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HEPATITIS B SURFACE ANTIGEN/IGM COMPLEXES: RELATION TO RECEPTORS FOR POLYMERIZED HUMAN SERUM ALBUMIN, HEPATITIS B VIRUS (HBV) DNA POLYMERASE ACTIVITY AND HBV MARKERS

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Summary. — The presence and the level of hepatitis B surface antigen (HBsAg)/IgM complexes were determined in 54 chronic HBsAg carriers in relation to receptors for polymerized human serum albumin (pHSA-R) tested by specific radioimmunoassay, and to hepatitis B virus-DNA polymerase (HBV-DNAp). HBsAg/IgM complexes, correlated significantly with the HBsAg concentration but, at a similar HBsAg concentration, significant highest values of HBsAg/IgM complexes were found among HBeAg positive patients. In addition, a significant correlation was found between HBsAg/IgM complex levels, HBeAg titres and HBV-DNAp activity ($r = 0.628$, $p < 0.001$ and $r = 0.559$, $p < 0.001$, respectively). Moreover, a positive linear correlation was found when comparing HBsAg/IgM complexes and pHSA-R levels ($r = 0.848$, $p < 0.001$). Patients who were positive for HBsAg/IgM complexes had a significantly higher glutamate-pyruvate transaminase (GPT) level than those who did not show any complexes. In conclusion, HBsAg/IgM complexes seemed to be indirectly related to HBV replication.

Key words: HBsAg/IgM complexes; polymerized human serum albumin receptors; hepatitis B virus infection; HBV-DNA polymerase

Introduction

The presence of complexes between HBsAg and IgM (HBsAg/IgM) has been recently described in acute hepatitis B virus (HBV) infection and used as prognosis indicator of chronic course of the disease (Careoda *et al.*, 1982; Toti *et al.*, 1983). It has been suggested that the IgM coupled with HBsAg could be an anti-polyalbumin antibody bound to HBsAg through the polymerized human serum albumin (pHSA) molecule (Alberti *et al.*, 1984; Palla *et al.*, 1983).

Several reports have demonstrated the presence of polymerized human serum albumin binding activity, the so-called pHSA receptor (pHSA-R) in HBV serum particles (Hansson and Purcell, 1979; Imai *et al.*, 1979; Neurath and Strick, 1979; O'Neill, 1979). In addition, pHSA-R has been also described in human hepatocytes (Trevisan *et al.*, 1982; Thung and Gerber, 1983*a*). The pHSA binding sites could be involved in the attachment of HBV to hepatocytes (Thung and Gerber, 1983*b*). The presence of pHSA-R was specially associated with chronic HBsAg carriers who were positive for HBeAg (Thung and Gerber, 1981; Alberti *et al.*, 1982; Pontisso *et al.*, 1983). Recent studies show that this receptor forms a structural part (peptide p31) of the HBsAg molecule (Machida *et al.*, 1983). In addition, pHSA-R are related to high infectious inocula that are able to transmit HBV infection (Masuko *et al.*, 1985). Recently, Craxi *et al.* (1985) showed a close relation between pHSA-R, HBsAg/IgM complexes, HBsAg concentration and the expression of HBeAg in chronic HBsAg carriers. However, the possible relationship between HBsAg/IgM complexes, pHSA-R levels and the degree of HBV replication is still not clear. The aim of this study was to determine HBsAg/IgM complexes and pHSA-R in chronic HBV carriers, in relation to viral replication.

Materials and Methods

Patients. A total of 54 HBsAg-positive chronic carriers was included into the study: 41 had chronic active hepatitis (CAH), 8 chronic persistent hepatitis (CPH), and 5 liver cirrhosis (LC); all were histologically proven conditions. Serum samples for the serological tests were taken at the time of biopsy and stored at −20 °C; the patients were not treated prior to admission. The epidemiological, clinical and biochemical data of the patients under study are summarized in Table 1.

HBV-markers. HBsAg, HBeAg, antiHBs, antiHBc and antiHBe were determined by solid-phase radioimmunoassay (Abbott Laboratories, North Chicago, Ill.). HBsAg concentration was calculated according to a standard curve obtained with HBsAg of a known concentration (200.000 ng/ml). HBeAg titration was performed using serial dilutions of each serum (1 : 50, 1 : 100, 1 : 500, 1 : 1000, 1 : 2000 and 1 : 5000). HBeAg titre was defined as the highest dilution that was still positive by RIA.

The HBV-DNA polymerase (HBV-DNAp) activity was determined following the method of Marion *et al.* (1980). The specificity of the HBV-DNAp determination was confirmed if one sample treated with swine-antiHBs (Nordic Immunological Laboratories, B.V., The Netherlands) showed ≥50 % reduction of the DNAp activity, as compared with a similar untreated sample (Nath *et al.*, 1982).

HBsAg/IgM complexes were detected using a commercial radioimmunoassay (Au-IgM-K, Sorin Biomedica, Saluggia, Italy). The method, its specificity and sensibility have been described in detail elsewhere (Palla *et al.*, 1983; Toti *et al.*, 1983). The amount of HBsAg bound to the solid phase was expressed as a quotient of the cpm of the test sample (S) and the mean cpm of the negative control samples (N): S/N. Values of 2.1 or greater were considered positive for the test.

GPT level was determined using a standard method (SMAC-Technicon, N.Y.) and expressed in IU/ml (normal values: 0—50 IU/ml).

pHSA preparation. The pHSA was prepared according to the method of Lenkei *et al.* (1977). Briefly, 20 mg of HSA (Sigma Chemical Co. St Louis Mo.) were dissolved in 0.9 ml of phosphate-buffered saline (PBS), pH 6.8, to which 0.1 ml of 2.5 % glutaraldehyde was added. The solution was mixed at room temperature for 2 hr, dialyzed against PBS pH 7.4, another 3 hr at 4 °C, and chromatographed on a Sephadex G-200 column (2.6×100 cm) in PBS, pH 7.4. The leading

Table 1. Epidemiological, clinical and biochemical data of chronic HBsAg carriers

No. of patients	Epidemiology No. (%)					
	Acute hepatitis	Transfusion	Household contacts	Parenteral injection	Drug addicts	Unknown
54	5 (9)	4 (7)	9 (17)	13 (24)	2 (4)	21 (39)
		Clinical symptoms No. (%)		GPT (IU/ml) mean ± SEM		
		5 (9)		202.4 ± 37.7		

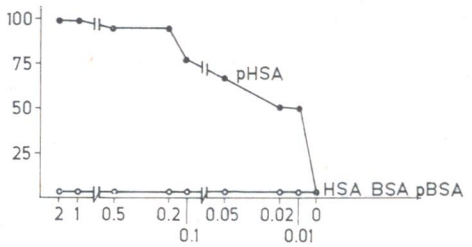

Fig. 1.
Inhibition of pHSA-R activity of serum, following the addition of serial concentrations of human serum albumin (HSA), polymerized HSA (pHSA), bovine serum albumin (BSA) and polymerized BSA (pBSA)
Abscissa: protein concentration (mg/ml); ordinate: % inhibition of pHSA-R activity.

protein peak, containing the albumin polymer, was pooled and adjusted to 4.5 mg/ml in PBS, pH 7.4.

Radioimmunoassay for pHSA-R. Polystyrene beads (diameter 6.5 mm, Northumbria Ltd., England) were incubated with pHSA prepared as described above (100 μg/ml in 0.1 mol/l carbonate buffer pH 9.5) during 4 hr at room temperature with gentle shaking. After three washes with PBS pH 7.4, the beads were additionally coated with 2.5 % PBS-bovine serum albumin (BSA) (Sigma) overnight at 4 °C. The beads were then washed extensively with PBS, air-dried and stored at −20 °C.

Polystyrene beads coated by pHSA and then by BSA were incubated with 200 μl of serum diluted 1 : 100 in 50 % PBS-foetal calf serum (FCS) overnight at room temperature. After three washes with distilled water, 200 μl of ^{125}I-antiHBs (supplied as a part of the Ausria-II kit, Abbott) was added and incubated for 2 hr at 37 °C. The beads were then washed three times each with distilled water and counted in a Gamma counter. The results are expressed as S/N ratio. Sera giving values of 2.1 or greater were regarded as positive in the pHSA-R assay.

Specificity of the pHSA-R assay. In order to demonstrate the specific binding of HBsAg to pHSA-coated beads, an inhibition test was performed using several dilutions of pHSA. In addition, HSA, BSA and polymerized BSA (pBSA), prepared in a similar way as pHSA, were used in the pHSA-R assay.

Statistical analysis. Student's t, Chi-square and linear correlation tests were used for statistical comparisons.

Results

Specific detection of pHSA-R

The specific binding of HBsAg to pHSA-coated beads was blocked by pHSA added to the serum sample in the pHSA-R assay. Concentrations as low as 0.01 mg/ml of pHSA decreased the cpm incorporation by 50 %. This percentage reached 100 % when using 1 mg/ml of pHSA whilst similar or greater concentrations of HSA, BSA or pBSA, added in like manner, did not inhibit the reaction appreciably (Fig. 1).

HBsAg/IgM complexes, pHSA-R and HBV-markers

Patients were divided into three groups according to the serological pattern of HBV-markers:

I —HBeAg positive, HBV-DNAp positive: n = 23
II —HBeAg positive, HBV-DNAp negative: n = 7 and
III—AntiHBe positive, HBV-DNAp negative: n = 24.

HBsAg/IgM complexes were detected in 29 out of 30 (97 %) HBeAg positive patients and only in 7 (29 %) antiHBe positive cases ($p < 0.001$).

Table 2. HBsAg/Ig M complexes, pHSA-R and HBV markers in HBsAg chronic carriers

	HBsAg/IgM complexes[a] + (%)	S/N (mean ± SEM)	pHSA-R[b] + (%)	S/N (mean ± SEM)
GROUP I HBV-DNAp +, HBeAg + n = 23	23 (100)	7.0 ± 0.6	23 (100)	21.6 ± 1.5
GROUP II HBV-DNAp −, HBeAg + n = 7	6 (86)	3.9 ± 1.0	7 (100)	12.4 ± 2.5
GROUP III HBV-DNAp −, antiHBe + n = 24	7 (29)	1.8 ± 0.2	19 (79)	6.0 ± 1.0

a: cut-off value S/N ≧ 2.1

b: cut-off value S/N ≧ 2.1
S: test sample cpm

In addition, the mean value of HBsAg/IgM complexes was significantly higher among patients in group I than those in groups II ($p < 0.005$) and III ($p < 0.001$) (Table 2). A significant correlation between HBsAg/IgM complex levels and HBsAg concentrations was demonstrated ($r = 0.588$, $p < < 0.001$). However, when HBeAg and antiHBe positive patients were classified according to their HBsAg concentration for the purpose of clarifying the influence of HBsAg concentration on HBsAg/IgM complex levels, it was observed that, at a similar HBsAg concentration, HBeAg positive patients had significantly higher HBsAg/IgM complex levels than those who were positive for antiHBe (Table 3). Furthermore, a significant correlation between HBsAg/IgM complex levels and HBeAg titres was also obtained

Table 3. HBsAg/IgM complexes in HBeAg and antiHBe positive patients showing a similar HBsAg concentration

	HBsAg/IgM complexes[a] (ratio S/N) (mean ± SEM)			
HBsAg ng/ml × 10^3 : 0 − 50		51 − 100	101 − 150	≧151
HBeAg posit. (n = 30)	n = 6 4.62 ± 1.52*	n = 8 4.59 ± 0.67**	n = 3 7.20 ± 1.22	n = 13 7.91 ± 0.68
antiHBe posit. (n = 24)	n = 17 1.83 ± 0.27*	n = 7 1.83 ± 0.31**	—	—

a: cut-off value S/N ≧ 2.1
(*): t-test: $p < 0.05$
(**): t-test: $p < 0.005$

S: test sample cpm
N: mean cpm of the negative controls

Table 2 continued

HBsAg concentration ng/ml (mean ± SEM)	HBeAg titre log (mean ± SEM)	HBV-DNAp[c] dpm (mean ± SEM)
206 621 ± 39 283	3.1 ± 0.1	3 170 ± 611
60 357 ± 11 909	2.5 ± 0.3	257 ± 47
33 617 ± 9 715	—	168 ± 14

c: cut-off value ≧ 500 dpm
N: mean cpm of the negative controls

($r = 0.628$, $p < 0.001$). Besides, when considering HBeAg positive patients, the highest values of HBsAg/IgM complexes were found in those who were also positive for HBV-DNAp.

In order to determine the possible relationship between HBsAg/IgM complexes and HBV replication, a linear correlation test with respect to the HBV-DNAp activity was performed. As a result, a significant correlation between both ($r = 0.559$, $p < 0.001$) was observed. The presence of pHSA-R was detected in all HBeAg positive patients in groups I and II, and in 19 out of 24 (79 %) patients in group III ($p < 0.05$). In addition, the mean value of pHSA-R was significantly higher among patients from group I than in those from groups II ($p < 0.005$) and III ($p < 0.001$) (Table 2). The pHSA-R levels correlated significantly with HBsAg concentration ($r = 0.587$, $p < 0.001$), HBeAg titre ($r = 0.468$, $p < 0.01$) and HBV-DNAp activity ($r = 0.471$, $p < 0.001$).

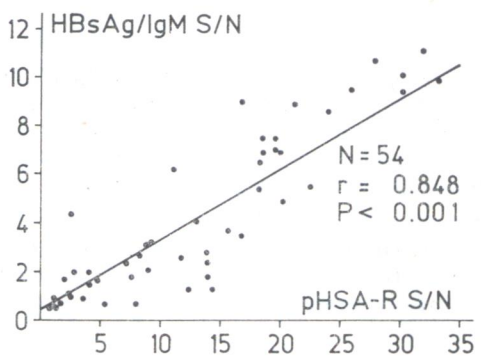

Fig. 2.
Correlation between pHSA-R and HBsAg/IgM complex levels in chronic HBsAg carriers
S/N: quotient of the test sample cpm (S) and the mean cpm of the negative control samples (N).

Table 4. Relation between HBsAg/IgM complexes, pHSA-R and the histological diagnosis of chronic HBsAg carriers

Histological diagnosis	HBsAg/IgM complexes[a] + (%)	HBsAg/IgM complexes[a] S/N (mean ± SEM)	pHSA-R[b] + (%)	pHSA-R[b] S/N (mean ± SEM)
Chronic active hepatitis n = 41	29(71)	4.5 ± 0.5*	37(90)	14.7 ± 1.5**
Chronic persistent hepatitis n = 8	4(50)	4.3 ± 1.3	8(100)	10.9 ± 3.3
Liver cirrhosis n = 5	3(60)	2.5 ± 0.7*	4(80)	7.4 ± 2.2**

[a]: cut-off value S/N $\geq$ 2.1
[b]: cut-off value S/N $\geq$ 2.1
(*): t-test, $p < 0.05$
(**): t-test, $p < 0.005$
S: test sample cpm
N: mean cpm of the negative controls

The comparison between HBsAg/IgM complexes and pHSA-R, in all studied patients, showed a good linear correlation ($r = 0.848$, $p < 0.001$) (Fig. 2).

HBsAg/IgM complexes, pHSA-R and liver damage

The GPT level was significantly higher among HBsAg/IgM positive patients than in those who gave negative results: mean value ± standard error of the mean (SEM): 254.6 ± 57.4 versus 120.2 ± 33.1 IU/ml; $p < 0.05$. In addition, pHSA-R positive patients had higher GPT level than those with negative results: mean value ± SEM: 203.8 ± 41.5 versus 186.0 ± 86.9 IU/ml. However, the latter differences were not of statistical significance.

With respect to the histological diagnosis, HBsAg/IgM complexes and pHSA-R levels were significantly higher in chronic active hepatitis patients than in cirrhotic ones ($p < 0.05$ and $p < 0.005$, respectively) (Table 4).

Discussion

A significantly higher HBsAg/IgM complex level was found among HBeAg positive patients than in those who were positive for antiHBe. This finding suggests that HBsAg/IgM complexes are more frequently detected in patients with an active virus replication. In addition, a significant correlation was also demonstrated with HBeAg titres. Furthermore, HBsAg/IgM complexes and HBV-DNAp activity levels showed good correlation. All these data suggest that the quantity of HBsAg/IgM complexes indirectly correlated with the presence of circulating Dane particles. If this is the case, the HBsAg/IgM complexes probably could be used as an infectivity marker and possibly to control antiviral therapy; although, these aspects require further research.

In our study, a significant correlation between HBsAg concentration and HBsAg/IgM complex levels was demonstrated. This result is similar to that obtained by Craxi *et al.* (1985). In addition, we also found that at similar HBsAg concentration, HBeAg positive patients had significantly higher HBsAg/IgM complex levels than those positive for antiHBe. This result together with the above mentioned relation between HBsAg/IgM complexes and HBV-DNAp, suggests that the IgM complexed with the HBsAg could be an antibody directed against a target in the Dane particle. Taking into account that a close relation between pHSA-R and HBsAg/IgM complexes was observed, it might be supposed that the IgM complex with HBsAg may be an anti-pHSA-R antibody. In a previous study, it has been demonstrated that the staining linear pattern in sheep's red blood cells coated with pHSA is due to an IgM antibody (Mora *et al.*, 1986). This finding could probably be due to an antipolyalbumin antibody or to an anti-receptor antibody cross--reacting with pHSA. Franklin *et al.* (1984) described the presence of IgM antibody against pHSA in sheep sera. Finally, specific blocking of HBsAg/ /IgM by pHSA has been reported by Toti *et al.* (1983).

In the other hand, Troisi *et al.* (1985) recently have demonstrated the presence of a human-IgM-antiidiotype against antiHBs using anti-IgM coated beads and ^{125}I-antiHBs. In this report the existence of HBsAg/IgM complexes was not totally excluded. Our results support this finding since at a similar HBsAg concentration, HBsAg/IgM complexes were significantly more frequently detected among HBeAg-positive patients. This suggests that the IgM could be in part independent of the HBsAg concentration (antiidiotype).

Another interesting finding is the relation between pHSA-R and HBV--DNAp acitvity. This correlation has been demonstrated using specific radioimmunoassay to detect pHSA-R. Our results confirm previous reports in which such relation was demonstrated by haemagglutination (Mora *et al.*, 1986) or enzyme immunoassay (Tsuji *et al.*, 1984). In addition, this finding supports the relation between pHSA-R and the infectivity of HBsAg carriers, reported by Masuko *et al.* (1985).

The highest GPT level detected in patients who were positive for HBsAg/ /IgM complexes or pHSA-R may be probably due to the high level of virus replication of these patients and, thus due to the liver damage that has been described in such situation (Realdi *et al.*, 1980). The fact that the level of HBsAg/IgM complexes or pHSA-R was significantly lower in cirrhotic patients than in those with chronic active hepatitis might be explained by the course of chronic HBV infection, chronic active hepatitis being the first step related to the high level of virus replication. In contrast, cirrhosis might be a later phase in evolution of the disease usually associated with the presence of antiHBe (Realdi *et al.*, 1980).

In conclusion, HBsAg/IgM complexes seem to be related to HBV replication. In addition, the significance of HBsAg/IgM complexes as indirect infectivity marker and their usefulness for antiviral therapy, require further research.

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