0270-9139/82/0204-0449$02.00/0
HEPATOLOGY

Vol. 2, No. 4, pp. 449-454, 1982
Printed in U.S.A.

Dane Particle-Associated Hepatitis B e Antigen in Patients with Chronic Hepatitis B Virus Infection and Hepatitis B e Antibody

GIOVANNI RAIMONDO, SERAFINO RECCHIA, CARLA LAVARINI, OSVALDO CRIVELLI, AND MARIO RIZZETTO

Department of Gastroenterology, Ospedale Maggiore di San Giovanni Battista, Torino 10126, Italy

A commercial radioimmunoassay was adapted to detect serum Dane particle-associated HBeAg in patients whose sera contained the homologous antibody. HBeAg was released from Dane particles with guanidine HCl.

Dane particles were separated from anti-HBe by gel-filtration (Sepharose 4B) and ultracentrifugation of the eluate. Dane particle-HBeAg was tested in 45 HBsAg carriers with anti-HBe and was present in 8 (18%) carriers, all of whom had chronic liver disease. By contrast, HBeAg was not found in 10 carriers with normal liver histology. Serum or liver HBcAg was found in 6 of 8 patients with Dane particle-HBeAg. None of the carriers without Dane particle-HBeAg had other markers of hepatitis B virion synthesis.

We conclude that Dane particle-HBeAg provides a sensitive index of active hepatitis B virus replication, a guide to the presence of chronic hepatitis in HBsAg carriers with anti-HBe, and a noninvasive method to follow infection in these patients.

The majority of individuals chronically infected by the hepatitis B virus (HBV) either express HBe antigen (HBeAg) in the blood or circulate the homologous antibody (anti-HBe) (1).

The presence in serum of HBeAg is associated with synthesis of the Dane particle (2), now regarded as the HB virion (3), and identifies highly infectious carriers of HB surface antigen (HBsAg) (4) who are likely to have liver disease (5–8). There is controversy on the significance of anti-HBe. The assumption that it identifies healthy noninfective carriers (5–8) has been disputed by reports of liver disease in 31 to 44% of carriers with anti-HBe (9–11) and by transmission of HBV to chimpanzees by sera containing this antibody (12, 13).

In previous studies, HBV replication was assessed by measurement of serum Dane particle-specific DNA polymerase activity or enumeration of circulating Dane particles by electron microscopy; a negative reaction or count was correlated with lack of infectivity of the carrier (14–16).

Recently, it was shown that radioimmunoassay (RIA) of sera for an immunological marker, the Dane particle-associated HB core antigen (HBcAg) (17), provides an index of HBV replication which is more sensitive and specific than the enzymatic and morphological tests. Based on the observation of Takahashi et al. (18) that HBeAg may be found within the Dane particle core, presumably representing a structural virion component whose excess is released in blood as soluble-free HBeAg, we developed a RIA for Dane particle-associated HBeAg using a standardized commercial kit.

Dane particle-HBeAg was determined in HBsAg carriers with anti-HBe. The results were compared with other markers of HBV, and the presence of HB virions in serum was correlated with clinical status.

MATERIALS AND METHODS

SERA AND BIOPSIES

The sera examined in this study were collected over several years from 45 HBsAg carriers with anti-HBe undergoing clinical evaluation of hepatic status. A liver biopsy was performed in 35 carriers; they included 10 healthy carriers with normal liver histology, 7 with chronic persistent hepatitis, 9 with chronic active hepatitis with and without cirrhosis, and 9 with inactive cirrhosis. Part of each liver biopsy was frozen for immunofluorescence staining of HBcAg. Histological liver disease was diagnosed according to Geall et al. (19). Each of the 10 patients in whom a biopsy was not done was considered to have chronic liver disease on the basis of

Received October 9, 1981; accepted March 9, 1982.
Address reprint requests to: Mario Rizzetto, M.D., Department of Gastroenterology, Ospedale Maggiore di San Giovanni Battista, Cs. Bramante 88, Torino 10126, Italy.

persistently elevated alanine-aminotransferase (ALT) levels.

All of the carriers had antibody to HBcAg (anti-HBc) at titers between $1:10^4$ and $1:10^6$. None had antibody to HBsAg (anti-HBs) or was positive for δ-antigen-antibody system associated with HBV (20). Two follow-up serum specimens collected over several months were available from each of two patients. Serum specimens were stored at −30°C for up to 5 years before use.

Assays and Reagents

Sera were tested for HBsAg, anti-HBs, anti-HBc, HBeAg, and anti-HBe by commercial RIA (Ausria II, Ausab, Corab, Kit HBe, Abbott Laboratories, North Chicago, Ill.). Anti-HBe was titered to endpoint in 2-fold dilutions, anti-HBc in 10-fold dilutions. Titers were expressed as the reciprocal dilution inhibiting the binding of 50% of radioactivity in the negative control.

Purified anti-HBc immunoglobulin G (IgG) and anti-HBe IgG (containing 5 mg protein per ml) were prepared by DE-52 chromatography. Anti-HBc IgG was obtained from a human serum containing anti-HBc at a titer of $1:10^6$ and devoid of anti-HBs and anti-HBe. Anti-HBe was obtained from a human serum containing high titers of anti-HBe and anti-HBc and devoid of anti-HBs; purified anti-HBe IgG contained anti-HBe at a titer of 1:1,024 and anti-HBc at a titer of $1:10^4$.

HBcAg immunofluorescence was performed by staining of frozen ether-fixed liver sections with fluorescein-ated anti-HBc IgG (20).

Purified HBsAg (10 μg protein per ml), a rabbit pre-inoculation anti-HBs negative serum and a postinoculation hyperimmune antiserum to HBsAg were reference reagents kindly provided by Dr. J. L. Gerin, Georgetown University, Rockville, Md. Sera used as negative controls were obtained from 10 healthy individuals without HBV antigen-antibody markers.

Preparation of Dane Particles

Dane particles were purified (21) from a human serum which contained Dane particle-specific DNA polymerase activity and a high titer of HBeAg (positive over negative ratio: 22).

Preparation of Serum Pellets after Gel Filtration

Sera were tested for Dane particle-associated HBcAg and HBeAg after gel filtration (17). Ten milliliters of Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) were packed in a 10-ml syringe and equilibrated with phosphate-buffered saline (pH 7.4, 0.1 *M*, plus 1% bovine serum albumin). Two milliliters of test sera were applied on the column, the void volume eluate was discarded, and the next 2.75-ml volume was collected, layered on 20% (w/v) sucrose, and centrifuged for 5 hr at 4°C and 40,000 rpm in the Beckman S.W. 50.1 rotor; the final pellet (hereafter mentioned as pellet) was resuspended in 200 μl (1/10 original serum volume) of phosphate-buffered saline plus 1% bovine serum albumin.

Solid-Phase RIA for Dane Particle-Associated HBcAg. Dane Particle-Associated DNA Polymerase Activity

HBcAg in Dane particle preparation was measured by solid-phase RIA using beads coated with anti-HBc and the trays of the Abbott Laboratories' RIA kits. Polystyrene beads (¼ inch, Spherotech AG, Zurich, Switzerland) were incubated for 4 hr at room terperature with a $1:10^3$ dilution of anti-HBc serum. The beads were washed, incubated overnight in phosphate-buffered saline plus 1.5% bovine serum albumin, dried and stored at −20°C until used. For the test, 150-μl aliquots of the Dane particles preparation were incubated in wells containing the beads, with the addition of 50 μl of 0.4% Nonidet. After 12 hr at 4°C, the trays were washed and 200 μl of ^{125}I-anti-HBc IgG (6×10^5 cpm) were applied to each well. After 4 hr, the beads were washed and counted for residual radioactivity in a LKB gamma-counter. The anti-HBc IgG fraction was radiolabeled by the chloramine-T method (22).

Dane particle-HBcAg in pellets was measured by a previously described microtiter RIA (17). Polyvinyl plates (Cooke Laboratories, Inc., Alexandria, Va.) were coated with a $1:10^3$ dilution of the anti-HBc serum. Single 50-μl aliquots of each resuspended pellet were inoculated in microtiter wells followed by 5 μl of 1% Nonidet to a

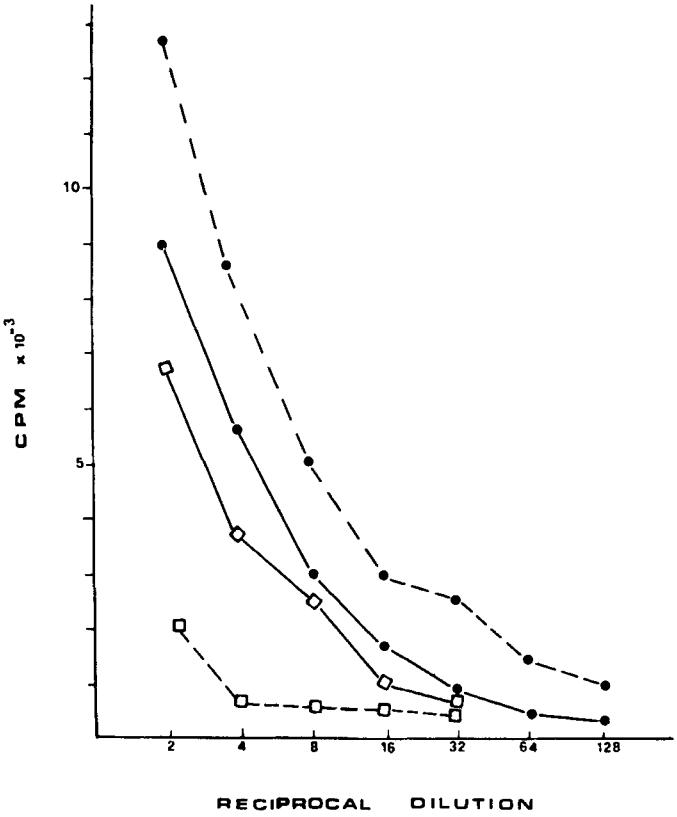

FIG. 1. Titration curves of Dane particles with the HBeAg RIA after addition of 1.5 *M* guanidine HCl (●- - -●) or 0.1% Nonidet P40 (●——●), and with the HBcAg RIA after addition of 1.5 *M* guanidine HCl (□- - -□) or 0.1% Nonidet P40 (□——□). For the test, 50 μl of 6 *M* guanidine or 50 μl of 0.4% Nonidet P40 were added to 150 μl of serial 2-fold dilutions of Dane particles.

final detergent concentration of approximately 0.1%. After 12 hr at 4°C, the plates were repeatedly washed with phosphate-buffered saline and 70 μl (2×10^5 cpm) of ^{125}I-anti-HBc IgG were applied to each well. Plates were then incubated for 4 hr at 37°C, washed, and counted for residual radioactivity. The titers of Dane particle-HBcAg were expressed as the ratio (P/N) of cpm of the test sample (P) to the average cpm of pellets from negative controls (N); a test sample was considered positive if the titer (P/N) was 2.1.

DNA polymerase activity was tested by the method of Kaplan et al. (23) in 20-fold concentrated serum pellets. Dane particle specificity of the DNA polymerase was confirmed by immunoprecipitation as described by Kaplan et al. (24).

Solid-Phase RIA for Dane Particle-Associated HBeAg

Dane particle-HBeAg RIA was performed with Abbott Laboratories' HBe RIA. Dane particle preparation and controls were tested in 150-μl aliquots. Dane particle preparation was tested with addition of 50 μl of phosphate-buffered saline, or 0.4% Nonidet P40 or different concentrations of guanidine HCl. Controls were tested with addition of 50 μl of 6 *M* guanidine.

For assaying Dane particle-HBeAg in pellets, the remaining 150 μl of each resuspended pellet were incubated into wells of Abbott Laboratories' trays with addition of 50 μl of 6 *M* guanidine. The titers of Dane particle-HBeAg were expressed as the ratio of positive over negative (P/N).

RESULTS

RIA for Dane Particle-HBeAg

Positivity was detected by HBeAg RIA in the untreated original preparation of Dane particles (titer of 5); no reactivity was observed with HBcAg RIA.

Strong reactivities were observed when Dane particles were tested for HBcAg and HBeAg with addition of Nonidet to a final concentration of 0.1% (titers of 45 and 60). Dane particles incubated with guanidine to a final molarity of 0.5, 1.5, and 3 yielded, in the HBeAg RIA, titers of 26, 98, and 65, respectively; the same guanidine extracts of Dane particles were negative or yielded only a borderline positivity when tested with HBcAg RIA. The titration curves of Dane particles incubated with Nonidet or guanidine and tested with HBcAg and HBeAg RIA are shown in Figure 1.

Fifty microliters of purified anti-HBc IgG or anti-HBe IgG were added to 150-μl aliquots of Dane particles. The preparations were treated with 20 μl of 2% Nonidet or 40 μl of 8 *M* guanidine and tested with the HBcAg and HBeAg RIA. Activity recovered with HBcAg and HBeAg RIA after incubation of Dane particles with Nonidet was completely blocked by anti-HBc IgG or anti-HBe IgG (which contained anti-HBc). Activity recovered with HBeAg RIA after incubation of Dane particles with guanidine was slightly inhibited by anti-HBc (titers from 87 to 65), but completely blocked by anti-HBe IgG.

One-milliliter aliquots of Dane particle preparation diluted 1:6 in phosphate-buffered saline (150 μl of which yielded a titer of 10 in the HBeAg RIA with 1.5 *M* guanidine and a titer of 3 in the HBcAg RIA with 0.1% Nonidet) were incubated with 1 ml of a 1:5 dilution of anti-HBs negative and hyperimmune rabbit antiserum to HBsAg, brought to 5 ml with phosphate-buffered saline, and left overnight at 4°C. The precipitates obtained by centrifugation for 20 min at 3,000 rpm were resuspended in 400 μl of phosphate-buffered saline plus 1.5 *M* guanidine.

The precipitate with anti-HBs negative serum yielded a titer of 8 in the HBeAg RIA and a titer of 5 with HBcAg RIA, the precipitate with hyperimmune serum to HBsAg yielded a titer of 35 with HBeAg RIA and a titer of 11 with HBcAg RIA.

No reactivity was observed when 10 pellets from normal controls or purified HBsAg were tested in the HBeAg RIA with addition of guanidine to a final molarity of 1.5.

Dane Particle-HBeAg in Serum of HBsAg Carriers with Anti-HBe. Correlation with Diagnosis, Serum Dane Particle-HBcAg and DNA-Polymerase Activity, and Liver HBcAg

None of the pellets contained detectable anti-HBe.

The presence of Dane particle-HBeAg in serum of 45 anti-HBe positive carriers with and without liver disease is shown in Figure 2. The antigen was not found in the 10 healthy carriers without histological evidence of liver disease. It was present in 8 carriers with chronic liver disease; 3 had chronic persistent hepatitis, 2 chronic active hepatitis, 2 inactive cirrhosis at histology; in another carrier, no liver biopsy was available, and the diagnosis was based on persistently abnormal ALT levels

The mean anti-HBe titer in carriers with Dane particle-HBeAg (1:2,048) was similar to that of Dane particle-HBeAg negative carriers (1:1,024). Serum Dane particle-

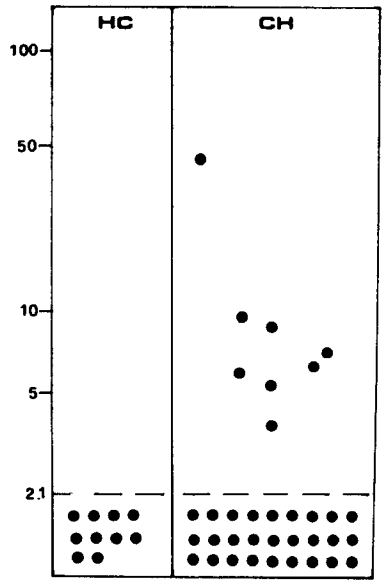

FIG. 2. Titers of Dane particle-HBeAg in 45 HBsAg carriers with serum anti-HBe divided according to clinical status. HC, healthy carriers; CH, chronic hepatitis. Values below 2.1 are negative.

HBcAg and DNA polymerase activity and intrahepatic HBcAg were only found in carriers with Dane particle-HBeAg. The diagnosis of these patients, the presence and correlation of HB virion markers, and the titer of anti-HBe is shown in Table 1. Dane particle-HBcAg was found in 2 patients with chronic persistent hepatitis, 1 with chronic active hepatitis, and in the patient with elevated serum ALT activity. A low level of DNA polymerase activity was detected in two patients (nos. 6 and 7); the Dane particle specificity of the reaction could not be confirmed due to nonspecific precipitation of activity by anti-HBs negative control serum.

HBcAg was observed in the liver of 5 of 7 patients whose biopsy was examined. HBcAg was localized only in hepatocyte nuclei in two patients (nos. 3 and 5); it was localized in the nuclei but predominantly in the cytoplasms (Figure 3) in the other three patients (nos. 1, 4, and 6).

Anti-HBe was reported positive in serum samples taken from Patients 4 and 5, one and three years previously. Two follow-up serum specimens taken 8 and 18 months apart were available from Patients 6 and 7. The first and second samples of Patient 6 contained anti-HBe at a titer of 1:1,024, Dane particle-HBeAg at titers of 6

TABLE 1. DIAGNOSIS AND HBV MARKERS IN EIGHT HBsAg CARRIERS WITH ANTI-HBe AND DANE PARTICLE-HBeAg IN SERUM

Case no.[a]	Sex	Age	Diagnosis[b]	HBV markers					
				Serum				Liver	
								HBcAg	
				Anti-HBe (titer)[c]	Dane particle-HBeAg (titer)[d]	Dane particle-HBcAg (titer)[d]	HBV DNA polymerase[d]	Nuclear	Cytoplasmic
1	M	29	CPH	64	40	10	NT[e]	+	+
2	M	43	CAH	32	4	—	—	−	−
3	M	23	CPH	8,192	10	3	—	+	−
4	M	40	IC	127	5, 5	—	—	+	+
5	F	27	CAH	8,192	9	6	—	+	−
6	F	40	IC	1,024	5	—	633[f]	+	+
7	M	35	CPH	1,024	6	—	712[f]	−	−
8	F	6	persistently elevated enzymes	1,024	7	3	NT	NT	

[a] P/N ratio.
[b] CPH, chronic persistent hepatitis; CAH, chronic active hepatitis; IC, inactive cirrhosis.
[c] Reciprocal serum dilution.
[d] Expressed as cpm.
[e] NT, not tested.
[f] Not confirmed as HBV-specific.

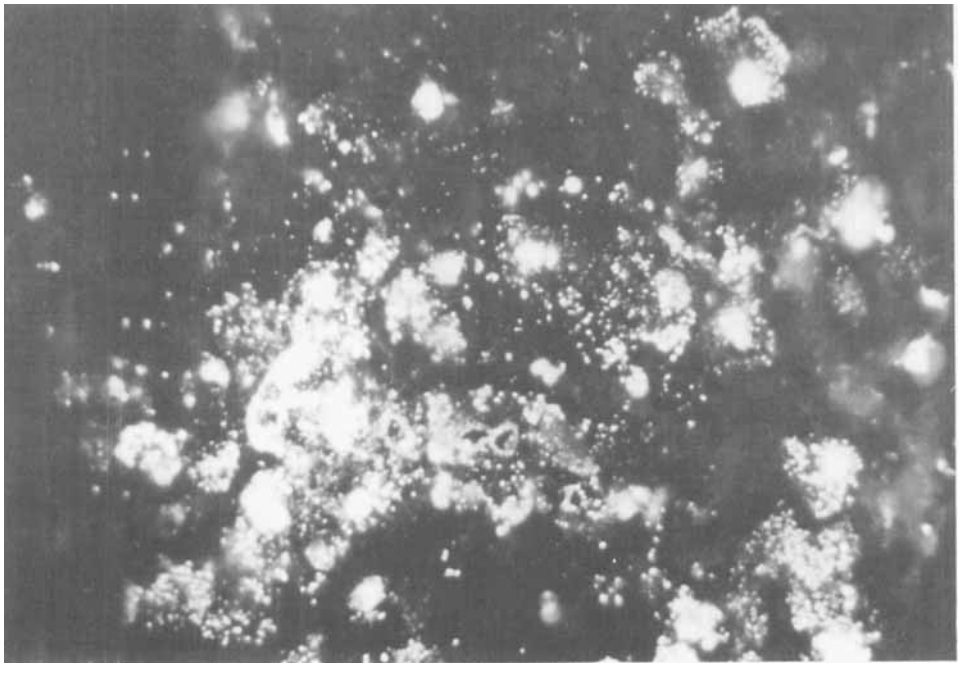

FIG. 3. HBcAg immunofluorescence in liver cell nuclei and cytoplasms of Patient 4, Table 1.

and 5. The first and second samples of Patient 7 contained anti-HBe at a titer of 1:2,048 and 1:1,024 and Dane particle-HBeAg at a titer of 6.

DISCUSSION

The HBeAg observed in the original preparation of Dane particles presumably represents contaminating-free antigen from serum which had a high titer of reactivity.

Incubation of Dane particles with Nonidet P40, which exposes HBcAg on the core particle (25), revealed an activity detectable both with the Abbott Laboratories' HBeAg RIA and HBcAg RIA. Incubation with guanidine, a dissociating agent which denatures the core antigen (26) but maintains the antigenicity of HBeAg (27), yielded a strong activity with HBeAg assay but none with HBcAg RIA. The activity recovered with HBeAg RIA after treatment with detergent was blocked by anti-HBc, whereas the activity recovered with the same assay after guanidine was not inhibited by anti-HBc but only by anti-HBe. We interpret these results as indicating that the Abbott Laboratories' HBeAg RIA also detects HBcAg but, after addition of guanidine, it measures a cryptic form of HBeAg released from core particles disrupted by the dissociating agent (18). The localization of extractable HBeAg in the HB virion is also shown by increased recovery of HBcAg and HBeAg in the immunoprecipitate between Dane particles and anti-HBs. The determination of Dane particle-HBeAg in sera containing anti-HBe required complete removal of the antibody, which would have otherwise interfered with the test. Anti-HBe was filtered through Sepharose 4 B, a procedure previously shown to separate anti-HBc from Dane particles, regardless of high titers of this antibody in serum (17).

In demonstration of the high sensitivity of the HBeAg RIA in clinical testing, the other serum and liver markers of the HB virion were found only in individuals positive for Dane particle-HBeAg. As in a previous study (17), DNA polymerase activity appeared to be the least sensitive marker, being detected only in 2 of 6 carriers with Dane particle-HBeAg; the specificity of the reaction, moreover, could not be confirmed in either patient.

The lack of HBcAg in 4 of 8 sera which were positive for Dane particle-HBeAg does not establish superiority of the HBeAg assay in the clinical setting; due to the paucity of serum available from most patients, the HBcAg test was performed in microtiter plates. The sample volume was 3-fold smaller than that used for the HBeAg RIA. The good correlation between titration curves of HBcAg and HBeAg in the preparation of Dane particles suggests that the two assays are of similar sensitivity. It is likely, therefore, that the Abbott Laboratories' HBeAg RIA may be used also as a method for serum Dane particle-HBcAg; variations between different batches, however, may be expected when assaying for core antigen, as the kit is optimized for HBeAg.

Based on the failure to detect DNA polymerase activity or Dane particles in sera with anti-HBe, it was postulated that anti-HBe indicates a noninfectious type of HBV replication reduced to synthesis of the HBsAg (16). Contradictory to this hypothesis, studies in experimental infectivity in chimpanzees reveal that HBV may be transmitted from inocula containing anti-HBe (12, 13), suggesting that the antibody may coexist with infectious, presumably complete forms of the HB virion.

This study demonstrates that HB virion synthesis, reflected by the presence of Dane particle-HBeAg, occurs in sera containing anti-HBe. The finding of Dane particle-HBeAg in 18% of patients examined suggests that a distinct proportion of carriers with anti-HBe are potentially infective and indicates that this antibody may be no safeguard of absent or diminished replication of the HB virion. Previous discrepancies of the significance of anti-HBe may be explained by the low levels of Dane particles circulating in anti-HBe positive sera. These were not detected by less sensitive assays but are detectable by the new RIAs for immunological markers of the HB virion or by an equally sensitive radioassay for HBV DNA, low to moderate amounts of which were found in four carriers with chronic persistent hepatitis despite absence of conventional markers of HBV replication and the presence of anti-HBe (28). Negative results in carriers with anti-HBe were obtained by Takahashi et al. (29) using a serum HBcAg RIA similar to ours: these authors examined only symptomless anti-HBe positive carriers. We also failed to detect productive HBV infection in these patients.

Our data show that HBeAg may circulate in the blood hidden from the homologous antibody in analogy with the HBcAg/antibody system. Anti-HBe was reportedly positive in sera of two patients taken years before the specimen in which HBeAg and anti-HBe were demonstrated. Both reactivities were detected in each of the specimens taken months apart from two other carriers, suggesting that coexistence of Dane particle-HBeAg with anti-HBe represents a persistent phenomenon of chronic HBV infection rather than a temporary dynamic equilibrium between antigen and antibody.

Liver damage in patients with anti-HBe may be influenced by the degree of infectious HBV replication, as chronic hepatitis was found in each carrier with Dane particle-HBeAg; hepatitis, however, was also present in many of the anti-HBe carriers without this marker, suggesting that an active synthesis of the HB virion may be but one of the factors contributing to the liver disease. Chronic HBV hepatitis is currently regarded as the clinical expression of a deficient immune response of the host to the virus, which is eliminated only partially from the body (30): some carriers with anti-HBe and liver disease possibly represent the extreme of this spectrum. Whether in this situation, anti-HBe is a secondary epiphenomenon or plays a specific role in modulating host-virus interactions is unknown. Interestingly, in 3 of 5 anti-HBe positive carriers with HBcAg fluorescence, intrahepatic antigen was observed in an unusual cytoplasmic localization, possibly related to abnormal retention of core particles which are not released as mature virions to the blood (31) or to synthesis of HBcAg which is not translated to the nucleus for assembly in core particles (32).

REFERENCES

1. Mushahwar IK, Overby LR, Frosner G, et al. Prevalence of hepatitis B e antigen and its antibody as detected by radioimmunoas-

says. J Med Virol 1978; 2:77–87.
2. Miyakawa Y, Mayumi M. Characterization and clinical significance of HBeAg. In: Vyas GN, Cohen SN, Schmid R, eds. Viral hepatitis. Philadelphia: The Franklin Institute Press, 1978: 193–201.
3. Krugman S, Gocke DJ. Viral hepatitis, etiology and pathogenes. In: Smith LH Jr, ed. Viral hepatitis. Philadelphia: W. B. Saunders, 1978: 6–12.
4. Grady GF. Relation of e antigen to infectivity of HBsAg positive inoculations among medical personnel. Lancet 1976; 2:492–493.
5. Magnius LO, Lindholm A, Lundin P, et al. A new antigen-antibody system: clinical significance in long term carriers of hepatitis B surface antigen. JAMA 1975; 231:356–359.
6. Nielsen JO, Dietrichson O, Juhl E. Incidence and meaning of the e determinant among hepatitis B antigen positive patients with acute and chronic liver diseases. Lancet 1974; 2:913–915.
7. Eleftheriou N, Heathcote J, Thomas H, et al. Incidence and clinical significance of e antigen and antibody in acute and chronic clinical liver disease. Lancet 1975; 2:1171–1173.
8. Trepo C, Magnius LO, Schaefer RA, et al. Detection of e antigen and antibody: correlations with hepatitis B core antigen liver disease and outcome in hepatitis B infections. Gastroenterology 1976; 7:804–808.
9. El Sheikh N, Woolf IL, Galbraith RM, et al. e antigen-antibody system as indicator of liver damage in patients with hepatitis B antigen. Br Med J 1975; 4:252–253.
10. Pagliaro L, D'Amico G, Di Piazza S, et al. HBeAg/anti-HBe in chronic liver disease. Ital J Gastroenterol 1978; 10:79–80.
11. Realdi G, Alberti A, Rugge M, et al. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology 1980; 79:195–199.
12. Shikata T, Karasawa T, Abe K, et al. Hepatitis B e antigen and infectivity of hepatitis B virus. J Infect Dis 1977; 4:571–576.
13. Berquist KR, Maynard JE, Murphy BL. Infectivity of serum containing HBsAg and antibody to e antigen. Lancet 1976; 1:1026–1027.
14. Stannard LM, Moodie J, Keen A, et al. Electron microscopic study of the distribution of the Australia antigen in individual sera of serologically positive blood donors and two patients with serum hepatitis. J Clin Pathol 1973; 26:209–216.
15. Alberti A, Diana S, Scullard GH, et al. Full and empty Dane particles in clinical hepatitis B virus infection. Gastroenterology 1978; 75:869–874.
16. Trepo C, Vitvitski O, Hantz B, et al. Nature and significance of HBeAg specificities. In: Bianchi L, Gerock W, Sickinger K, et al., eds. Virus and the liver. Lancaster, England: MTP Press, 1980: 49–57.
17. Rizzetto M, Shih JWK, Verme G, et al. A radioimmunoassay for HBcAg in the sera of HBsAg carriers: serum HBcAg, serum DNA polymerase activity and liver HBcAg immunofluorescence as markers of chronic liver disease. Gastroenterology 1981; 80:1420–1427.
18. Takahashi K, Akahane Y, Gotanda T, et al. Demonstration of hepatitis B e antigen in the core of Dane particles. J Immunol 1979; 122:275–279.
19. Geall MG, Schonfield LJ, Summerskill WHJ. Classification and treatment of chronic active hepatitis. Gastroenterology 1968; 55:724–727.
20. Rizzetto M, Canese MG, Arico S, et al. Immunofluorescence detection of a new antigen-antibody system (delta/anti-delta) associated with the hepatitis B virus in the liver and in the serum of HBsAg carriers. Gut 1977; 18:997–1003.
21. Kaplan PM, Ford EC, Purcell RH, et al. Demonstration of subpopulations of Dane particles. J Virol 1976; 17:885–893.
22. Hunter WM, Greenwood FC. Preparation of iodine-131 labeled human growth hormone of high specific activity. Nature 1962; 194:495–496.
23. Kaplan PM, Greenman RL, Gerin JL, et al. DNA polymerase associated with human hepatitis B antigen. J Virol 1973; 12:995–1005.
24. Kaplan PM, Gerin JL, Alter HJ. Hepatitis B specific DNA polymerase activity during post-transfusion hepatitis. Nature 1974; 249:762–763.
25. Almeida JD, Rubenstein D, Stott EJ. New antigen-antibody system in Australia antigen positive hepatitis. Lancet 1971; 2:1225–1227.
26. Crivelli O, Rizzetto M, Lavarini C, et al. Enzyme-linked immunosorbent assay for detection of antibody to the HBsAg associated delta antigen. J Clin Microbiol 1981; 14:173–177.
27. McAuliffe VJ, Purcell RH. Current status of tests for HBeAg and anti-HBe. In: Vyas GN, Cohen SN, Schmid R, eds. Viral hepatitis. Philadelphia: The Franklin Institute Press, 1978: 161–171.
28. Bonino F, Hoyer B, Nelson J, et al. Hepatitis B virus DNA in the sera of HBsAg carriers: a marker of active HBV replication in the liver. Hepatology 1981; 1:386–391.
29. Takahashi K, Imai M, Tsuda F, et al. Association of Dane particles with e antigen in the serum of asymptomatic carriers of hepatitis B surface antigen. N Engl J Med 1976; 117:102–105.
30. Bianchi L, Gudat F. Immunopathology of hepatitis B. In: Popper H, Shaffner F, eds. Progress in liver disease, Vol VI. New York: Grune and Stratton, 1979: 371–392.
31. Gudat F, Bianchi L. Evidence for phasic sequences in nuclear HBcAg formation and cell membrane-directed flow of core particles in chronic hepatitis B. Gastroenterology 1977; 73:1194–1197.
32. Goudeau A, Maupas P, Coursaget P, et al. Hepatitis B virus antigens in human primary hepatocellular carcinoma tissue. Int J Cancer 1979; 24:421–429.