Serum Hepatitis B Antigen Particles in Various Liver Diseases

Relation to Immunological Tests for Hepatitis B Antigen

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The morphological form of the hepatitis B antigen (HBAg) in the serum of patients with hepatitis B antigenemia is known at present to be of three types when classified electron-microscopically. Initially, Bayer et al.[1] reported 20 nm spherical particles and tubular forms of varying length, and subsequently Dane et al.[2] reported large 42 nm double shelled particles (Dane particles) with a 27 nm electron-dense inner core. It has also been known that these particles have a common antigenicity with one another. On the other hand, HBAg is generally detected by observing the immune reaction between the antibody to HBAg and HBAg, and the electron-microscopic detection of these particles is exceptional. While various immunological techniques for detecting HBAg have been divised[3-9], at present radioimmunoassay technique[10,11] is considered to be most sensitive. However, it has recently been pointed out that it is not sufficient to detect all infectious donors even by radioimmunoassay[12-16] and the specificity of this method itself is also presenting a problem[17,18,19].

Under such circumstances, we detected the HBAg particles in the serum of patients with various liver diseases by electron microscopy, and studied the results in relation to those obtained by radioimmunoassay for HBAg. In addition, we investigated the incidence of HBAg in various liver diseases using electron-microscopic method.

MATERIALS AND METHODS

The search for the antigen particles was made in 196 serum samples obtained from a total of 183 cases including 58 of acute hepatitis, 8 of prolonged hepatitis, 60 of chronic hepatitis, 30 of liver cirrhosis, 21 of hepatoma and 6 of fulminant hepatitis.

Counterelectrophoresis[4] and solid-phase radioimmunoassay[11] were employed as immunological methods. The electron microscopy was performed by the method described by Almeida and Waterson[20] with minor modification. To 1 ml of the serum the same volume of phosphate-buffered saline (P.B.S) was added, and this was centrifuged at 18,000 r.p.m. for two hours. The pellet so obtained was suspended in 2 ml of P.B.S., and centrifuged again at 18,000 r.p.m. for two hours. The resultant pellet was resuspended in 0.2 ml of distilled water. A drop of this suspension was then mixed with an equal volume of 3% phosphotangstic acid adjusted to pH 6.0, and after mounting the mixture on a 400-mesh carbon/formvar grid it was examined in a JEM 100 KV Scanning-Transmitting Type electron microscope at a magnification of 90,000.

Apart from the above, as a control, electron microscopic observations were performed in the cases previously ascertained by counterelectrophoresis as positive for HBAg which included 6 of acute hepatitis and 5 of chronic hepatitis, and in the cases as negative for HBAg which included 10 of normal subjects and 5 of obstructive jaundice. In addition, some serum samples were used for observing the formation of antigen-antibody complexes. To 0.25 ml of serum sample was added 0.05 ml of anti-HBAg rabbit serum (available from Behringwerke Corp.), and the mixture was incubated for 1 hour at 37°C and then left at 4°C overnight. After this it was observed by the method described above.

RESULTS

HBAg particles were observed as reported hitherto[1,2,21] in all of the 6 cases of acute hepatitis and the 5 cases of chronic hepatitis which were confirmed to be positive for HBAg by counterelectrophoresis previously. These particles could be seen either singly or in aggregates of varying size (Fig. 1, 2, and 3). The single particles could be aggregated by adding anti-HBAg serum, and the particle aggregates were essentially similar to naturally occurring aggregates. The particles could be seen in none of the 10 cases of normal subjects and the 5 cases of obstructive jaundice. In the 6 cases of acute hepatitis the number of the particles was reduced gradually and in parallel with clinical improvement. In 4 of the 6 cases the particles disappeared within one month after HBAg became undetectable by radioimmunoassay, whereas the remaining 2 cases exhibited small quantities of the particles even at ten months after HBAg became undetectable by radioimmunoassay (Fig. 4).

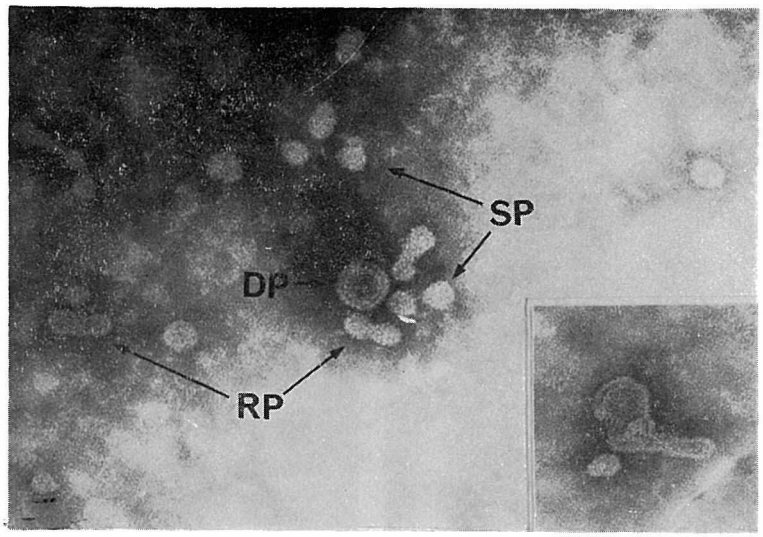

Fig. 1. Electron micrograph showing 20 nm spherical particles (SP) and rod-like (tubular) particles (RP), and 42 nm Dane particles (DP).
Note Dane particle with a tail-like projection similar to rod-like form in inset. ×180,000

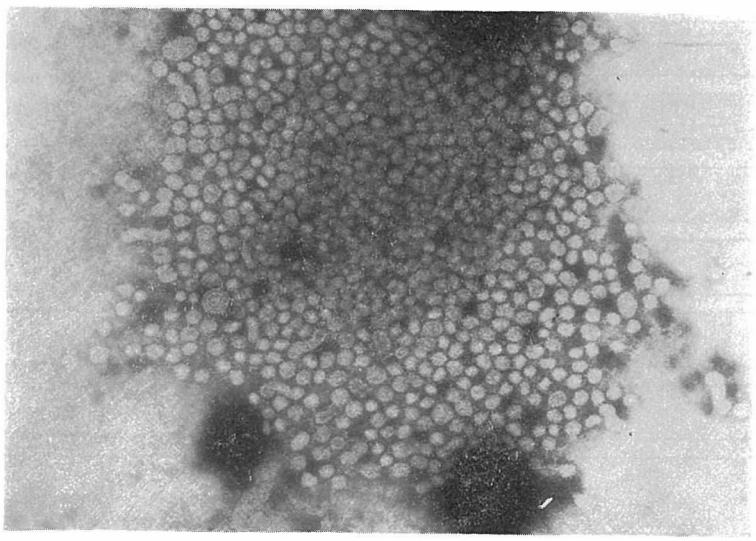

Fig. 2. Electron micrograph showing a particle aggregate consisting mainly of 20 nm spherical particles. ×87,500

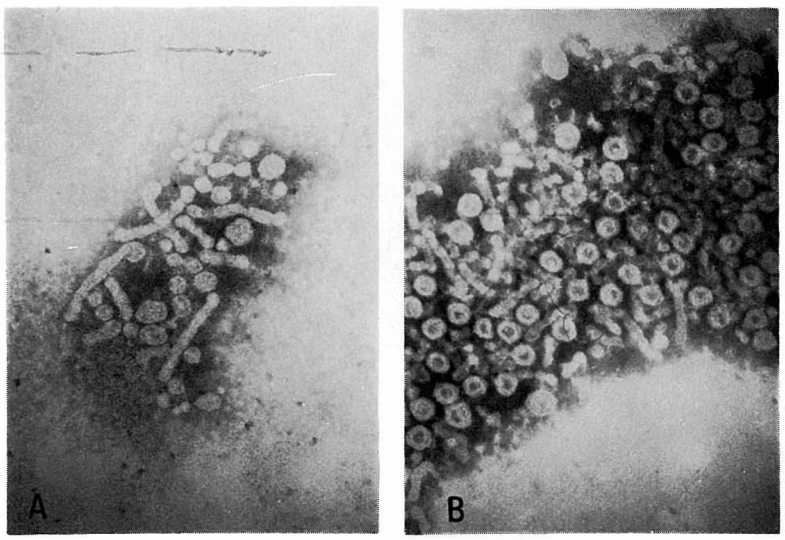

Fig. 3. Electron micrographs showing particle aggregates.
A) Aggregate showing a random distribution of three morphological forms of HBAg. ×84,000
B) Aggregate consisting predominantly of Dane particles and tubular particles. ×90,000

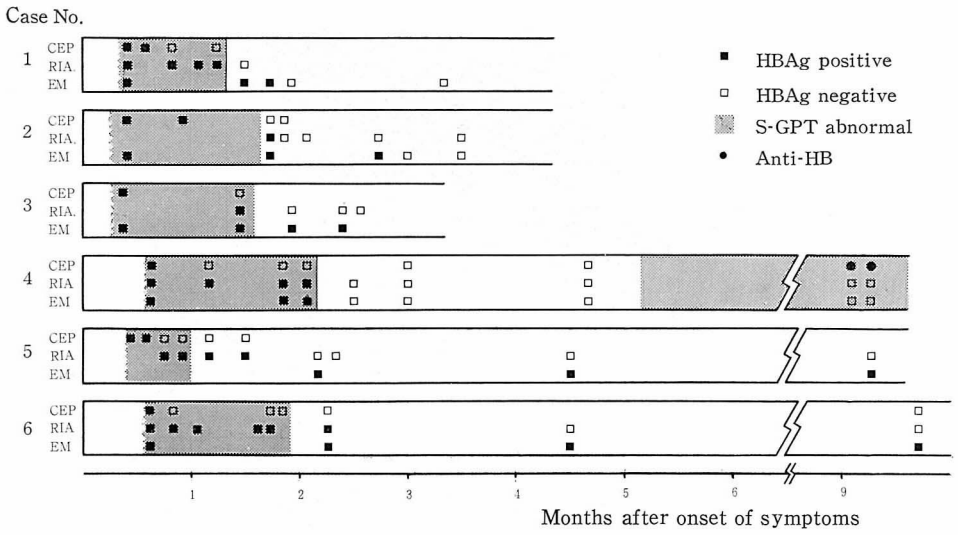

Fig. 4. Comparison of the Duration of HBAg Positivity by Counterelectrophoresis (CEP), Radioimmunoassay (RIA) and Electron Microscopy (EM) in 6 Patients with Acute Hepatitis

The relation between the HBAg determined by radioimmunoassay and the HBAg paticles detected by electron microscopy is shown in Table 1. Positive reaction by radioimmunoassay was seen in 86 of the 196 serum samples, and HBAg particles were not observed in 3 of the 86 serum samples. On the other hand, 115 of the 196 samples exhibited the particles, and 32 of the 115 samples were negative for HBAg by radioimmunoassay. The discrepancy between the results from radioimmunoassay and those from electron microscopy was seen in 19.9% of the total samples.

When the particles are observed in the samples negative for HBAg by radioimmunoassay, and all these particles are those of the 20 nm spherical particles and exist singly, the differentiation of the particles from certain lipoproteins may be morphologically difficult. Therefore, in the present study, various concentrations of anti-HBAg rabbit serum were added to such serum samples, and the samples which exhibited a particle agglutination were regarded as positive for HBAg particle. Of 32 serum samples which were negative for radioimmunoassay but showed the particles, 24 contained only single particles, 7 contained single particles and aggregates, and 1 contained only aggregates. The serum samples which were positive for radioimmunoassay but did not exhibit the particles were reexamined after adding anti-HBAg rabbit serum, and the particles were not seen either singly or in aggregates.

Table 2 represents the incidences of HBAg in various liver diseases by counterelectrophoresis, radioimmunoassay and electron microscopy. The incidence of positive HBAg determined by electron microscopy was recorded at 63.7% (37/58) for acute hepatitis, at 60.0% (36/66) for chronic hepatitis including 66.6% (26/39) for aggressive form, at 50.0% (15/30) for liver cirrhosis and at 71.4% for hepatoma, and these values were more than 10% higher than those obtained by radioimmunoassay in all of the diseases. In addition, in view of classification by disease, hepatoma tended to show a high incidence.

Table 1. Correlation of HBAg by Radioimmunoassay with HBAg Particles by Electron Microscopy

HBAg by radioimmunoassay	HBAg particles by electron microscopy		Total
	Positive	Negative	
Positive	83	3	86
Negative	32	78	110
Total	115	81	196

Table 2. Frequency of HBAg by Counterelectrophoresis, Radioimmunoassay and Electron Microscopy among Patients with Liver Diseases

Patient group	No. of cases	Counterelectro-phoresis	Radioimmuno-assay	Electron microscopy
Acute hepatitis	58	24(41.3)	30(51.7)	37(63.7)
Prolonged hepatitis	8	3	5	6
Chronic hepatitis	60	23(38.2)	29(48.2)	36(60.0)
Chr. persistent hepatitis	21	8(38.1)	8(38.1)	9(42.7)
Chr. aggrgssive hepatitis	39	15(38.4)	21(53.8)	26(66.6)
Liver cirrhosis	30	7(23.3)	12(40.0)	15(50.0)
Hepatoma	21	7(33.3)	11(52.3)	15(71.4)
Fulminant hepatitis	6	0	1	2

( ), %

DISCUSSION

In the results of our present study, considerable discrepancy was found between the results from radioimmunoassay for HBAg and those from electron-microscopic observation of HBAg particles. It was noteworthy facts that the serum samples which were negative for HBAg by radioimmunoassay but exhibited the particles electron-microscopically were found in 16.3% (32/196) of the total serum samples. The failure to demonstrate HBAg from the particle positive samples by immunological techniques may partly be attributable to the presence of antigen-antibody complexes. While the antigen-antibody complex is observed morphologically as an aggregation of the particles[20], the 32 serum semples which were negative for HBAg by radioimmunoassay but exhibited the particles were investigated in this respect, and the particle aggregates were observed only in 8 of 32 samples. Hence, the majority of these serum samples were found to contain too small amount of HBAg to be detected by radioimmunoassay technique. Recently the occurrence of Type B hepatitis has been reported in some of the recipients of blood which was negative for HBAg by radioimmunoassay[12,14,16]. These facts indicates that current immunological tests for HBAg, including radioimmunoassay, cannot detect all HBAg-containing sera and are not adequate to detect all sera from the donors capable of transmitting HBAg-positive hepatitis. While at present nothing is more sensitive than radioimmunoassay as a practical immunological technique for detecting HBAg, the test to detect the antibody to the core component of Dane particle has been noted recently as an additional test

for detecting hepatitis B virus-containing serum, missed by radioimmunoassay for HBAg. Being detected in almost all of sera positive for HBAg by radioimmunoassay and also in part of sera negative for HBAg by radioimmunoassay, this is said to be a more sensitive indicator of virus replication than radioimmunoassay for HBAg[16,22].

83 of the 86 serum samples positive for HBAg by radioimmunoassay showed HBAg particles, and this indicates that the HBAg circulating in the serum is localized at these particles. In addition, the serum samples which were free of the particles were considered to correspond to the false positive cases as reported by Prince et al.[17] rather than the cases resulting from a technical error.

It is now well known that HBAg is closely associated with the infective agent of long-incubation hepatitis and is detected at considerable frequency in the sera of patients with a variety of chronic liver disease and hepatoma. And an etiological relation between the persistence of HBAg and the development of chronic liver disease and hepatoma has been emphasized by a number of reports[23-31]. The results of our electronmicroscopic study made for investigating the incidence of HBAg in various liver diseases also positively supported a number of the findings described previously. Especially, the 71.4% incidence of HBAg in hepatoma was worthy of note. It is known that acute viral hepatitis with HBAg can in some cases progress through chronic hepatitis and postnecrotic cirrhosis to the development of hepatoma[24,25,27,32], and hepatoma not infrequently occurs in patients with pre-existing liver cirrhosis[33-35]. Therefore, one may predict that the patient with chronic liver disease who are positive for HBAg, especially for both HBAg and α-fetoprotein, may be at greater risk for the development of hepatoma than the patient with chronic liver disease who are negative for HBAg.

SUMMARY

Immunological detection of HBAg and the electron-microscopic observation of HBAg particles were performed on 196 serum samples collected from a total of 183 cases consisting of 58 of acute hepatitis, 8 of prolonged hepatitis, 60 of chronic hepatitis, 30 of liver cirrhosis, 21 of hepatoma and 6 of fulminant hepatitis, and the results obtained by both techniques were studied comparatively. As the result, the discrepancy between both techniques was noted in 19.9% (35/196) of the total serum samples, and almost all of the discrepancy was seen in the serum samples which were negative for HBAg by radioimmunoassay but exhibitid the particles electron-microscopically. The incidence of HBAg in various liver diseases

determined electron-microscopically was recorded at 63.7% (37/58) for acute hepatitis, at 60% (36/60) for chronic hepatitis including 66.6% (26/39) for aggresive form, at 50% (15/30) for liver cirrhosis and at 71.4% (15/21) for hepatoma, and these values were more than 10% higher than those determined by immunological techniques. These results indicated that electron microscopy was a more sensitive method than immunological tests for detecting HBAg and supported the hypothesis that HBAg may play some role in the development of chronic liver disease and hepatoma.

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