Correlations between the detection of e antigen or antibody and electron microscopic pattern of hepatitis B surface antigen (HBsAg) associated particles in the serum of HBsAg carriers

C. TREPO¹, R. G. BIRD², AND A. J. ZUCKERMAN²

From the Lindsley F. Kimball Research Institute of the New York Blood Center, 310 East 67th Street, New York, New York 10021 and the London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London WC1E 7HT, England²

SUMMARY The distribution of HBsAg associated particles, especially the presence of Dane particles, was studied by electron microscopy in coded sera of 68 chronic HBsAg carriers. Results were correlated with the detection of eAg or Ab and clinical diagnosis. Sera from haemodialysis and chronic hepatitis patients showed a high prevalence of e antigenaemia (9/13, 69.2% and 8/19, 42.1%) and Dane particles (11 and 16 respectively, 84%). By contrast, out of 36 chronic asymptomatic carriers of HBsAg, 28 (77.7%) were positive for e antibody but only 1 (2.7%) had eAg. Dane particles were found in 13/36 (36.1%). A statistically significant correlation was observed between the detection of eAg and the presence of Dane particles (94.4%) in the serum. However, Dane particles were still observed in 10/28 (35.7%) of anti-e positive sera. The data suggest that eAg may be linked to complete HB virions.

Previous reports have shown that e antigen (eAg) is frequently detected in the serum of hepatitis B surface antigen (HBsAg) carriers with chronic active hepatitis or undergoing haemodialysis whereas anti-e is associated with the healthy carrier state (Magnius and Espmark, 1972; Eleftheriou *et al.*, 1975; El Sheikh *et al.*, 1975; Feinman *et al.*, 1975; Trepo *et al.*, 1976). Other data also indicate that eAg may be associated with infectivity (Magnius and Espmark, 1972; Magnius *et al.*, 1975; Okada *et al.*, 1976; Skinhøj *et al.*, 1976), detection of hepatitis B core antigen in liver nuclei (Trepo *et al.*, 1976; Murphy *et al.*, 1976), higher titre of HBsAg (Trepo *et al.*, 1976), and a greater proportion of Dane particles in the serum (Nielsen *et al.*, 1974; El Sheikh *et al.*, 1975).

Although many reports have been devoted to the study of HBsAg associated particles by electron microscopy (EM) in acute or chronic hepatitis or in silent HBsAg carriers (Nielsen *et al.*, 1973; Stannard *et al.*, 1973), there have been very few

systematic studies comparing the changes in patterns of particle distribution in various conditions (Almeida, 1972; Couleru *et al.*, 1973; Yamada, 1974; Zuckerman, 1975). None of these was performed under code.

To investigate further the clinical significance of the eAg/Ab system and its relationship with HBsAg associated particles we examined by electron microscopy coded sera from 68 chronic HBsAg carriers and correlated the results with detection of eAg or Ab as well as the clinical status of the patients.

Material and methods

PATIENTS STUDIED

Sera were obtained from 68 subjects, all of whom had been found to be persistent HBsAg carriers for more than a year. Nineteen patients had chronic hepatitis, five with the persistent type and 14 with the active type as defined by an international committee (De Groote *et al.*, 1968). Thirteen HBsAg patients were undergoing chronic haemodialysis for renal insufficiency and 36 were asymptomatic volunteer blood donors with repeatedly normal serum alanine

¹Present address: Pavillon H, Hopital Edouard-Herriot, 69374 Lyon Cedex 2, France

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aminotransferase detected as carriers (CAC) by routine HBsAg screening, performed by reverse passive haemagglutination (Prince *et al.*, 1975). Evidence of liver disease was assessed by routine clinical and laboratory evaluation.

SEROLOGICAL METHODS

The available sera were initially retested for HBsAg by radioimmunoassay (RIA) (Ausria II, Abbott Laboratories). The specificity of all positive results for HBsAg was established by repeating the RIA test after neutralisation with known human anti-HBs positive sera (Prince *et al.*, 1973).

eAg and anti-e were tested in parallel by counter-electrophoresis and immunodiffusion as described by Magnus and Espmark (1972). Counterelectrophoresis was carried out using the same agarose medium as for immunodiffusion with veronal buffer pH 8.6 and a constant current of 10 mA for 45 minutes. Plates were read after 24 hours' incubation at 28°C in a humid chamber.

The specificity of samples positive for eAg was confirmed by immunodiffusion after five-fold concentration of the serum.

A coded serum specimen from each of the 68 HBsAg carriers was sent frozen to the London School of Hygiene and Tropical Medicine for EM studies. Specimens were processed and examined after negative staining by methods which have been described elsewhere (Zuckerman, 1970, 1975).

Results

SEROLOGY FOR eAg AND ANTI-e

Results of eAg and anti-e testing among the various groups of HBsAg carriers are summarised in Table 1. The highest proportion of eAg positivity was found among haemodialysis (69.2%) and chronic hepatitis (42.1%) patients, whereas only one out of 36 asymptomatic blood donors (2.7%) was found positive for eAg; in contrast, anti-e was detected in 28/36 (77.7%) of these but in none of the chronic hepatitis or haemodialysis patients.

ELECTRON MICROSCOPY

Examination by EM of samples from 68 HBsAg

carriers revealed particles characteristic of HBsAg in 53 (Table 2). In the 15 others, very few particles could be seen on first testing and identification was difficult. These samples were therefore retested by immune electron microscopy after incubation with anti-HBs. Eleven of these were definitely found to contain aggregated HBsAg particles and four contained particles of all sizes not identified by EM as hepatitis B antigen.

Six patterns of particle distribution were observed (Table 3):

- 1 scattered 22 nm particles and filaments
- 2 scattered particles including 43 nm Dane particles
- 3 aggregated particles without Dane particles
- 4 aggregated particles with Dane particles
- 5 HBsAg particles demonstrable by immune EM only
- 6 particles which could not be definitely identified as HBsAg

A good correlation was found between the distribution of particles and the clinical diagnosis: scarce 22 nm particles without Dane particles and filaments requiring the use of immune EM for their identification were observed among 13/36 (36.1%) CAC and restricted to them with the exception of one haemodialysis and one chronic hepatitis case. By contrast, Dane particles were also found in 13/36 CAC (36.1%), in 11/13 (84.6%) of haemodialysis, and 16/19 (84.2%) of chronic hepatitis patients.

COMPARISON BETWEEN eAg/Ab DETECTION, EM FINDINGS, AND CLINICAL STATUS

As shown in Table 4, a strong correlation was found between detection of eAg and the presence of Dane particles in the serum. Dane particles were indeed found in 17/18 (94.4%) of eAg positive sera, in 13/22 (59.1%) of sera without eAg or Ab, and in 10/28 (35.7%) of those with anti-e.

With the exception of one haemodialysis case, all cases positive for eAg had easily detectable Dane particles by EM. However, Dane particles were detected in many more instances than was eAg (Tables 1, 3, and 5). Out of 68 HBsAg carriers, 40 (58.8%) had Dane particles in the serum compared with 18 (26.4%) who were found positive for

Table 1 Detection of eAg and anti-e by immunodiffusion and counterelectrophoresis in 68 chronic HBsAg carriers according to their clinical status

Diagnosis	Number of cases	eAg positive (%)	anti-e positive (%)	Neither eAg nor anti-e positive (%)
Chronic hepatitis	19	8 (42.1)	0	11 (57.2)
Haemodialysis	13	9 (69.2)	0	4 (30.7)
Asymptomatic blood donors	36	1 (2.7)	28 (77.7)	7 (19.4)

Table 2 *Electron microscopic findings in various groups of HBsAg carriers*

Electron microscopic findings	Diagnosis (no. studied)		
	Haemodialysis (13)	Chronic hepatitis (19)	Asymptomatic blood donors (36)
Scattered 22 nm and filaments	1	2	8
Scattered 22 nm and filaments and Dane particles	7	15	11
Aggregated 22 nm and filaments	0	0	2
Aggregated 22 nm and filaments and Dane particles	4	1	2
Particles identified only by immune EM	0	0	11
Failure to identify HBsAg	1	1	2

Table 3 *Detection of particles in various groups of HBsAg carriers*

Diagnosis	Number	Number (and percent) of subjects with		
		Dane particles	No Dane particles	Few particles
Haemodialysis	13	11 (84.6)	2 (15.3)	1 (7.6)
Chronic hepatitis	19	16 (84.2)	3 (45.7)	1 (5.2)
Asymptomatic blood donors	36	13 (36.1)	23 (63.8)	13 (36.1)

Table 4 *Correlation between eAg and anti-e and detection of Dane particles in HBsAg carriers*

Serological findings	Proportion with Dane particles detected (%)	
eAg	17/18	(94.4) ¹
Neither eAg nor anti-e	13/22	(59.1)
Anti-e	10/28	(35.7) ¹

¹P < 0.001

eAg. Among the 13 HBsAg carriers negative for both eAg and Ab who had Dane particles, there were 2/13 (15.3%) haemodialysis, 8/19 (42.1%) chronic hepatitis, and 3/7 (42.8%) CAC.

No significant difference in clumping of Dane particles or other particles was observed, either between the different clinical categories of carriers or between those with e or anti-e.

Discussion

The distribution of HBsAg particles, especially the frequency of Dane particles, has been found to be similar in chronic hepatitis and haemodialysis patients but is quite different in CAC (Nordenfelt and Kjellen, 1975). Striking differences in the infectivity of these various types of carriers has been observed (Magnius and Espmark, 1972; Heathcote *et al.*, 1974) and the remarkable abundance of Dane particles in haemodialysis and chronic hepatitis is well documented (Almeida, 1972; Couleru *et al.*, 1973; Yamada, 1974). However, the frequency of detection of Dane particles in CAC

remains controversial. Although absent for some authors, in the serum of CAC with normal liver function tests and histology (Nielsen *et al.*, 1973), Dane particles were still detectable in 28/34 cases of another long-term prospective follow-up study (Holtermüller *et al.*, 1975). Dane particles were detected in 36.1% of CAC in *this* series, a proportion also reported previously (Stannard *et al.*, 1973). Since our results were obtained by EM and not by immune electron microscopy, this is an underestimate of the real frequency of Dane particles in CAC. In this study, the prevalence of eAg in chronic hepatitis and haemodialysis patients and of eAb among asymptomatic blood donors is similar to that reported by Magnius *et al.* (1975). HBsAg particle patterns differed dramatically between eAg and eAb positive chronic HBsAg carriers. Comparison of eAg/Ab detection with EM findings indicates a possible link between eAg and Dane particles. Like others, we observed a statistically significant correlation between the detection of eAg and the presence of Dane particles. Neurath *et al.* (1976) have presented evidence that eAg may be an additional antigen present on the surface of Dane particles and large filaments but absent from the 22 nm particles. As the Dane particles may be the circulating form of hepatitis B virus, this finding could explain the correlations between eAg, presence of Dane particles in the serum, and the apparent greater infectivity of HBsAg carriers with eAg and/or Dane particles (McAuliffe *et al.*, 1976; Neurath *et al.*, 1976).

The prevalence of Dane particles in anti-e positive sera is still controversial. Nielsen *et al.* (1974) failed to detect any Dane particles in those sera but El Sheikh *et al.* (1975) observed a small proportion of Dane particles by immune EM in sera from five out of 27 patients with e antibody, nine of whom had chronic hepatitis. Dane particles were detected in this EM study in 10/28 anti-e positive sera from CAC with repeatedly normal serum alanine aminotransferase values and liver function tests. Agglutinated particles were seen in only two cases. These latter findings do not seem to support the suggestion that eAg could be another surface Ag of the Dane particle. Although it may be speculated that production of anti-e in large excess (Yamada, 1974) and/or of low avidity could preclude aggregation of Dane particles, these discrepancies may more likely reflect differences in antigenic specificity of the e complex (McAuliffe *et al.*, 1976). The existence of defective Dane particles representing a deletion mutant of hepatitis B virus (Gerin *et al.*, 1975) which may lack eAg could be an alternative hypothesis. The detection of Dane particles in a significant proportion of anti-e sera helps to explain why some anti-e HBsAg positive sera may still retain infectivity (Berquist *et al.*, 1976).

The greater proportion of sera with fewer particles among anti-e positive CAC may be explained by the significantly lower HBsAg serum titres observed in such carriers (Trepo *et al.*, 1976).

The quantitative and qualitative correlations found between HBsAg associated particles, eAg or Ab detection, and the clinical status of the carriers are puzzling and call for a specific link of eAg to the complete hepatitis B viron itself. Further studies are needed to clarify the exact relations of the e antigens and antibodies to the morphological forms of HBsAg and clinical or immune status of the host. In the present state of knowledge, the EM may still provide clinically valuable additional information on the potential evolution and contagiousness of hepatitis B infections.

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