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Relation between HBsAg Binding with Polymerized Human Serum Albumin and HBV Replication

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Summary. The binding between hepatitis B surface antigen (HBsAg) and polymerized human serum albumin (poly-HSA) was studied in HBsAg-negative (25) and HBsAg-positive (92) sera by a sensitive enzyme-linked immunosorbent assay technique, and a correlation of binding activity was made with HBe-markers and hepatitis B virus-specific DNA polymerase. The binding could be detected only in HBsAg-positive sera and was found to be independent of the presence of HBe-markers and DNA polymerase activity. Further, binding was noted in significantly higher proportions of sera samples from the patient group compared with the healthy carrier group ($p < 0.01$).

The demonstration of receptors for polymerized human serum albumin (poly-HSA) on hepatitis B surface antigen (HBsAg) particles and hepatocyte membrane documents that poly-HSA plays a vital role in facilitating hepatitis B virus (HBV) entry into hepatocytes by acting as a 'linker molecule' between viral particles and cell membrane. In recent studies, the binding activity between HBsAg and poly-HSA was shown to be maximum in the presence of HBeAg, and either reduced or absent after seroconversion of HBeAg into anti-HBe [1]. However, there are other reports which contradict these findings [2]. Thus the association between binding activity and HBeAg still remains controversial. The present study, therefore, was undertaken to evaluate the binding activity of poly-HSA with HBsAg particles in the presence of HBV-specific DNA polymerase to resolve the debate relating to the degree of binding and viral replication.

Serum samples from 57 patients having HBV infections and 35 healthy HBV carriers were collected and stored at –70° prior to analysis. The patient groups included acute viral hepatitis (22), fulminant hepatitis (17),

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cirrhosis of liver (12) and chronic active hepatitis (6). In addition, serum samples from 25 healthy persons negative for HBsAg and DNA-polymerase activity were also analyzed. In all these samples, HBsAg was tested by the micro-enzyme-linked immunosorbent assay (ELISA) technique [3]. HBeAg and anti-HBe were tested by the EIA-kits from Abbot Laboratories. HBV-specific DNA-polymerase activity was tested by the modified technique of Fang et al. [4]. Polymerized human serum albumin was prepared according to the method of Milich et al. [5]. Poly-HSA with a molecular weight of 500,000 was used in the present study. The binding between HBsAg and poly-HSA was determined by a newly developed ELISA technique using a poly-HSA-coated microtiter plate as the solid phase and anti-HBs-HRPO as conjugate. A brief description of the technique is that a polyvinyl microtiter plate was coated with poly-HSA by adding 100 µl of poly-HSA solution (1 mg/ml) in carbonate buffer (pH 9.6) to each well. After overnight incubation (usually 18–20 h) at room tempeature (25 °C), the plate was washed with phosphate-buffered saline (PBS) containing 0.5% Tween-20 (PBS-T) followed by post-coating with 1% bovine serum albumin. Then, 100 µl of test serum, after 1:10 dilution (unless specified) with PBS, (pH 7.2) was added to each well, incubated at 37 °C for 2h, and the plate was washed. Finally, 100 µl of anti-HBs-HRPO conjugate was added to each well and incubation was continued for 1 h at 37 °; the plate was washed, and the color was produced by adding 100 µl of 0-phenylene diamine solution (0.4 mg/ml) in 0.1 *M* phosphate citrate buffer, (pH 5.0). The reaction was stopped by adding 100 µl of 4 *N* sulphuric acid to each well and absorbance was read at 492 nm. The poly-HSA binding activity was reflected by the optical density (OD) ratio of the test serum to the control blank, where the control blank is the average OD of 25 HBsAg-negative serum samples. Based on the OD ratio values for an additional 20 serum samples from healthy persons, a cutoff value (equivalent to the mean + SD) was calculated and found to be 1.8. Any test serum showing an OD ratio of more than 2.0 was labelled as positive for poly-HSA binding activity. Serum samples with positive binding activity usually showed OD ratios of more than 10.

The ELISA method was found to be highly sensitive and specific. The minimal concentration of HBsAg detected by this technique was 0.1 µg/ml, which is less than that [0.2 µg/ml] reported by Milich et al. [5] using a radioimmunoassay method. The specificity of this method was confirmed by the quantitative inhibition of binding activity by preincubating sera with poly-HSA. Similarly, addition of anti-HBs before incubation with anti-HBs-HRPO conjugate suppressed the binding activity. However, pretreatment with anti-HBe-positive serum or various other normal human serum components could not suppress the activity.

The binding activity between HBsAg and poly-HSA was detected in HBsAg-positive sera but not in HBsAg-negative sera. The values of OD ratios in relation to HBe-markers in different groups are shown in table I. The difference in OD ratios between $HBeAg^+$ and $anti\text{-}HBe^+$ sera is nonsignificant. Further, the binding activity decreased on diluting serum samples in a dose-dependent manner. Both $HBeAg^+$ as well as $anti\text{-}HBe^+$ sera showed an identical pattern of decrease in binding activity with dilution of sera. The final dilutions with positive binding activity in both $HBeAg^+$ as well as anti-

Table I. Values of optical density ratios in relation to HBe-markers

Group	Number of Sera	OD ratios mean
Healthy-HBsAg carriers		
HBeAg (+)	4	12.2
Anti-HBe (+)	10	11.6
Both (−)	17	14.2
Acute viral hepatitis		
HBeAg (+)	10	42.6
Anti-HBe (+)	12	37.4
Both (−)	22	38.7
Fulminant hepatitis		
HBeAg (+)	6	28.7
Anti-HBe (+)	2	28.7
Both (−)	6	31.9
Cirrhosis of liver		
HBeAg (+)	3	21.8
Anti-HBe (+)	1	18.5
Both (−)	6	21.8
Chronic active hepatitis		
HBeAg (+)	2	22.3
Anti-HBe (+)	0	–
Both (−)	2	18.7
Healthy controls	25	1.8

HBe^+ sera were nearly the same. Binding activity was found to be present irrespective of the presence of HBV-specific DNA polymerase and HBeAg status in serum samples from various HBV-infected patients (table II). Although binding activity could be documented in both HBV-infected patients with liver diseases and in asymptomatic HBV-carriers, it was demonstrated in a higher proportion of patients compared with carriers ($p < 0.01$).

Thus, our data further support the recently established role of poly-HSA in binding, and thus facilitating the attachment of HBsAg particles (and hence HBV) to the hepatocyte membrane. However, the present study could not substantiate earlier reports which demonstrated a high binding activity in the presence of HBeAg. Binding activity was found to be independent of markers of viral replication and could be detected both with and without these markers. In addition, the relative binding activity (OD ratio values) in each disease group, both with and without

Table II. Binding between HBsAg and poly-HSA in HBsAg-positive sera from different groups

Groups	HBeAg-positive		HBeAg/anti-HBe-negative		Anti-HBe-positive	
	DNA-P^+[a]	DNA-P^-	DNA-P^+	DNA-P^-	DNA-P^+	DNA-P^-
Acute viral hepatitis	4/5 (80%)[b]	3/5 (60%)	4/5 (80%)	1/4 (25%)	3/3 (100%)	–
Fulminant hepatitis	3/3 (100%)	1/2 (50%)	2/2 (100%)	4/5 (80%)	4/5 (60%)	–
Cirrhosis	3/5 (60%)	–	2/2 (100%)	1/4 (25%)	–	1/1 (100%)
Chronic active hepatitis	2/4 (50%)	–	1/1 (100%)	0/1 (0%)	–	–
Healthy HBV-carriers	1/5 (20%)	–	1/9 (11.11%)	1/10 (10%)	1/10 (10%)	0/1 (0%)

[a] DNA-P^+, DNA polymerase positive; DNA-P^-, DNA-polymerase negative.
[b] Values in parentheses show the percent positivity of poly-HSA binding.

HBe-markers, was noted to be nearly the same. Since the HBsAg level is usually high in the presence of HBeAg compared with anti-HBe, high binding activity in the presence of HBeAg was expected. However, the present data showing identical relative binding with and without HBe-markers contradict the earlier hypothesis that high binding activity in the presence of HBeAg is due to high HBsAg concentration. The ELISA method used in the present study is very sensitive, which is the probable reason for detecting binding activity irrespective of the status of viral multiplication and thus of HBsAg concentration in the sera. Low binding activity in absence of HBeAg, as reported earlier might be due to the less sensitive techniques used. We conclude that the binding activity does not depend on viral replication but on the nature of HBsAg particles. These specific HBsAg particles are possibly large size HBsAg particles with some associated serum proteins.

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