 1/200; ●), and assayed for HBsAg/IgM (diluted 1/100; △). Sucrose concentration was determined by refractometry (□).

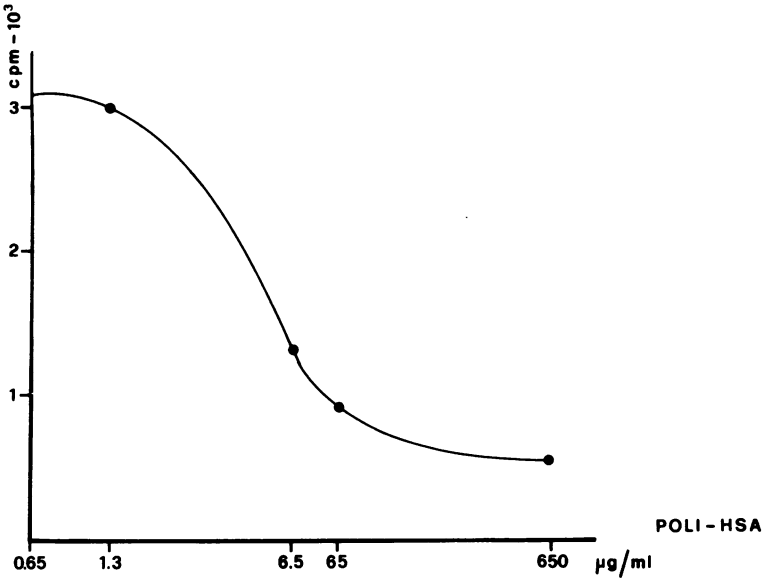

FIG. 4. Aliquots (100 μl) of PBS plus 1% bovine serum albumin containing increasing amounts of poly-HSA (0.0001 to 1 mg/ml) were added to HBsAg/IgM-positive serum. Addition of poly-HSA (●) inhibited the reaction progressively, starting from a concentration of 0.001 mg/ml.

PBS, containing various amounts (0.0001 to 1.0 mg/ml) of human albumin cross-linked by glutaraldehyde (15). Addition of poly-human serum albumin (HSA) inhibited the reaction progressively, starting from a concentration of 0.001 mg/ml (Fig. 4). The HBsAg/IgM reaction was not blocked when poly-HSA was added before or after incubation of the positive serum on the beads. Addition of poly-HSA to the sample did not significantly modify the total HBsAg counts in radioimmunoassay.

HBsAg/IgM in different subsets of HBsAg carriers and in chimpanzees experimentally infected with HBV. Twenty-four of 25 patients with acute hepatitis B (96%) were positive for HBsAg/IgM at hospital admission; the persistence of HBsAg/IgM during a 30-day follow-up is shown in Table 1 in comparison with persistence of other HBV markers. Clearance of HBsAg/IgM from serum and seroconversion to anti-HBs were observed within 30 days in the 18 patients who recovered from disease. All of them were still HBsAg positive at that time. In contrast, a positive HBsAg/IgM reaction persisted at 30 days in six carriers who did not clear the HBsAg and developed a form of chronic hepatitis by histology; all of them still circulated HBsAg/IgM and HBeAg 6 months after onset of clinical disease.

TABLE 1. Relationship of interval of time after hospital admission to prevalence of HBeAg, anti-HBe, and HBsAg/IgM in 25 patients with acute type B hepatitis

Days after hospital admission	No. (%) patients with:		
	HBeAG	Anti-HBe	HBsAg/IgM
1	21 (84)	3 (12)	24 (96)
5	21 (84)	3 (12)	22 (88)
15	11 (44)	4 (16)	16 (64)
20	8 (32)	8 (32)	13 (52)
30	6 (24)	11 (44)	6 (24)

Patterns of HBsAg/IgM in a patient with acute self-limited hepatitis and in one whose hepatitis progressed to chronicity are shown in Fig. 5A and B.

HBsAg/IgM was not detected in 12 asymptomatic carriers with normal liver function tests. It was present in 87% of patients with minimal liver changes by histology.

A liver biopsy and the IFL analysis of intrahepatic HBsAg, HBcAg, and δAg were available in 103 HBsAg-positive patients with liver disease; their histological diagnosis and IFL patterns are summarized in Table 2.

All of the patients with intrahepatic HBcAg had HBsAg/IgM in serum (Table 2); 31 (75%) of them were HBeAg positive and 9 (22%) were anti-HBe positive. Also positive for HBsAg/IgM were 31 (88%) of the patients with intrahepatic δAg, 20 (90%) of those with intracytoplasmic HBsAg, and 10 (38%) of those with negative IFL. No relationship was observed between the histological severity of liver disease and the persistence of HBsAg/IgM in serum.

To evaluate the possible relationship between

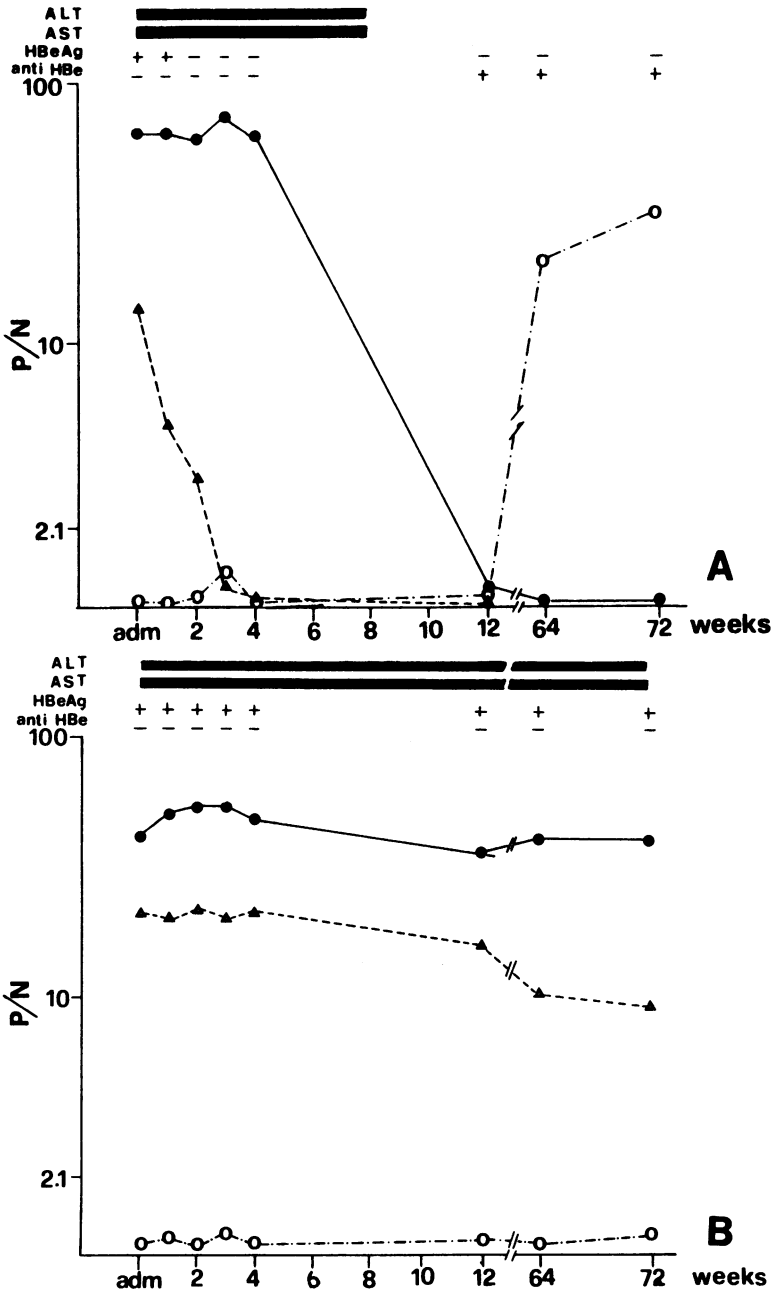

FIG. 5. Patterns of HBsAg/IgM in a patient with acute self-limited hepatitis (A) and in a patient with acute hepatitis which progressed to chronicity (B). Presence (+) or absence (−) of HBeAg, anti-HBe, and elevated aminotransferase (ALT) and aspartate aminotransferase (AST) (▬) is shown. Symbols: Titer of serum HBsAg (●); anti-HBs (○); HBsAg/IgM (△), expressed as the positive/negative ratio (P/N). Values of P/N higher than 2.1 were considered positive.

the concentration of HBsAg in the sample and the HBsAg/IgM reactivity, 22 sera were titrated by counterimmunoelectrophoresis (16) for HBsAg and by the standard procedure for HBsAg/IgM. HBsAg titers (range, 1/16 to 1/256) were unrelated to the positive response for HBsAg/IgM, as assessed by total counts per minute.

Two chimpanzees were inoculated with the serum (1 ml undiluted) of a human HBsAg

TABLE 2. Relationship of type of hepatitis to prevalence of intrahepatic markers of viral infection and serum HBsAg/IgM complexes[a]

Histological diagnosis	No. positive for indicated antigen					Total (%)
	HBcAg	HBcAg + δAg	δAg	HBsAg only	None	
Minimal changes						
HBsAg/IgM +	6	0	0	5	4	15 (88.2)
HBsAg/IgM −	0	0	0	0	2	2 (11.8)
Persistent hepatitis						
HBsAg/IgM +	6	0	2	4	1	13 (76.5)
HBsAg/IgM −	0	0	2	0	2	4 (23.5)
Chronic active hepatitis						
HBsAg/IgM +	20	4	22	5	4	55 (87.3)
HBsAg/IgM −	0	0	2	0	6	8 (12.7)
Chronic active hepatitis with cirrhosis						
HBsAg/IgM +	3	0	3	3	1	10 (76.9)
HBsAg/IgM −	0	0	0	1	2	3 (23.1)
Inactive cirrhosis						
HBsAg/IgM +	2	0	0	3	0	5 (50.0)
HBsAg/IgM −	0	0	0	1	4	5 (50.0)
Total (%)						
HBsAg/IgM +	37 (100)	4 (100)	27 (87.1)	20 (90.9)	10 (38.5)	98 (81.7)
HBsAg/IgM −	0	0	4 (12.9)	2 (9.1)	16 (61.5)	22 (18.3)

[a] Intracytoplasmic HBsAg was present in some liver specimens with nuclear HBsAg or δAg IFL (data not shown).

carrier (subtype *ay*) with chronic hepatitis. Both animals developed HBsAg antigenemia 1 week after inoculation and intrahepatic HBcAg 3 weeks later. This was followed by a self-limited hepatitis; HBV antigens disappeared from liver and serum, followed by seroconversion to anti-HBc, anti-HBe, and anti-HBs. The course of immunological, serological, and biochemical events in one chimpanzee is shown in Fig. 6. Circulating HBsAg/IgM was detected during the acute phase of infection coincident with intrahepatic expression of HBcAg. The clearance of the complexes from the serum occurred at the same time as the disappearance of HBcAg from liver and clinical recovery from hepatitis.

DISCUSSION

Incubation of sera from patients with HBV-associated liver disease on beads coated with anti-human IgM resulted in adsorption of HBsAg to the solid phase. The adsorption of the antigen to the solid phase appeared to be specifically mediated through the reaction of IgM with anti-μ, as the reactivity was inhibited by addition of IgM from control sera and did not occur when anti-IgG, anti-IgA, or antibodies to HBV specificities other than HBsAg were substituted for the anti-IgM coating the solid phase.

The buoyant density of HBsAg/IgM was similar to that of the 22-nm form of HBsAg, since the activity banded at a density of 1.22 g/cm^3 in CsCl. The sedimentation rate of HBsAg/IgM in sucrose was faster than that of free HBsAg, consistent with the increased size of an HBsAg/IgM complex. Two peaks of activity were obtained: the first was comprised mostly of 22-nm particles and the second contained larger complexes of HBsAg with filamentous forms. These data indicate that in sera of patients with HBV part of the HBsAg may circulate complexed with IgM globulins.

This phenomenon possibly may be explained by any of the following hypotheses: (i) nonspecific adsorption of HBsAg to preformed IgM immune complexes; (ii) formation of specific immune complexes between HBsAg and IgM antibody to it; (iii) non-immunological affinity of IgM molecules for the HBsAg produced under conditions involving liver damage; and (iv) specific binding of IgM class antibodies to polymerized albumin bound to HBsAg particles.

Although circulating immune complexes are found in many viral and nonviral liver diseases (3, 28, 30, 34), their role in the phenomenon under study appears excluded by lack of HBsAg/IgM reaction after incubation of purified HBsAg with cryoglobulins or immune complexes from non-A, non-B hepatitis.

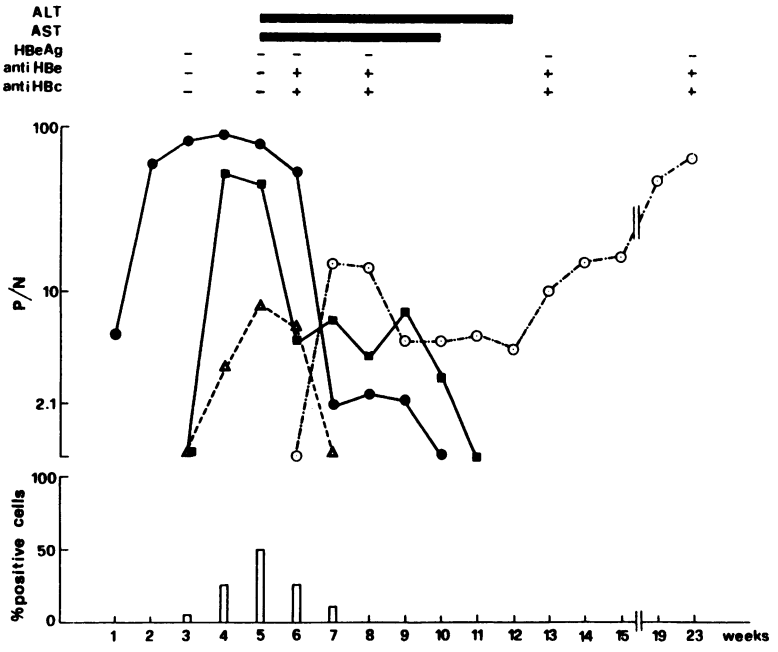

FIG. 6. Course of HBV infection and HBsAg/IgM in chimpanzee no. 36 after inoculation with HBsAg-positive serum. (Top) Presence (+) or absence (−) of HBeAg, anti-HBe, anti-HBc, elevated aminotransferase (ALT) and aspartate aminotransferase (AST) (▬). (Middle) Titers of HBsAg (●), HBcAg (■), anti-HBs (○), and HBsAg/IgM (△), expressed as the positive/negative ratio (P/N). (Bottom) Intrahepatic expression of HBcAg (open vertical bar) by IFL.

The HBsAg/IgM reaction might reflect a primary IgM anti-HBs response neutralized by an excess of circulating HBsAg (2, 3, 6–8, 12, 13, 21, 22, 25). Free anti-HBs IgM antibodies, however, were only detected in one of the patients convalescent from acute hepatitis, elution experiments were unsuccessful in dissociating IgM antibodies with anti-HBs specificity, and the persistence of HBsAg/IgM in patients with long-standing infection suggests that this phenomenon does not represent a temporary equilibrium between HBsAg and the homologous antibody at the phase of seroconversion. A prolonged IgM response to continued antigenic stimulation is a possible explanation, however.

IgM might bind to the HBsAg because of a physicochemical affinity similar to the adsorption of other normal or altered plasma protein on this antigen (23, 24, 27). An interaction of this type was shown to occur between aggregated HSA (poly-HSA) and receptors to it localized on the surface of hepatitis B virions (Dane particles) and 22-nm forms of the HBsAg (15, 18, 26, 29, 35–38); a mechanism of selective affinity of the HBsAg for class-specific IgM might also explain the HBsAg/IgM phenomenon.

HBsAg/IgM might be related to the anti-Dane antibody, described by Alberti et al. (1), which appears to be directed against poly-HSA receptors (A. Alberti, personal communication); this reactivity, however, was found only in patients convalescent from hepatitis B, whereas HBsAg/IgM is a phenomenon associated with progression of hepatitis to chronicity.

Alternatively, the IgM component of the complex might bind to poly-HSA fixed to the HBsAg particles and possibly represent antibody to the denatured plasma protein. Since previous experiments have shown that poly-HSA does not bind to immunoglobulin through an affinity for the Fc receptor (15), the blocking of the HBsAg/IgM reaction obtained with poly-HSA could be due to steric hindrance of the HBsAg/IgM complex by high-molecular-weight aggregates of poly-HSA, HBsAg, and possibly anti-poly-HSA antibodies. More likely, the phenomenon is explained by binding of IgM antibody to poly-HSA on HBsAg particles.

Interestingly, patients whose HBsAg combines with poly-HSA are similar to those who exhibit the highest titers of HBsAg/IgM activity: carriers with liver disease. In patients with hepatitis B the HBsAg/IgM test may provide complementary evidence of transition of the infection to chronicity (9).

LITERATURE CITED

1. **Alberti, A., S. Diana, G. H. Sculard, A. L. W. F. Eddleston, and R. Williams.** 1978. Detection of a new antibody

system reacting with Dane particles in hepatitis B virus infection. Br. Med. J. 2:1056–1058.
2. **Almeida, J. D., and A. P. Waterson.** 1969. Immunocomplexes in hepatitis. Lancet ii:983–986.
3. **Anh-Tuan, N., and E. Novak.** 1980. Detection and quantitation of hepatitis B surface antigen immunocomplexes (HBsAg-ICs) by an antigen-specific method. J. Immunol. Methods 33:293–295.
4. **Ankerst, J., P. Christensen, L. Kjellen, and G. Kronvall.** 1974. A routine diagnostic test for IgA and IgM antibodies to rubella virus: absorption of IgG with Staphylococcus aureus. J. Infect. Dis. 130:268–270.
5. **Best, J. J., J. F. Banatvala, and D. Watson.** 1969. Serum IgM and IgG responses in postnatally acquired rubella. Lancet ii:65–69.
6. **Bonino, F., B. Filippone, C. Lavarini, S. Recchia, O. Crivelli, A. Arrigoni, and P. Trinchero.** 1980. Circulating immunocomplexes in acute type B hepatitis as detected by a solid phase radioimmunoassay for C1q binding activity, p. 202. *In* L. Chiandussi and S. Sherlock (ed.), Systemic effects of HBsAg immunocomplexes.
7. **Brzosko, W. J., K. Madalinski, Krawczynski, H. Skawarska, and A. Nowoslaski.** 1971. Australia antigen immunocomplexes in patients with different forms of hepatitis. J. Infect. Dis. 123:251–256.
8. **Carella, G., M. Digeon, G. Feldmann, P. Jungers, J. Dronet, and J. F. Bach.** 1977. Detection of hepatitis B antigen in circulating immunocomplexes in acute and chronic hepatitis. Scand. J. Immunol. 6:1297–1303.
9. **Careoda, F., R. DeFranchis, A. Monforte, M. Vecchi, E. Rossi, M. Primignani, M. Palla, and N. Dioguardi.** 1982. Persistence of circulating HBsAg/IgM complexes in acute viral hepatitis, type B: an early marker of chronic evolution. Lancet ii:358–360.
10. **Dietz, W. H., O. Porcell, T. E. Moon, C. J. Peters, and R. H. Purcell.** 1976. IgM levels and IgM mediated immune responses in patients with acute hepatitis A, acute hepatitis B and chronic HB antigenemia. Clin. Exp. Immunol. 23:69–72.
11. **Duermeyer, W., F. Wielaard, and J. Van der Veen.** 1979. A new principle for detection of specific IgM antibodies applied in an ELISA for hepatitis. Am. J. Med. Virol. 4:25–27.
12. **Fresco, G. F.** 1978. Detection of circulating immunocomplexes in hepatitis by means of a new method employing 125-I-antibody. Acta Hepatogastroenterol. 25:185–187.
13. **Fye, K. H., M. J. Becker, A. N. Theofilopoulos, H. Moutsopolos, J. L. Feldman, and N. Talal.** 1977. Immunocomplexes in hepatitis B antigen-associated periarteritis nodosa: detection by antibody-dependent cell-mediated cytotoxicity and the Raji cell assay. Am. J. Med. 62:783–785.
14. **Hansson, B. G., J. K. Calhoun, D. C. Wong, S. H. Feinstone, R. H. Purcell, C. S. Pannuti, J. L. Pereira, R. S. Koff, J. L. Dienstag, and S. Iwarson.** 1981. Serodiagnosis of viral hepatitis A by solid phase radioimmunoassay specific for IgM antibodies. Scand. J. Infect. Dis. 13:5–7.
15. **Hansson, B. G., and R. H. Purcell.** 1979. Sites that bind polymerized albumin on hepatitis B surface antigen particles: detection by radioimmunoassay. Infect. Immun. 26:125–130.
16. **Holland, P. V., R. H. Purcell, H. Smith, and J. H. Alter.** 1972. Subtyping of hepatitis-associated antigen (HBAg); simplified technique with counterelectrophoresis. J. Immunol. 109:420–422.
17. **Hunter, W. M., and F. C. Greenwood.** 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) 194:495–497.
18. **Imai, M., Y. Yanase, and T. Nojiri.** 1979. A receptor for polymerized human and chimpanzee albumins on hepatitis B virus particles co-occuring with HBeAg. Gastroenterology 76:241–247.
19. **Kryger, P., L. R. Mathiesen, A. M. Møller, J, Aldershvile, B. G. Hansson, and J. O. Nielsen.** 1981. Enzyme-linked immunosorbent assay for detection of imunoglobulin M antibody to hepatitis B core antigen. J. Clin. Microbiol. 13:405–409.
20. **Lemon, S. M., N. L. Gates, T. E. Simms, and W. H. Bancroft.** 1981. IgM antibody to hepatitis B core antigen as a diagnostic parameter of acute infection with hepatitis B virus. J. Infect. Dis. 143:803–809.
21. **Madalinski, K., and I. Bragiel.** 1979. HBsAg immunocomplexes in the course of infection with hepatitis B virus. Clin. Exp. Immunol. 36:371–373.
22. **Meltzer, M., and E. C. Franklin.** 1966. Cryoglobulins clinical and laboratory study. Cryoglobulins with rheumatoid factor activity. Am. J. Med. 40:828–830.
23. **Milich, D. R., P. K. Bhatnagar, E. D. Papas, and G. N. Vyas.** 1981. Interactions between polymerized human albumin, hepatitis B surface antigen and complement. II. Involvement of C1q in or near the hepatitis B surface antigen receptor for polyalbumin. J. Med. Virol. 7:193–204.
24. **Milich, D. R., E. D. Papas, P. K. Bhatnagar, and G. N. Vyas.** 1981. Interactions between polymerized human albumin, hepatitis B surface antigen and complement. I. Binding of polyalbumin to C1q. J. Med. Virol. 7:181–192.
25. **Millman, I., W. T. London, A. I. Sutnick, and B. S. Blumberg.** 1970. Australia antigen-antibody complexes. Nature (London) 226:83–84.
26. **Neurath, A. R., and N. Strick.** 1979. Radioimmunoassay for albumin-binding sites associated with HBsAg: correlation of results with the presence of e-antigen in serum. Intervirology 11:128–132.
27. **Neurath, A. R., N. Strick, C. Y. Huang, and A. M. Prince.** 1979. Antigenic sites related to human serum proteins in HBsAg: isolation from normal human serum of a high-molecular-weight glycoprotein reacting with anti-albumin. J. Med. Virol. 2:231–233.
28. **Nydegger, U. E., P. H. Lambert, H. Gerber, and P. A. Miescher.** 1974. Circulating immunocomplexes in the serum in systemic lupus erythematosus and in carriers of hepatitis B antigen: quantitation by binding to radiolabelled C1q. J. Clin. Invest. 54:297–299.
29. **O'Neill, S. P.** 1979. Interaction of hepatitis B surface antigen with polymerized human albumin. J. Med. Virol. 4:177–185.
30. **Pernice, W., C. P. Sodomann, G. Luben, F. R. Seiler, and H. H. Sedlacek.** 1979. Antigen-specific detection of HBsAg containing immunocomplexes in the course of hepatitis B virus infection. Clin. Exp. Immunol. 37:376–378.
31. **Purcell, R. H., J. L. Gerin, and J. B. Almeida.** 1974. Radioimmunoassay for the detection of the core of the Dane particle and antibody to it. Intervirology 2:231–243.
32. **Rizzetto, M., H. G. Canese, J. L. Gerin, W. T. London, L. D. Sly, and R. H. Purcell.** 1980. Transmission of the hepatitis B virus associated delta antigen to chimpanzees. J. Infect. Dis. 141:590–602.
33. **Rizzetto, M., J. W.-K. Shih, and J. L. Gerin.** 1980. The hepatitis B virus associated delta antigen: isolation from liver, development of solid phase radioimmunoassays for delta and anti-delta and partial characterization of delta. J. Immunol. 125:318–324.
34. **Santoro, F., P. Wattre, J. P. Dessaint, and A. Capron.** 1977. Hepatitis B circulating immunocomplexes. Characterization by radioimmunoprecipitation PEG assay. J. Immunol. Methods 15:201–203.
35. **Shih, J. W.-K., P. L. Tan, and J. L. Gerin.** 1980. Relationship of large hepatitis B surface antigen polypeptide to human serum albumin. Infect. Immun. 28:459–463.
36. **Stevens, C. E., A. R. Neurath, and W. Szmuness.** 1978. Correlations between HBeAg/anti-HBe, HBsAg titer, HBsAg-associated albumin binding sites and infectivity, p. 211–213. *In* G. N. Vyas, S. N. Cohen, and R. Schmid (ed.), Viral hepatitis. The Franklin Institute Press, Philadelphia.
37. **Thung, S. N., and M. A. Gerber.** 1981. HBsAg associated albumin receptors and anti-albumin antibodies of patients with liver disease. Gastroenterology 80:260–272.

38. **Thung, S. N., and M. A. Gerber.** 1981. Presence of receptor for polymerized albumin in HBsAg-containing hepatocytes and hepatoma cell line. Hepatology **2**:132–136.
39. **Thung, S. N., and M. A. Gerber.** 1981. Specificities of albumin receptors and albumin antibodies. Infect. Immun. **32**:1292–1294.
40. **Wichman, A., and H. Borg.** 1977. Purification of human immunoglobulin M by affinity chromatography on protamine sepharose. Biochim. Biophys. Acta **490**:463–465.