JOURNAL OF CLINICAL MICROBIOLOGY, May 1980, p. 458-461 Vol. 11, No. 5
0095-1137/80/05-0464/04$02.00

Hepatitis B e Antibody Determination: Comparison and Evaluation of Four Different Methods

JAN ALDERSHVILE,[1]* BRUNO CASPANI,[2] AND GERT G. FRÖSNER[2]

Division of Hepatology, Hvidovre Hospital, University of Copenhagen, Second Department of Medicine, Kommunehospitalet, Copenhagen, Denmark, and Max von Pettenkofer-Institut, University of Munich, Federal Republic of Germany[2]

A radioimmunological neutralization assay for the determination of hepatitis B e antibody is described. The method is compared with a blocking and a competitive assay and with immunodiffusion. The neutralization assay proved to be the most sensitive method, and results obtained by this method were reproduced most easily. The frequency of nonspecific results may be up to 1% of the sera tested. Among patients with acute type B hepatitis, e antibody was found regularly soon after the clearance of hepatitis B e antigen by using the neutralization assay, whereas this antibody was found on the average several weeks later and in a lower percentage of patients by using the other methods. It is concluded that at present the neutralization assay is the method of choice.

Since the discovery of the hepatitis B e antigen-antibody system in 1972 (6, 7), this system has received increasing interest due to its obvious biological and clinical importance. However, the determination of hepatitis B e antigen (HB_eAg) and antibody (anti-HB_e) has been hampered by the relatively insensitive methods used but, recently, highly sensitive methods have been developed (2, 9). By means of these tests, HB_eAg is regularly found in acute type B hepatitis (1, 2, 5), and the persistence of this antigen for more than 10 weeks after onset of symptoms indicates a chronic HB_sAg carrier state with high risk for development of chronic liver disease (1, 10).

Previously, anti-HB_e was only found regularly in asymptomatic healthy hepatitis B surface antigen (HB_sAg) carriers (4, 8), and the presence of this antibody was associated with relatively low or non-infectivity of serum (3, 11). Further, the presence of anti-HB_e was considered to have some prognostic value in patients with chronic type B hepatitis. Therefore, sensitive and specific methods for the determination of this antibody seem important.

In the present study, a neutralization assay for the determination of anti-HB_e is described and compared to three other methods for anti-HB_e determination. Sera from patients with acute type B hepatitis were used for evaluation.

MATERIALS AND METHODS

A total of 28 consecutive patients with biopsy-verified acute hepatitis and circulating HB_sAg in serum at the time of admittance to the hospital were included in the study. From 24 of these patients, weekly serum samples were available for the first 4 weeks after admittance, and from 27 patients serum samples were available from later follow-up examinations.

Further serum samples from 224 healthy staff members, all HB_sAg negative, were tested.

HB_sAg, anti-HB_s, and anti-HB_c. HB_sAg and antibody (anti-HB_s) and antibody to the hepatitis B core antigen (anti-HB_c) were determined by solid-phase radioimmunoassays (Ausria II, Ausab and Corab, Abbott Laboratories, North Chicago, Ill.).

HB_eAg. HB_eAg was determined by a solid-phase radioimmunoassay (2) and by immunodiffusion (2).

Anti-HB_e. The following four methods were used.

(i) Neutralization assay. For the neutralization assay, an HB_eAg-positive serum of human origin was used as an HB_eAg source. The HB_eAg titer of this serum was between 1:800 and 1:1,600, determined by radioimmunoassay. For the anti-HB_e determination, 100 μl of a predetermined dilution (1:150) of the HB_eAg-positive serum was incubated overnight with 100 μl of the serum to be tested. The amount of HB_eAg in the initial incubation was selected to give specific binding of 5,000 to 10,000 cpm of ^{125}I-labeled anti-HB_e immunoglobulin G in the test when incubated with a serum negative for anti-HB_e. After the first overnight incubation, an anti-HB_e–coated bead was added to the mixture. After a second overnight incubation, the bead was washed and incubated overnight with 200 μl of ^{125}I-labeled anti-HB_e immunoglobulin G preparation. All incubations were carried out at room temperature. The bead was washed, and the bound radioactivity was counted. All serum samples that neutralized more than 50% of the predetermined quantity of HB_eAg were considered positive for anti-HB_e.

(ii) Blocking assay. For the blocking assay, 200 μl of the serum to be tested was incubated overnight with an HB_eAg-coated bead. After being washed, the bead was incubated overnight with 200 μl of ^{125}I-labeled anti-HB_e immunoglobulin G preparation. After a second washing, the bound radioactivity was

counted. The method has been described in detail elsewhere (2).

(iii) Competitive assay. For the competitive assay, 20 μl of the test serum, 200 μl of ^{125}I-labeled anti-HB_e immunoglobulin G preparation, and an HB_eAg-coated bead were incubated overnight, washed, and counted. For detail, see elsewhere (2).

(iv) Immunodiffusion. Immunodiffusion was performed as previously described (2).

RESULTS

Sensitivity. Four anti-HB_e-positive sera with different titers in immunodiffusion were selected for the comparison of methods. All tests were run in such a way that they were read on the same day, and the same [^{125}I]anti-HB_e label was used.

All dilutions were run in triplicates called A, B, and C. Endpoint titers of the four serum samples are shown in Table 1. The endpoint titers are given as the last positive dilution before the first negative result was seen in the titration. In the blocking assay, it was sometimes observed that after the dilutions had become negative, they became positive again on further dilution and then finally negative. This phenomenon was only observed very seldom in the competition assay and never in the neutralization assay and may be due to a greater variation in the test reproducibility.

Generally, the neutralization test was the most sensitive method, followed by the blocking test, the competition test, and finally the immunodiffusion test. If the sensitivity of immunodiffusion was considered to be 1, the sensitivity of the competition test was about 10 times higher, that of the blocking test was about 150 times higher, and that of the neutralization test was about 250 times higher.

Reproducibility. The same four serum samples were run in the same way as described above on another day with a [^{125}I]anti-HB_e label made on another day (Table 2). With the exception of the blocking test, all results showed good agreement between the two runs.

Correlation with other hepatitis B virus markers. Serum samples from 224 healthy staff members were tested for anti-HB_s, anti-HB_c, and anti-HB_e by both the blocking and neutralization assays. A total of 64 staff members were found to have anti-HB_s, anti-HB_c, or both in serum, and 32 of these had anti-HB_e by both the blocking and the neutralization assays. By the blocking assay an additional one, and by the neutralization assay an additional eight, of the 64 staff members were found to have anti-HB_e. Thus, agreement between the two tests was found in 86% of the 64 staff members, but the neutralization method was significantly ($P < 0.05$; the sign test) more sensitive than the blocking method. In the serum of four additional staff members without other hepatitis B virus markers, anti-HB_e was found in two by the blocking assay and in two by the neutralization assay. The specificity of these results is therefore unclear.

Anti-HB_e in acute hepatitis B. Weekly serum samples taken during the first 4 weeks after admittance of the patients to the hospital were available from 24 of the 28 patients with circulating HB_sAg and biopsy-verified acute hepatitis. The development of anti-HB_e in relation to time, as determined by three different methods, is shown in Table 3. Generally, anti-HB_e was

TABLE 1. *Endpoint anti-HB_e titers as determined by four different methods*[a]

Serum	Test	Titer			
		Immunodiffusion	Competition	Blocking	Neutralization
458339	A	1:2	1:20	1:500	1:500
	B	1:2	1:20	1:500	1:500
	C	1:2	1:40	1:200	1:500
433836	A	1:4	1:40	1:200	1:1,000
	B	1:4	1:20	1:200	1:1,000
	C	1:4	1:40	1:200	1:1,000
448768	A	1:12	1:40	1:500	1:2,000
	B	1:12	1:40	1:4,000	1:2,000
	C	1:12	1:40	1:500	1:2,000
MWW	A	1:24	1:320	1:4,000	1:6,000
	B	1:24	1:320	1:4,000	1:4,000
	C	1:24	1:160	1:4,000	1:4,000

[a] All dilutions were run in triplicates designated A, B, and C.

TABLE 2. *Reproducibility of endpoint anti-HB_e titers as determined by four different methods (compare with Table 1)*[a]

Serum	Test	Titer			
		Immunodiffusion	Competition	Blocking	Neutralization
458339	A	1:2	1:40	1:1,000	1:1,000
	B	1:2	1:20	1:500	1:1,000
	C	1:2	1:40	1:500	1:1,000
433836	A	1:4	1:20	1:500	1:1,000
	B	1:4	1:40	1:500	1:1,000
	C	1:4	1:40	1:500	1:1,000
448768	A	1:12	1:40	1:1,000	1:2,000
	B	1:12	1:40	1:1,000	1:2,000
	C	1:12	1:40	1:500	1:2,000
MWW	A	1:24	1:160	1:4,000	1:4,000
	B	1:24	1:320	1:8,000	1:4,000
	C	1:24	1:160	1:4,000	1:4,000

[a] All dilutions were run in triplicates designated A, B, and C.

TABLE 3. *Development of anti-HB_e in 24 patients with acute viral hepatitis*

Serum	Test	No. of patients showing positive reaction/total no. tested				
		0–7[a]	8–14	15–21	22–28	>28
HB_sAg-positive		24/24	22/24	20/24	16/24	5/24
HB_eAg-positive	Radioimmunoassay	14/24	4/24	2/24	2/24	1/24
	Immunodiffusion[b]	2/24	2/24	1/24	1/24	1/24
Anti-HB_e-positive[c]	Neutralization	4/24	7/24	13/24	17/24	18/24
	Blocking	0/24	0/24	1/24	3/24	12/24
	Immunodiffusion	0/24	0/24	1/24	1/24	2/24
No HB_e markers		6/24	13/24	9/24	5/24	5/24

[a] Days after admission to the hospital.

[b] These patients were also HB_eAg positive by radioimmunoassay.

[c] The patients positive for anti-HB_e by immunodiffusion were also positive by both blocking and neutralization methods. Patients positive for anti-HB_e by blocking were also positive by the neutralization assay.

detected by the neutralization test more frequently and much earlier in the course of the disease than it was by the blocking test. Among the remaining 4 of the 28 patients, 1 was followed for 3 weeks and was at that time anti-HB_e positive only, by the neutralization assay. The other three patients were followed from 5 to 12 months. Of these, the first available serum sample from one patient had no HB_e markers, whereas samples from the two others were anti-HB_e positive only by the neutralization test. In the last available serum sample, two patients were positive for anti-HB_e by both the blocking and neutralization assay, whereas one was positive only by the neutralization assay.

DISCUSSION

In the clinical evaluation of patients with hepatitis B virus infections, the HB_e system seems to be important due to its value in evaluation of infectivity and its probable prognostic value.

In the present report, three different radioimmunological methods and immunodiffusion were compared for the determination of anti-HB_e. The neutralization assay was the most sensitive followed closely by the blocking test and then by the competitive test and immunodiffusion. Further, the neutralization assay was the most reproducible, showing maximally a twofold variation in the titers. The greater bead-to-bead variation found for the competition and blocking assays was probably due to the more difficult double coating of the beads (first with anti-HB_e, then with HB_eAg) used in these tests. Therefore, at present the neutralization assay seems to be the method of choice.

It has been reported previously that the neutralization assay is about 6,000 times more sensitive than rheophoresis (9), an increase in sensitivity which was not found in this study when the neutralization procedure was compared to immunodiffusion.

Among the 224 healthy staff members, 64 had circulating anti-HB_s, anti-HB_c, or both and, of these, 62.5% were found to be positive for anti-HB_e in the neutralization assay compared to 51.5% in the blocking assay. This difference is probably due to the difference in sensitivity between the two methods, as all persons except one positive by the blocking assay were also positive by the neutralization assay. Among four sera negative for other hepatitis B virus markers, two were positive for anti-HB_e by the neutralization assay and two by the blocking assay. Whether these four sera were nonspecifically positive is not known. The frequency of nonspecific results obtained with each method could thus be as high as 1%.

Among patients with acute type B hepatitis, anti-HB_e was found regularly soon after the clearance of HB_eAg when the neutralization test was used. Using the blocking method, anti-HB_e was demonstrable later and only in about one-half of the patients. Previously, anti-HB_e has been found in a varying number of patients with acute type B hepatitis (2, 10), and this variation may not only be caused by a difference in sensitivity between the methods used but also by factors such as the avidity of the antibody, which may belong in part to the immunoglobulin M class.

In conclusion, we believe that the neutralization anti-HB_e method is the method of choice until purified HB_eAg and specific anti-HB_e sera of animal origin become available.

ACKNOWLEDGMENTS

This study was supported by the Danish Medical Research Council (no. 512-10225, 512-10136), Københavns Kommune-

hospitals 100 års jubilæumsfond, Ebba Celinders Foundation, Købmand i Odense Johann og Hanne Weimann f. Seedorff's Foundation, and the Deutschen Forschungsgemeinschaft (Fr. 400/6). Patients with acute type B hepatitis were included in the Copenhagen Hepatitis Acuta Programme.

LITERATURE CITED

1. **Aldershvile, J., G. G. Frösner, J. O. Nielsen, F. Hardt, F. Deinhardt, P. Skinhøj, and the Copenhagen Hepatitis Acuta Programme.** 1980. Hepatitis B e antigen and antibody measured by radioimmunoassay in acute HBsAg positive hepatitis. J. Infect. Dis. February issue.
2. **Frösner, G. G., M. Brodersen, G. Papaevangelou, U. Sugg, H. Hass, I. K. Mushahwar, C. M. Ling, L. R. Overby, and F. Deinhardt.** 1978. Detection of HBeAg and anti-HBe in acute hepatitis B by a sensitive radioimmunoassay. J. Med. Virol. **3**:67-76.
3. **Grady, G. F., U. S. National Heart and Lung Institute Collaborative Study Group and Phoenix Laboratories Division, Bureau of Epidemiology, Center for Disease Control.** 1976. Relation of e antigen to infectivity of HBsAg-positive inoculations among medical personnel. Lancet **ii**:492-494.
4. **Hess, G., J. O. Nielsen, W. Arnold, and K. H. Meyer zum Büschenfelde.** 1977. e-System and intrahepatocellular HBcAg and HBsAg in HBsAg positive patients with liver disease and healthy carriers. Scand. J. Gastroenterol. **12**:325-330.
5. **Krugman, S., L. R. Overby, I. K. Mushahwar, C. M. Ling, G. G. Frösner, and F. Deinhardt.** 1979. Viral hepatitis, type B. New Engl. J. Med. **300**:101-106.
6. **Magnius, L. O., and Å. Espmark.** 1972. A new antigen complex co-occurring with Australia antigen. Acta Pathol. Microbiol. Scand. Sect. B **80**:335-337.
7. **Magnius, L. O., and Å. Espmark.** 1972. New specificities in Australia antigen positive sera distinct from the Le Bouvier determinants. J. Immunol. **109**:1017-1021.
8. **Magnius, L. O., A. Lindholm, P. Lundin, and S. Iwarson.** 1975. A new antigen-antibody system. J. Am. Med. Assoc. **231**:356-359.
9. **Mushahwar, I. K., L. R. Overby, G. Frösner, F. Deinhardt, and C. M. Ling.** 1978. Prevalence of hepatitis B e antigen and its antibody as detected by radioimmunoassays. J. Med. Virol. **2**:77-87.
10. **Norkrans, G., G. Frösner, and S. Iwarson.** 1979. Determination of HBeAg by radioimmunoassay: prognostic implications in hepatitis B. Scand. J. Gastroenterol. **14**:289-293.
11. **Okada, K., I. Kamiyama, M. Inomata, M. Imai, Y. Miyakama, and M. Mayumi.** 1976. e Antigen and anti-e in serum of asymptomatic carrier mothers as indicator of positive and negative transmission of hepatitis B virus to their infants. N. Engl. J. Med. **294**:746-749.