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Hepatic Glomerulonephritis

Role of Hepatitis B Surface Antigen (HBsAg)

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Summary. A possible role for HBsAg in hepatic glomerulonephritis was evaluated in kidney specimens from 104 patients with various liver diseases. Huang's method using formalin-fixed and paraffin-embedded sections was applied to the kidney from 65 cases. Only 4 (3.8%) with liver cirrhosis had glomerular HBsAg deposition; 3 on frozen and one on paraffin sections. In one case of crescentic glomerulonephritis, HBsAg appeared to have had a pathogenetic role with glomerular immunofluorescence dominant for IgM, less intense IgG and negative IgA. The glomerular HBsAg in the remaining 3 patients with hepatic IgA glomerulonephritis was likely to be concomitant or superimposed. One of them had a nephrotic syndrome for which corticosteroid treatment was given, resulting in a near-complete remission and disappearance of HBsAg in the glomeruli, but the glomerular immunohistology was unaffected by the treatment. The present findings suggest that HBsAg has only a minor role.

Key words: HBsAg – Hepatic IgA glomerulonephritis – Liver cirrhosis – Formalin-fixed, parraffin-embedded section

Introduction

Hepatitis B surface antigen (HBsAg) has been shown to play a pathogenetic role in immune complex glomerulonephritis (Combes et al. 1971), polyarteritis (Gocke et al. 1970), arthritis (Onion et al. 1971), mixed cryoglobulinaemia (Levo et al. 1977) and urticaria (Dienstag et al. 1978). Recently, HBcAg (Ślusarczyk et al. 1980), and HBeAg (Takekoshi et al. 1979) have also been implicated. Although the associated liver disease is usually chronic active hepatitis (Myers et al. 1973; Knieser et al. 1974; Kohler et al. 1974), its severity ranges from asymptomatic carrier or minimal inflammation (Brzosko et al. 1974; Hirschel et al. 1977) to progressive cirrhosis (Bajtai et al. 1975; Moriyama et al. 1976).

However it is known that chronic liver disease is often accompanied by glomerular lesions, these having been termed hepatic (cirrhotic) glomerulone-

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phritis (Fisher and Perez-Stable 1968) or glomerulosclerosis (Bloodworth and Sommers 1959; Sakaguchi et al. 1965). Immunofluorescent studies have revealed dominant participation of IgA in the form of immune complexes (Manigand et al. 1970; De Werra et al. 1973; Callard et al. 1975; Nochy et al. 1976; Berger et al. 1977). We have also confirmed that the major type of hepatic glomerular lesion is IgA-associated glomerulonephritis. However, there have been few systematic searches of the role of hepatitis B virus antigens in hepatic glomerulonephritis. The present study was undertaken to clarify the significance of HBsAg.

Materials and Methods

A total of 104 kidney specimens from the following liver diseases were examined for glomerular deposition of HBsAg; 12 cases with fulminant or subacute hepatitis, 6 with acute hepatitis, 12 with chronic hepatitis and 74 with liver cirrhosis. The glomerular histology was classified as previously described as (1) no change, (2) mild to moderate mesangial proliferation (A), (3) moderate to marked mesangial proliferation with local circumferential mesangial interposition (CMI) (B), (4) moderate to marked mesangial proliferation with diffuse CMI (MPGN), (5) membranous nephropathy (MN), (6) crescentic glomerulonephritis (RPGN) and (7) diffuse proliferative and exudative glomerulonephritis (AGN).

Fifteen livers were also studied for HBsAg; one with fulminant hepatitis, 4 with chronic hepatitis and 10 with hepatic cirrhosis.

The production of monospecific anti-HBs serum labeled with fluorescein-isothiocyanate (FITC) was supervised by Dr. Hiroshi Yoshizawa (1977). Anti-HBs serum was raised in albino rabbits. The animals were repeatedly immunized with purified HBsAg emulsified with complete Freund adjuvant. After being absorbed with HBsAg-negative human serum insolubilized by 2.5% glutaraldehyde according to Avrameas' method (1969), the antiserum was confirmed to be monospecific by the micro-Ouchterlony method and immunoelectrophoresis. The gamma globulin fraction of antiserum was labeled with FITC by the method of Kawamura (1969), and the resultant dye/protein ratio was 1.21. This FITC-labeled anti-HBs serum when undiluted and diluted to 1:8 gave an exclusively cytoplasmic stain to the known HBsAg-positive and negative liver specimens without false negatives and false positives. The undiluted antiserum was employed for the following staining processes.

39 kidney specimens and 15 liver tissues were snap-frozen in n-Hexane precooled at $-70°$ C in acetone-dry ice bath, then cut at 4 micra in cryostat. In the remaining 65 kidney tissues, paraffin sections were used by the method of Huang (1975). Briefly, after deparaffinizing, the sections were incubated with 0.1% pronase solution at 37° C for 2 h, then washed with phosphate buffered saline for 5 to 12 h. Both unfixed frozen- and paraffin sections were processed for direct staining

Table 1. Glomerular histology in liver disease with examination for glomerular HBsAg

	Glomerular histology[a]							Total
	No change	A	B	MPGN	MN	RPGN	AGN	
Fulminant or subacute hepatitis	12	2	0	0	0	0	0	12
Acute hepatitis	4	2	0	0	0	0	0	6
Chronic hepatitis	7	1	1	0	1	1	1	12
Liver cirrhosis	22	19	24	8	0	1	0	74
			(1)	(2)		(1)		

[a] see text for the abbreviations

( )cases with positive HBsAg in the glomeruli

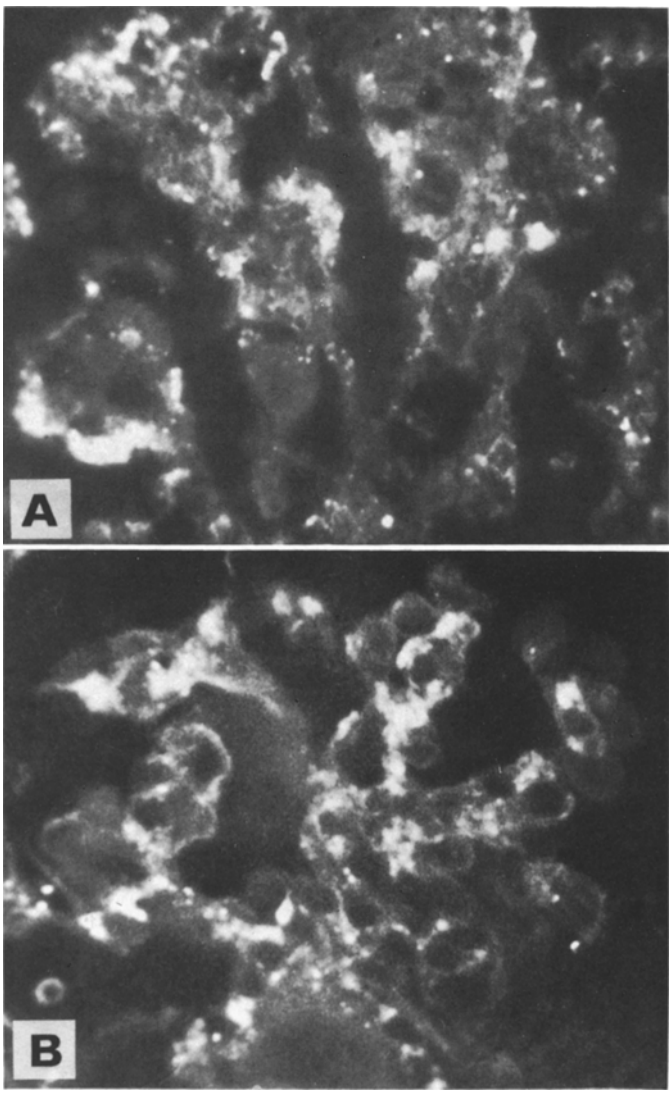

Fig. 1 A, B. Immunofluorescence of HBsAg deposited in the glomeruli from case 1, T.F. 42 years old male (Table 2). **A** unfixed frozen section. **B** formalin-fixed, paraffin-embedded section. HBsAg in a granular fashion distributed from mesangial region to subendothelial space in both photographs. The fluorescence in the paraffin section was slightly less intense

with FITC-labeled anti-HBs serum according to Kawamura (1969). Positive and negative controls were run simultaneously. The former included HBsAg positive liver tissue and renal sections known to be positive for immunoglobulins for a comparison, while the latter included rat, or occasionally normal human, liver and kidney specimens. Positive HBsAg staining was specifically abolished by absorption of FITC-labeled anti-HBs serum with purified HBsAg.

Routine immunofluorescent studies were performed using FITC-labeled monospecific antisera for IgG, IgA, IgM, C_3 and fibrinogen (Behringwerke, West Germany) with positive and negative controls as previously described (Nakamoto et al. 1978).

Table 2. Clinical and pathological findings in four cases with positive HBsAg in the glomeruli

Case No.	Hepatic disease	Nephritic urinary changes	Glomerular pathology	Serum		Intrahepatic HBsAg	Source of renal tissue	Glomerular immunohistology				
				HBsAg	Anti-HBs			IgG	IgA	IgM	C_3	fibrinogen
1. T.F. 42, m	Liver cirrhosis	+	MPGN	+	−	−	1st biopsy	++	+++	++	+++	+
							2nd biopsy	++	+++	+	++	++
2. S.F. 58, m	Liver cirrhosis	+	MPGN	−	−	ND	biopsy	+	++	+	++	+
3. S.S. 16, m	Liver cirrhosis	+	RPGN	+	+	+	autopsy	+	−	++	++	+
4. S.Y. 42, f	Liver cirrhosis	+	moderate mesangial proliferation with local CMI	−	+	ND	biopsy	+	+	+	+	+

ND = not done

HBsAg in the serum was measured by radioimmunoassay (Abbott Laboratories, U.S.A.) and anti-HBs by passive haemagglutination method (Imai et al. 1974).

Statistical analysis was performed by chi-square test or Fisher's exact probability test.

Results

HBsAg in the serum was positive in 28 (50.9%) of 55 subjects examined. Five (33.3%) of 15 liver specimens studied showed the presence of HBsAg in the hepatocytes, and the incidence among seropositive 11 specimens was 45.5%. In contrast, glomerular HBsAg was positive in only 4 (3.8%) out of 104 cases, as shown in Table 1. The incidence of glomerular HBsAg was significantly lower than that in the serum ($P < 0.001$) and liver tissue ($P < 0.01$). Of 4 with positive HBsAg in the glomeruli, 3 patients were detected by the unfixed frozen sections (7.7% among 39 specimens) and the last one by the paraffin sections (1.5% among 65 specimens). This difference was not significant.

The two types of section, the frozen and the paraffin, were made in 13 patients. Two patients positive and 11 negative for glomerular HBsAg by frozen section exhibited the same result by the paraffin sections, although immunofluorescence in the latter was slightly less intense (Fig. 1).

Table 2 presents the details of 4 cases positive for glomerular HBsAg. Case 3 manifested massive proteinuria and rapidly deteriorating renal function. At the same time, elevated levels of hepatic enzymes and positive HBsAg were noted in the serum. The renal biopsy disclosed crescentic glomerulonephritis (RPGN) in which glomerular HBsAg was not sought, nor was liver biopsy

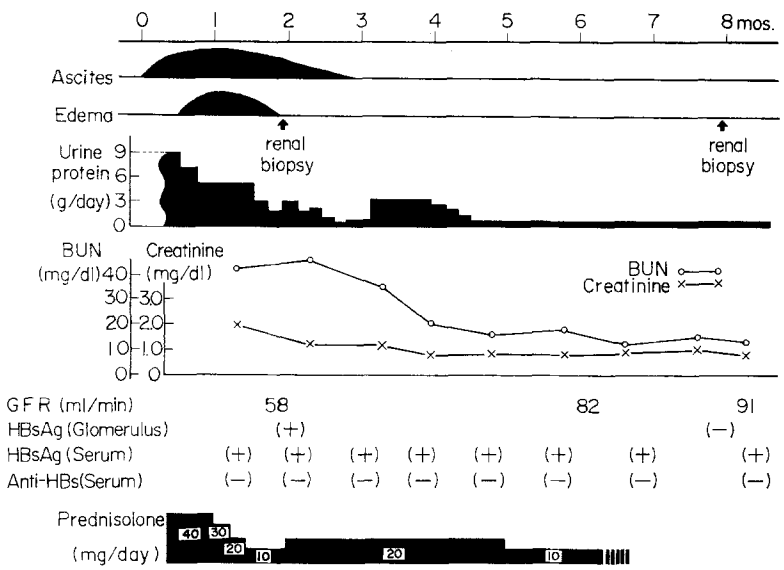

Fig. 2. Clinical course of case 1, T.F. 42 years old male (Table 2); see text for details

planned. He was placed on intermittent peritoneal dialysis for about one year until he died of peritonitis complicated by cerebral haemorrhage. Autopsy revealed early cirrhosis of the liver and the presence of HBsAg in both liver and kidney. Polyarteritis was not verified. The glomerular immunohistology showed an IgM dominant pattern with less intense IgG and negative IgA.

The other 3 cases had a history of antecedent liver disease. Peritoneoscopy and/or liver biopsy displayed well-developed liver cirrhosis in all of them. Case 2 was negative for both HBsAg and anti-HBs in the serum, while isolated HBsAg and anti-HBs were present in cases 1 and 4, respectively. The glomerular pathology, accompanied by nephritic urinary changes, was moderate or marked mesangial proliferation with local or diffuse CMI (MPGN). In addition, the glomerular immunofluorescence was IgA dominant or IgA equal in amount to other immunoglobulins, this finding being consistent with the so-called hepatic IgA glomerulonephritis. Case 1 was treated with corticosteroids for the nephrotic syndrome (Fig. 2). Following treatment for 4 months, proteinuria decreased to a trace, and BUN and serum creatinine were normalized with a rising GFR. In the second renal biopsy obtained 8 months after the onset of nephrotic syndrome, glomerular HBsAg had disappeared despite persistently positive HBsAg in the serum. The glomerular immunohistology was essentially same as that in the initial biopsy.

Discussion

Although 50.9% and 33.3% of the patients examined in the present series were positive for HBsAg in the blood and the liver, respectively, only 4 (3.8%) of 104 cases with various liver diseases were found to have HBsAg deposited

in the glomeruli. Several reasons for this unexpectedly lower incidence of glomerular HBsAg have to be considered. The staining titer of undiluted FITC-labeled anti-HBs serum used was judged to be high enough, because dilution to 1:8 gave a definite stain with known positive specimens, without false negatives. We applied Huang's method (1975) to the kidney tissue, which was originally devised to detect HBsAg or HBcAg in formalin-fixed and paraffin-embedded liver specimens. Huang (1975) had already noticed that positive immunofluorescence seen on the paraffin sections was somewhat less intense than on frozen ones, as was confirmed in our study. In addition, the positive rate of glomerular HBsAg on the frozen tissues in the present series was 7.7%, while it was 1.5% on the paraffin ones, although statistically this difference is not significant. However, at least 13 patients who were examined by both types of sections disclosed the same result, suggesting that a lower incidence of glomerular HBsAg on the paraffin sections is not necessarily the result of the methodology. It may be argued that HBsAg lodged nonspecifically in the diseased glomeruli. This is unlikely, firstly in view of the vigorous washing processes in the staining precedure, and secondly, the lower incidence of glomerular HBsAg itself would vote against the nonspecific lodgement.

The present series did not deal with HBcAg or HBeAg in the glomeruli. In the study by Ślusarczyk et al. (1980) who examined both HBsAg and HBcAg, HBcAg alone was present in one-third in the patients with glomerular deposits of HB virus antigens, while another one-third were accompanied by both HBsAg and HBcAg and the last one-third, with HBsAg alone. From these results, the participation of HB virus with HBsAg and HBcAg would be about 150% of the incidence of HBsAg, thus possibly raising our incidence from 3.8% to 5.8%. Recently, Takekoshi et al. (1979) observed 2 cases of membranous nephropathy with subepithelial deposits of IgG, C_3 and HBeAg, but unaccompanied by HBsAg and HBcAg. Since this is the first description of glomerular HBeAg deposition, there is no way to estimate the frequency of HBeAg as compared with HBsAg. The role of HBeAg in hepatic glomerulonephritis, therefore, remains to be explored.

However, it is noteworthy that the majority of hepatic glomerulonephritis associated with chronic liver disease displays IgA dominant immunofluorescence (Manigand et al. 1970; De Werra et al. 1973; Callard et al. 1975; Nochy et al. 1976; Berger et al. 1977; our preceding report). Nevertheless, the modality of antibody to all HB virus antigens is known to belong to the immunoglobulin class of IgG or IgM. In this sense, only case 3 mentioned above showed immunohistology consistent with HB virus infection. This patient thus appears to resemble the cases reported by Bajtai et al. (1975) and Moriyama et al. (1976). In contrast, the remaining 3 cases had glomerular deposits of IgA dominant or equal to IgG and IgM, this being characteristic of hepatic IgA glomerulonephritis. The positive glomerular HBsAg may be related to the concomitant IgG or IgM. Moreover, the glomerular immunofluorescence in case 1 remained in spite of the steroid treatment and the disappearance of glomerular HBsAg. Hirschel et al. (1977) described one patient whose second renal biopsy became negative for HBsAg despite the persistent nephrotic syndrome. They assumed a change from HBsAg to other HB virus antigen(s), but this assumption is

not applicable to our case because the nephrotic syndrome remitted. What role, then, might HBsAg have played in this case? It is conceivable that the occurrence of the nephrotic syndrome has been contributed to by probable HBsAg-associated immune complexes which were ultimately removed by the steroids. If so, the presence of glomerular HBsAg in the case 1 could be considered a simple superimposition on the preexisting hepatic IgA glomerulonephritis.

The lower incidence of glomerular HBsAg observed in the present series thus appears to be explained by the reasons mentioned above. We conclude that HBsAg plays only a minor role in hepatic glomerulonephritis.

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