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Replication of hepatitis B virus with corticosteroid therapy in hepatitis B virus related membranous nephropathy

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Summary. The therapeutic effect of corticosteroid in hepatitis B virus (HBV) related membranous nephropathy was investigated in a 29-year-old chronic HBV carrier. Prednisolone (60 mg/day) was given for eight weeks and gradually reduced over the subsequent four months. In the renal biopsies taken before and after corticosteroid therapy, light microscopy revealed progression of sclerosis. Immunofluorescent staining showed glomerular capillary deposition of hepatitis B core antigen (HBcAg) by polyclonal antisera and hepatitis B e antigen (HBeAg) by monoclonal antibodies. Electron microscopy revealed 40–50 nm diameter virus-like particles in the glomeruli only from the biopsy performed after corticosteroid therapy. The serum concentrations of alanine aminotransferase, HBeAg, and HBV DNA increased with corticosteroid therapy suggesting active viral replication despite the absence of overt clinical hepatitis. Renal function did not improve and corticosteroid therapy was apparently not helpful in this patient. Our results conflict with the earlier notion that short-term corticosteroid does not interfere with a favorable outcome of the infection of the related renal disease.

Key words: Hepatitis B virus – Membranous nephropathy – Corticosteroid – Viral replication

Introduction

The pathogenetic role of hepatitis B virus (HBV) in renal disease has attracted much attention, since Combes and coworkers (1971) originally reported glomerulonephritis with complexes of hepatitis B virus surface antigen (HBsAg) and its antibody (anti-HBs) in a patient infected with HBV. Various renal pathologies have been described and membranous nephropathy (MN) remains the best recognized glomerulopathy (Takekoshi et al. 1978; Sluzarczyk et al. 1980). A nephrotic syndrome and proteinuria are the commonest manifestations of HBV associated membranous nephropathy (Takekoshi et al. 1978; Sluzarczyk et al. 1980; Lai et al. 1987a) and treatment of these patients remains difficult.

Furthermore, the prognosis of this condition is uncertain but there have been reports of progression to renal insufficiency (Kohler et al. 1974; Hsu et al. 1983). One may attempt to treat these patients with corticosteroid and/or immunosuppressive therapy as in cases of idiopathic membranous nephropathy (Ponticelli et al. 1987) assuming that the nephropathy is not a etiologically related to HBV. A recent report suggested that short-term corticosteroid therapy, given at the onset of nephrotic syndrome, does not interfere with the normal favorable course of the infection and the related renal disorder (Cadrobbi et al. 1985). In this report, we present a patient with HBV related membranous nephropathy who demonstrated histopathological progression and probable sustained viral replication following corticosteroid therapy.

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Case report

A 29-year-old Chinese mechanic, previously healthy, with no history of blood transfusion or liver diseases, was admitted in November 1986 for investigation of asymptomatic proteinuria detected incidentally.

Physical examination revealed mild ankle oedema. Laboratory investigation revealed normal serum complement concentrations and immunoglobulin pattern. The rheumatoid factor and antinuclear factor were not detected. Phase-contrast microscopic examination of urine showed numerous glomerular red blood cells. The proteinuria was 2.9 gm/day and the endogenous creatinine clearance was 67 ml/min. The renal function

Table 1. Renal function test, liver function test and serology

	Before steroid therapy	3 months after commencing steroid therapy	2 months after stopping steroid therapy
Blood urea nitrogen [mmol/1(mg/dl)]	5.7 (16.0)	6.7 (19.0)	7.0 (19.6)
Serum creatinine [umol/1(mg/dl)]	92 (1.04)	99 (1.12)	81 (0.92)
Creatinine clearance [ml/min/1.73 m^2]	109	83	108
Proteinuria [g/24 h]	2.9	2.1	2.8
Serum albumin [g/1(g/dl)]	40 (4.0)	41 (4.1)	41 (4.1)
Alkaline phosphatase [U/1(K-A U/dl)]	93 (13.1)	91 (12.8)	73 (10.8)
Serum bilirubin [umol/1(mg/dl)]	8 (0.5)	16 (1.0)	5 (0.3)
Alanine aminotransferase [U/1]	15	70	40
HBsAg [titer]	32	32	32
anti-HBc [titer]	80	80	80
HBeAg [titer]	16	64	64
HBV DNA*	1+	3+	3+

* HBV DNA was detected by dot blot hybridization using an oligonucleotide probe (Lin et al. 1987). The concentration of HBV DNA was determined by radioautogram of nylon membrane to which serum specimens were applied and tested for HBV DNA. The density of the dot was scored 0, 1+, 2+, 3+ and 4+. Two fold dilution of sera was also assayed for HBsAg, anti-HBc, and HBeAg. The highest dilution which remained positive for each HBV marker was considered as the end point

tests, liver function tests, and hepatitis B virus antigen status on admission and in the later phases are shown in Table 1. An intravenous urogram showed two normal-sized kidneys with good excretion of contrast. Percutaneous renal biopsy was performed in January 1987 and revealed membraneous nephropathy. The patient initially received Prednisolone 60 mg/day for two months; the dosage was then gradually reduced over the subsequent four months. The total duration of corticosteroid therapy was six months. No clinical complications of corticosteroid treatment were observed and his proteinuria persisted. Mild elevation of serum alanine aminotransferase was detected during corticosteroid treatment but liver biopsy was not performed. A second renal biopsy was performed two months after the cessation of corticosteroid treatment.

Materials and methods

The renal biopsy specimens were processed for light microscopy, immunofluorescence studies, and electron microscopy by standard methods (Lai et al. 1987a). Four micron frozen sections were studied by indirect immunofluorescence technique for HBsAg, hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg) using polyclonal monospecific rabbit anti-human HBV antigen antisera (Behringwerke AG, West Germany; Dakopatts, Denmark; Abbott, USA). Antisera of 1:50 and 1:100 dilution were used for anti-HBsAg and anti-HBcAg antisera and absence of staining at 1:100 was judged as negative. Antiserum of 1:2 and 1:4 dilution were used for anti-HBeAg antiserum and absence of staining at 1:2 was judged as negative. The specificity of staining for HBsAg and HBcAg was controlled by blocking and absorption procedures as previously described (Lai et al. 1987b). Direct immunofluorescence for HBsAg, HBcAg, and HBeAg using murine monoclonal antibodies at 1:10 dilution was also performed (gifts from Professor M. Mayumi, Jichi Medical School, Japan). The specificity and properties of these monoclonal antibodies had previously been reported (Ito et al. 1981; Hirose et al. 1984).

HBsAg was determined by reversed passive haemagglutination (Auscell, Abbott, IL. USA) and enzyme immunoassay (Auszyme monoclonal, Abbott, IL. USA). Anti-HBs, antibody to hepatitis B core antigen (anti-HBc), HBeAg, and hepatitis B e antibody (anti-HBe) were determined by enzyme immunoassays (Corzyme (recombinant DNA origin), and HBe EIA, Abbott, IL. USA). The HBV DNA in serum was detected by dot blot hybridization using an oligonucleotide probe as previously described (Lin, Wu, Lai 1987).

Results

In the pre-treatment renal biopsy two of the 20 glomeruli (10%) studied by light microscopy show segmental mesangial expansion and sclerosis while the other glomeruli appear normal. All glomeruli exhibit uniform thickening of capillary basement membrane with spike and "chain-like" patterns by silver stain (Fig. 1 A). Crescent and vasculitic changes are not seen. The tubules are intact.

Immunofluorescence studies show diffuse granular deposits of IgG (4+), C3 (2+), and IgA (trace) along the capillary loops. Granular deposition of HBcAg is demonstrated along the capillary wall using polyclonal antiserum. HBeAg is not demonstrable by polyclonal antiserum.

Electron microscopy reveals a thickened glomerular basement membrane, largely in relation to the presence of well-circumscribed large subepithelial electron-dense deposits (Fig. 2). No virus-like particles are identified.

In the post-treatment renal biopsy. Four of the 24 glomeruli (17%) examined by light microscopy are completely sclerotic. Thickening of the capillary basement membrane and "spike" pattern are demonstrated. In addition, five glomeruli (21%) show segmental mesangial sclerosis with partial collapse of capillary lumen (Fig. 1 B). Aneurysmal dilata-

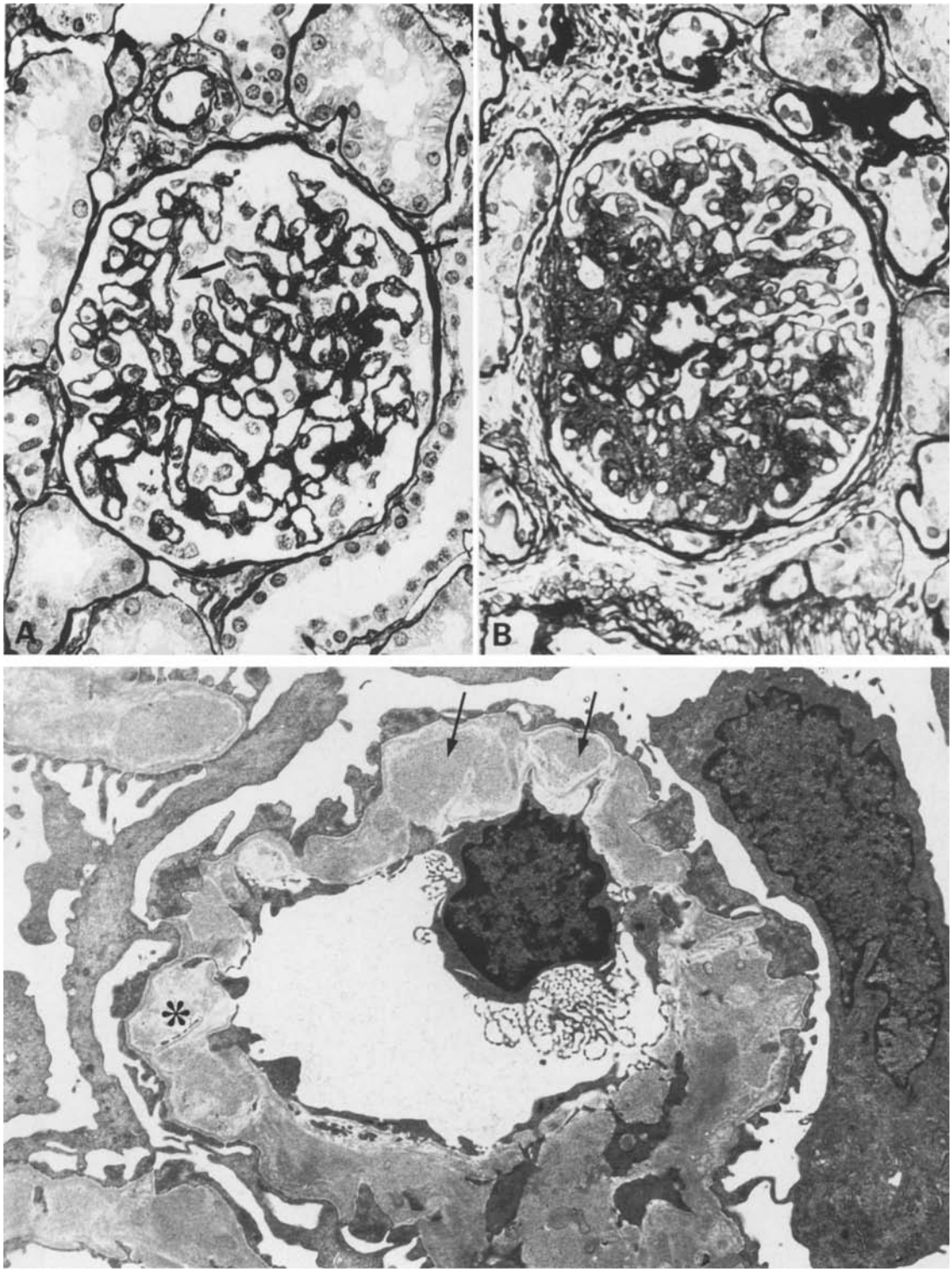

Fig. 1. (A) Glomerulus from first biopsy displaying uniform thickening of capillary basement membrane with argyrophilic spike and "chain-like" patterns (*arrows*). Periodic acid-methenamine silver stain (× 300). **(B)** Glomerulus from second biopsy demonstrating segmental mesangial sclerosis and capillary collapse. 21% of glomeruli in this biopsy revealed similar lesions. Periodic acid-methenamine silver stain (× 300)

Fig. 2. From first renal biopsy, the glomerular capillary basement membrane exhibited large subepithelial and intramembranous electron-dense deposit (*arrows*) and foci of electron-lucent granular deposits *. Lead citrate & uranyl acetate (× 6500)

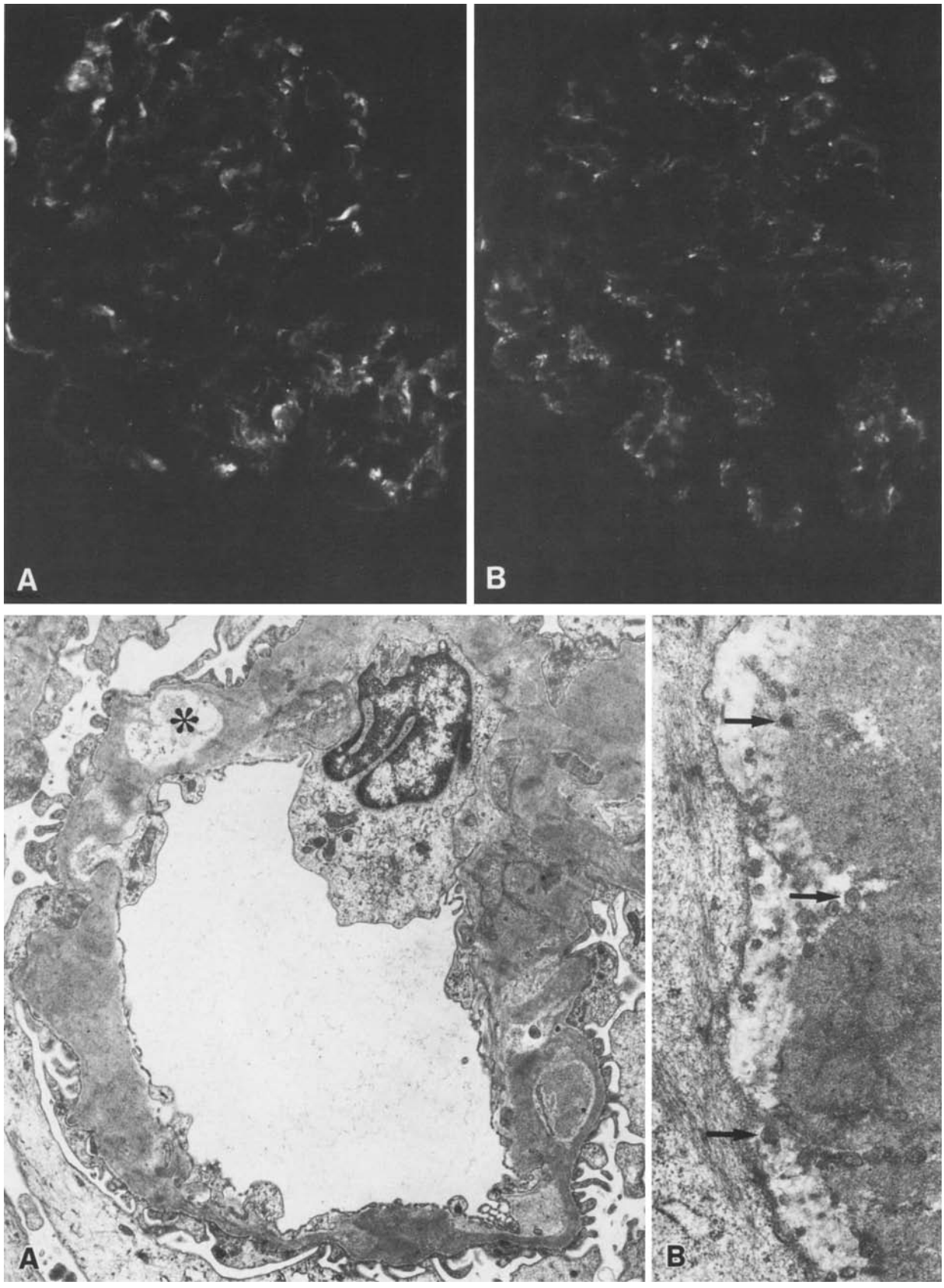

Fig. 3. (A) Positive glomerular capillary basement membrane staining for HBcAg. FTIC polyclonal rabbit antihuman HBcAg antiserum (× 300). **(B)** Fine granular HBeAg staining along the glomerular capillar basement membrane in the same biopsy. FITC monoclonal murine antihuman HBeAg $(Fab')_2$, (× 300)

Fig. 4 (A). From the second biopsy, irregular thickened glomerular capillary wall with persistence of intramembranous electron-dense deposits and granular electron-lucent deposition *. Lead citrate & uranyl acetate, (× 6400). **(B)** Numerous scattered 40–50 nm virus-like particles (*arrows*) were seen in extraglomerular capillary basement membrane where electron-dense deposits were also

tion of the capillary loops is observed in two glomeruli. Crescent and vasculitic changes are not seen. Hyalinization and medial thickening of arteriolar wall are detected. Atrophic changes and scarring involve 15% of the tubules.

Diffuse granular capillary stainings for IgG (3+) and C3 (2+) are demonstrated. Granular deposition of HBcAg and HBeAg are demonstrated along the capillary basement membrane using polyclonal antisera and monoclonal antibodies respectively (Fig. 3A and 3B).

Ultrastructural examination reveals thickened glomerular basement membrane with large subepithelial and intramembranous electron-dense deposits (Fig. 4A). Spherical virus-like particles of 40–50 nm in diameter are demonstrated within the electron dense deposits. Similar virus-like particles are seen in extraglomerular arteriolar basement membrane, which also exhibits the presence of dense deposits (Fig. 4B).

Discussion

Although the association of HBV infection and MN is strong in endemic area, the pathogenesis is not yet clear. Immune complexes containing HBV antigens are deposited in the glomerular capillaries but the nature of the HBV antigen remains uncertain. Glomerular staining of HBsAg (Takekoshi et al. 1978) and HBcAg (Sluzarczyk et al. 1980; Lai et al. 1987a), HBeAg (Hirose et al. 1984) have both been reported. This inconsistency in the nature of HBV antigens demonstrated is likely to be related to the reagents used; as shown in our patient, glomerular HBcAg deposits were demonstrated with polyclonal antisera and HBeAg deposits were revealed by monoclonal antibodies. Despite the frequent demonstration of glomerular HBV antigens, glomerular virus-like particles have, so far, only been identified in five patients by Hsu and coworker (1983).

A specific and rational treatment regimen for these patients with symptomatic nephrotic syndrome is still lacking. Spontaneous regression of nephrotic syndrome was reported in 30 to 60 percent of HBV associated MN, and these patients usually remained symptomatic for 12 months or longer (Hsu et al. 1983; Ito et al. 1981; Kleinknecht et al. 1979). The remaining patients had persistent proteinuria with fluid retention. Corticosteroid therapy used in idiopathic MN had been administered to some patients as a therapeutic trial for symptomatic relief (Kleinknecht et al. 1979; Cadrobbi et al. 1985). Furthermore, it is not uncommon for corticosteroids to be given to nephrotic children suspected of having steroid-responive lipoid nephrosis, and for subsequent serology and pathological examination to reveal the diagnosis of HBV associated MN (Takekoshi et al. 1978; Lai et al. 1987c). An isolated case report has suggested that short-term corticosteroid therapy, given at the onset of nephrotic syndrome, does not seem to interfere with the favorable course of the infection and related renal disorder (Cadrobbi et al. 1985).

A deleterous effect of corticosteroid with exacerbation of liver impairment following abrupt withdrawal of corticosteroid had been reported in patients with chronic HBV hepatitis (Hoofnagle et al. 1986). In contrast with patients with chronic active hepatitis, most patients with HBV associated MN may not have evidence of hepatic dysfunction and their liver biopsies may even be normal (Hsu et al. 1983; Lai et al. 1987b). Corticosteroid therapy was administered in our patient in an attempt to reduce the proteinuria and to prevent progressive renal impairment. The serology suggested that corticosteroid therapy in our patient was associated with active viral replication with increased serum concentrations of alanine aminotransferase, HBeAg and HBV DNA, although symptomatic liver dysfunction was not detected. Histopathological examination of post-treatment renal biopsy revealed histological progression. The increased glomerulosclerosis did not support a protective value for corticosteroid therapy in MN associated with HBV infection. Virus-like particles resembling Dane particles were not detected by careful ultrastructural examination of the pre-treatment renal biopsy. The appearance of virus-like particles in the glomeruli after corticosteroid therapy may support the serological evidence of active viral replication.

From our observations, the use of corticosteroid in HBV related MN may be undesirable; possible active viral replication and corticosteroid treatment was not associated with histological improvement. Our results do not support the earlier notion (Cadrobbi et al. 1985) that short-term corticosteroid does not interfere with the favorable outcome of the infection or the related renal disease. Further prospective trial is warranted to clarify the potential benefits and risks of corticosteroid therapy in HBV related MN.

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