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The role of hepatitis B surface antigen in the pathogenesis of glomerulopathies

J. NAGY, G. BAJTAI, H. BRASCH, T. SÜLE, M. AMBRUS, G. DEÁK and A. HÁMORI

Second Department of Medicine and Department of Pathology, University Medical School, Pécs and Blood Transfusion Service, Pécs, Hungary

Abstract. The frequency of hepatitis B surface antigen (HBsAg) has been studied in the sera and renal biopsies of 276 patients with various forms of glomerulonephritis (GN), the nephrotic syndrome and other nephropathies. Using a modified Hepanosticon method, HBs antigenemia was detected in 32 of 196 patients (16.3%) with immune complex (IC) GN and the nephrotic syndrome. Indirect immunofluorescence revealed HBsAg in 33 renal biopsy tissue specimens (16.8%). HBsAg was found in the sera of four of the 80 remaining patients with other renal diseases (5%), and in the renal biopsy tissues of another four (5%). Antibody against HBsAg could only be demonstrated in the serum of one glomerulonephritic patient. The sera of 18,799 normal blood donors were used as controls; of these 186 (0.99%) had positive tests for HBsAg. It is concluded that, in some patients with GN and the nephrotic syndrome, HBsAg-containing IC may be implicated in the development and/or progression of the disease.

Introduction

It is nearly a decade since it became known that, in the course of hepatitis B virus (HBV) infection, a lipoprotein substance now known as hepatitis B surface antigen (HBsAg) [Blumberg et al. 1965] is synthesized. Four HBV-associated antigens have now been identified: HBs, HBc, e-antigen and DNA polymerase [Editorial, Lancet 1975]. These viral proteins nearly always induce an immune reaction. In the presence of an unsatisfactory immune response the antigen may persist and lead to continuous formation of immune complexes. Under favourable conditions the circulating immune complexes may be deposited in certain organs, e. g. in the renal glomeruli, vascular walls, synovial membranes and other filter membranes and give rise to pathological processes. Several workers, including the present authors, have described glomerulonephritis (GN) [Bajtai et al. 1975, Bläker et al. 1975, Brzosko et al. 1974, Combes et al. 1971, Conte et al. 1975, Eknoyan et al. 1972, Favre et al. 1977, Guardia et al. 1975, Hirschel et al. 1977, Knieser et al. 1974, Kohler et al. 1974, Lagrue et al. 1974, Myers et al. 1973, Nagy et al. 1976, Nowoslawski et al. 1972, 1975], periarteritis nodosa (PAN) [Baker et al. 1972, Cream 1974, Gerber et al. 1972, Gocke et al. 1970, Krawczynski et al. 1974, Min et al. 1976, Prince 1971, Trepo 1970, 1972, 1974] and arthritis [Alarcon et al. 1973, Alpert et al. 1971, Duffy et al. 1976] associated with acute and chronic hepatitis with positive tests for HBV.

Individuals having clinical HBV infections and patients with repeated subclinical HBV infections who are still carrying the virus may be candidates for an immune complex (IC) disease. In the latter patients, the development of GN may be the first manifestation of the causative illness.

We therefore examined the incidence of HBsAg in 276 patients suffering from various types of GN leading to the nephrotic syndrome and other nephropathies. We also established the frequency of occurrence of HBsAg in the renal biopsy tissues in each case.

Materials and methods

From 1974 to 1977 we examined the incidence of positive tests for HBsAg in the sera and renal biopsy tissues of 276 patients with various forms of GN with the nephrotic syndrome and other nephropathies. The ages of the patients, 171 males and 105 females, varied between 14 and 72 years. The presence of HBsAg in the serum was established by passive hemagglutination (Hepanosticon, Organon) according to Hollós' modification 1975, and that of antibody against HBsAg by means of immunoelectroosmophoresis (IEOP) using the method of Vewalka as modified by

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Novák [1971]. Serum samples of 18,799 blood donors were used as control material.

The renal biopsy and necropsy tissue specimens were evaluated with light microscopy and with immunofluorescence (IF) in each case; in selected cases electron microscopy was also used.

Specimens for *light microscopy* were embedded in paraffin, cut in 2 μ sections, stained with hematoxylin and eosin, periodic acid-Schiff (PAS), periodic acid-silver-methenamine (PASM) and Congo red.

Material for *immunofluorescence microscopy* was quick frozen in CO_2. 5 μ sections were cut in a cryostat. The unfixed sections were stained with fluorescein-isothiocyanate FITC conjugated antisera to human IgG, IgA, IgM, IgE, C3, albumin and fibrinogen (Hyland, Costa Mesa, Ca.). HBsAg was studied by indirect IF; rabbit anti-Au/SH-antiserum (Behring, Marburg, Germany) was applied first, and subsequently the sections were treated with FITC-labelled goat anti-rabbit-7S-globulin (Cappel, Dowingtown, Pa.). The specificity of the reaction was checked by blocking experiments and by simultaneous staining of a liver section known to be positive for HBsAg. The sections were examined and photographed with a Fluoval (Zeiss) fluorescent microscope using appropriate excitation and absorption barrier filters [Bajtai et al. 1975, Nagy et al. 1976].

Tissue for electron microscopy was fixed in glutaraldehyde, post-fixed in osmium and embedded in Epon. Ultrathin sections were cut on an LKB ultramicrotome, stained with lead citrate and uranyl acetate and examined on a Tesla BS 613 electron microscope.

Results

The patients were divided into groups using a combination of the classifications proposed by Beregi [1977], Bohle et al. [1976], Churg et al. [1970] and Habib [1973] (Table 1). The group of "Minimal proliferative intercapillary GN without nephrotic

Table 1 HBsAg in sera and kidney biopsies of 276 adult renal patients.

	Number of cases studied	positive tests for HBsAg				
		sera	kidney biopsies			both
			Tr.	+–++	total	
I. Primary GN						
1. Diffuse GN Minimal proliferative intercapillary with nephrotic syndr.	14	–	–	–	–	–
without nephrotic syndr.	23	–	–	–	–	–
Endocapillary (acute) proliferative	1	–	–	–	–	–
Mesangioproliferative	55	11	2	4	6	3
Mesangioproliferative with crescents	7	–	1	–	1	–
Membranous	17	3	3	1	4	3
Membranoproliferative	26	6	2	4	6	3
Berger's disease	32	5	7	3	10	3
2. Focal GN	31	3	–	–	–	–
Focal sclerosing	9	2	1	–	1	–
II. GN associated with systemic diseases	7	–	1	1	2	–
SLE						
Schoenlein-Henoch syndr.	11	2	2	1	3	1
III. "End stage" kidney	12	1	1	1	2	1
IV. Renal diseases other than I–III	10	1	–	–	–	–
Chron. pyelonephritis						
Chron. interstitial nephritis	4	1	2	–	2	1
Secondary amyloidosis	3	–	–	–	–	–
Primary amyloidosis	2	–	–	–	–	–
Hereditary nephritis	9	1	–	–	–	–
Myeloma kidney	3	–	–	–	–	–
Total	276	36	22	15	37	15

The broken lines encompass immune-complex GN. Tr. = traces, + = minimal amount, ++ = moderate amount.

Fig. 1a

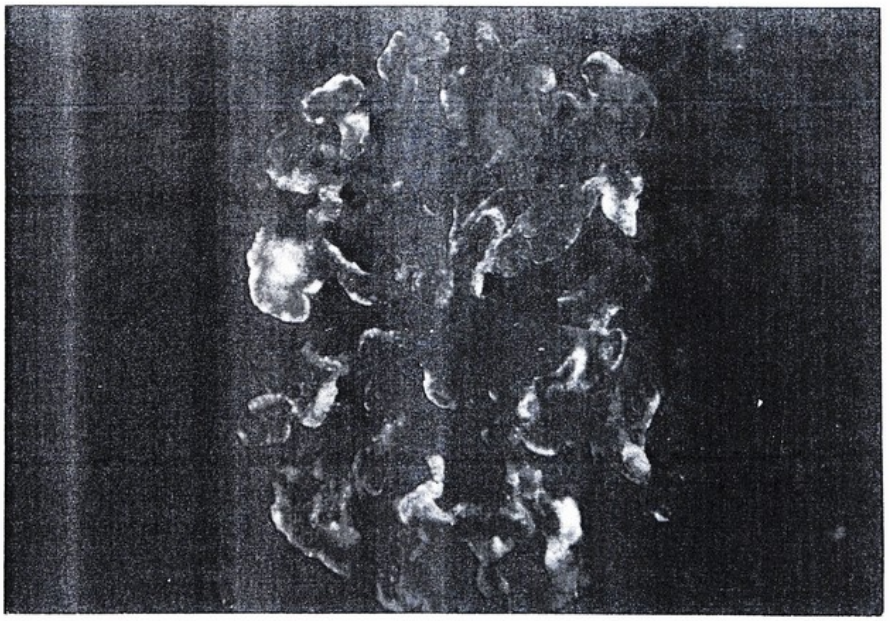

Fig. 1b

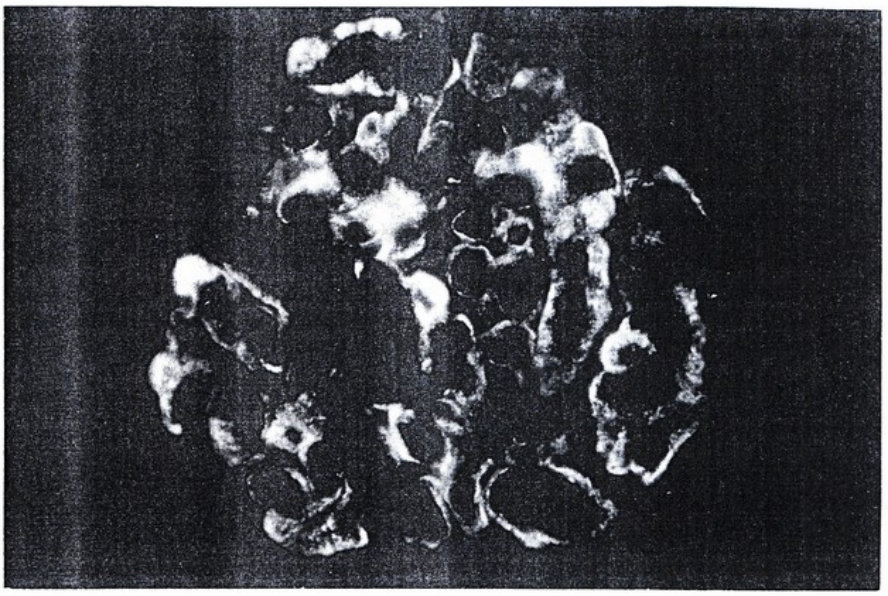

Fig. 1c

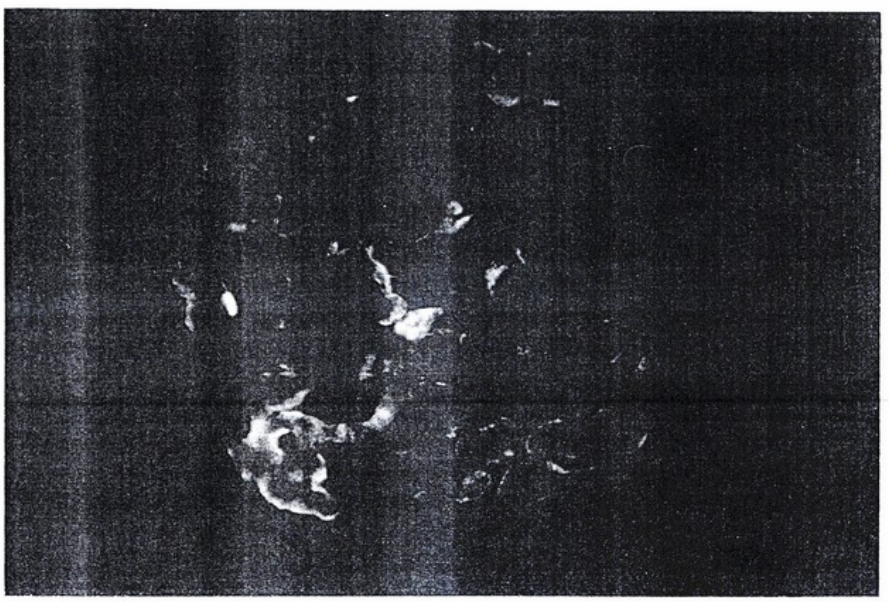

Fig. 1 Membranoproliferative glomerulonephritis. Granular fluorescence along the glomerular basement membranes and in the mesangium (a, HBsAg, ×500), (b, IgG, ×500), (c, C3, ×500).

syndrome" is made up of patients most of whom were referred to our Department after screening for proteinuria and in whose renal tissue only slight alterations were demonstrated by light microscopy and immunofluorescence. Most of these patients were completely free from complaints and only had minimal or moderate proteinuria and/or hematuria. In Table 1 the two broken lines encompass the cases of presumed immune complex origin. Theoretically some of the cases of "Minimal proliferating intercapillary GN without nephrotic syndrome" and of "End-stage kidney diseases" may have been of immune complex origin. But, as has been pointed out before, we have never seen marked granular staining in "Minimal proliferating intercapillary GN", and only rarely in the "End-stage kidney" group. We believe that this would not justify classification of these pathological conditions as immune complex diseases.

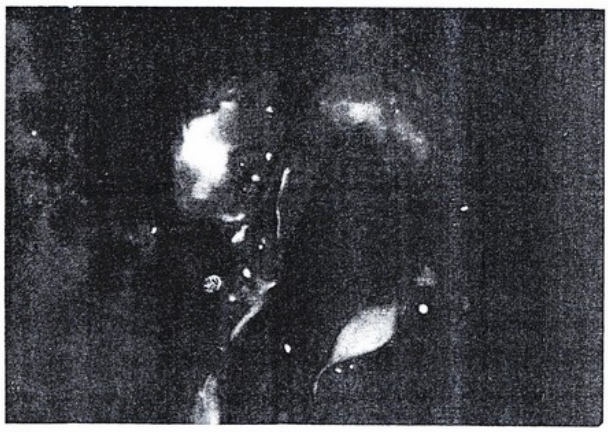

Fig. 2a

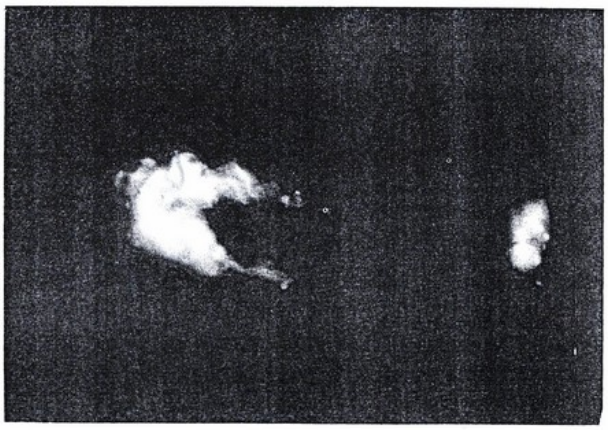

Fig. 2b

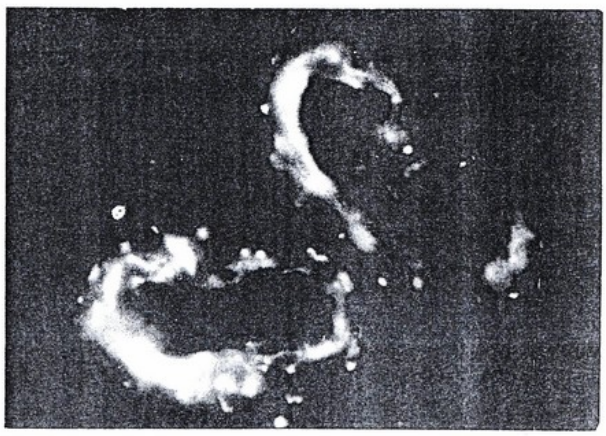

Fig. 2c

Fig. 2 Granular, partly confluent; fluorescence in the walls and lumina of arterioles (a, HBsAg, ×500), (b, IgM, ×500), (c, C3, ×500).

Table 1 shows the frequency of detection of HBsAg in the sera and renal biopsy tissues of all patients. HBsAg was identified in the sera of 36 patients (13.0%) and in the kidney biopsies of 37 patients (13.4%). In 15 patients (5.4%) serum HBsAg was found to coexist with positive tests in the tissues. Of 196 patients suffering from IC GN (primary GN without minimal proliferating intercapillary GN and GN associated with systemic diseases) 32 (16.3%) had HBs antigenemia; HBsAg was found in the renal biopsies of 33 (16.8%). The number of patients having HBs antigenemia coexisting with positive biopsies was 13 (6.6%). Patients with other renal diseases showed a much lower incidence of positive tests for HBsAg; 4 out of 80 (5%) had antigenemia and another 4 had positive biopsies. 186 (0.99%) of the 18,799 healthy blood donors had HBs antigenemia. Anti-HBsAg-antibody was detected in the sera of only one of the glomerulonephritic patients.

A chronic carrier state can only be said to exist if antigenic material has been demonstrable in the serum for more than six months. Therefore the examinations were repeated after four to ten months in 20 of the 32 patients with antigenemia; the remaining 12, some of whom had died, failed to appear for reexamination. Only ten cases were positive. Repeat tests in the 4 HBs antigenemia patients found in the group without IC deposition proved negative.

In 33 patients with IC GN indirect IF revealed HBsAg deposition in the renal biopsy tissues. Deposits were mainly situated in the glomeruli, in the walls of small arteries and arterioles and, less frequently, in the cytoplasm, basement membrane and lumina of the tubular cells as well as in the interstitium. Glomerular HBsAg deposits were encountered in 22 cases, vascular ones in 27 and tubular and interstitial deposits in four. As compared with immunoglobulins and complement component C3, the amount of HBsAg found in the kidney tissue was very small in 22 cases (Table 1). Generally its staining intensity failed to reach that of the immunoglobulins. With the exception of two cases, its localization was identical with that of the immunoglobulins (fig. 1a, b,

c). Glomerular deposits were chiefly associated with IgG deposition and less frequently with that of IgM and IgA. Outside the glomeruli, HBsAg was most often seen in the walls of arterioles and small arteries. In these biopsies no changes characteristic of PAN were encountered in any of the tissue specimens. The light microscopic picture was characterized by hyaline thickening of the vascular walls and sometimes by the deposition of fibrinoid material. The HBsAg was localized mainly in the endothelium and also in the vascular lumen where it was closely related to the endothelium. Its deposition was most often associated with IgM and C3 in similar localization (fig. 2a, b, c) and less frequently with IgA and IgG.

Electron microscopic examinations were performed in 4 cases showing deposits of HBsAg. No. 22 nm spherical or tubular particles corresponding to HBsAg were observed.

Six of the renal patients positive for HBsAg had previously received transfusions, and hepatitis was recorded in the case history of seven; six patients had been given immunosuppressive therapy. During the time of observation liver function tests (SGOT, SGPT and SLDH) of no more than five patients showed positivity.

Table 2 shows the average number of months that elapsed between the onset of the disease and the examination for HBsAg.

Discussion

Animal experiments and observations on humans have indicated that HBsAg-containing IC may be formed in the course of HBV induced acute and chronic hepatitis. In the presence of a good immune response, the HBsAg is eliminated at the latest four to six months after the infection. Since HBsAg contains several components of normal serum proteins, such as prealbumin, IgG, transferrin and apolipoprotein C, it may be moderately immunogenic. As a result of a poor antibody response in individuals with a pathologic immune system, immune complexes are constantly being produced. Following deposition in various organs, the circulating immune complexes may give rise to GN, PAN and arthritis.

Table 2 Interval between onset of disease and examination for HBsAg.

	Number of patients	Average number of months positive for HBsAg (sera and/or renal tissue)	negative for HBsAg (sera and/or renal tissue)
I. Primary GN			
1. Diffuse GN			
Minimal proliferating intercapillary with nephrotic syndr.	14	–	42
without nephrotic syndr.	23	–	6
Endocapillary (acute) proliferative	1	–	2
Mesangioproliferative	55	42	31
Mesangioproliferative with crescents	7	6	11
Membranous	17	31	9
Membranoproliferative	26	26	21
Berger's disease	32	37	31
2. Focal GN	31	30	22
Focal sclerosing	9	8	14
II. GN associated with systemic diseases	7	17	28
SLE			
Schoenlein-Henoch syndr.	11	13	12
III. "End stage" kidney	12	79	97
IV. Renal diseases other than I–III	10	47	84
Chron. pyelonephritis			
Chron. interstitial nephritis	4	21	17
Secondary amyloidosis	3	–	19
Primary amyloidosis	2	–	4
Hereditary nephritis	9	10	19
Myeloma kidney	3	–	12

Attention has often been called to the occurrence of GN associated with acute and chronic hepatitis [Benner et al. 1968, Eknoyan et al. 1972, Feizi and Gitlin 1961, MacLachlan et al. 1965, Mistilis and Blackburn 1970, Randall et al. 1971, Read et al. 1963, Sakaguchi et al. 1965, Silva et al. 1965], however, the causal relationship between the two diseases has not been established for very long. By demonstrating HBsAg at the site of the renal lesion in the glomeruli Combes [1971] was the first to prove the pathogenetic role of HBsAg in membranous GN following chronic hepatitis. The association of chronic hepatitis with chronic membranous GN in the presence of glomerular HBsAg has been observed in one case by Kohler [1974], in two by Bläker [1975] and in one by ourselves [Bajtai et al. 1975, Nagy et al. 1976]. Myers [1973], Favre [1977] and Hirschel [1977] described membranoproliferative GN originating under similar conditions, while one of Knieser's [1974] three patients suffered from focal sclerosing GN.

The large number of patients with asymptomatic HBV infection raises the question of how many had contracted a chronic IC disease (manifested as GN). Reversing the question, one may wonder how commonly symptomless HBsAg occurs in patients with chronic GN. The purpose of our investigations was to answer the latter question. Only a few communications have appeared in recent years and even these contain data which contradict each other in many respects. They are summarized in Table 3. Differences in the patients' ages, in geographic location, and the methods and immune sera used may account for the different results. Brzosko [1974] suggests that the large number of positive tests for HBsAg observed in his material may be due to transfusions, frequently given to children in Poland. Only six of our patients had previously been transfused, but several reported having injections and blood samples taken within the year preceding recognition of their renal disease.

Table 3 Comparison of data on routine tests for HBsAg positivity in glomerulonephritis.

	HBsAg positivities			
	sera examined/positive		kidney biopsies examined/positive	
	No.	%	No.	%
Conte [1975]	71/0	0	71/22	31
Guardia [1975]	105/8	7.8	N. D.	
Powell [1977]	21/3	14.2	N. D.	
Vos [1973]	182/37	20.3	N. D.	
Brzosko [1974]*	32/16	50.0	32/18	56.2
Lagrue [1974]*	161/10	6.8	N. D.	
Nagy [1978]*	196/32	16.3	196/33	16.8

N. D. = not done

* Only cases of immune complex glomerulonephritis were considered.

HBs antigenemia did not prove persistent in some of our patients. Thus it was possible for us to re-examine 20 patients of whom only 10 proved positive after four to ten months. Further studies will decide whether they were cases of intermittent antigenemia or genuine recoveries. The rapid disappearance of HBsAg from the circulation does not exclude simultaneous development of chronic GN, as has been shown by Györkey [1975] in monkeys. After the injection of HBV positive blood focal GN developed in the animals, but in patients a variety of forms of chronic GN have been observed.

In 13 of our patients with IC GN, positive tests for HBsAg in serum were associated with positive tests in tissue. The detection of antigen in the serum is influenced by the sensitivity of the method used, and by the proportion of antigen to antibody. With immunological equivalance and antibody predominance it often happens that the antigen only becomes demonstrable after the circulating IC have been split by enzymes [Millman et al. 1970]. With antibody predominance, mainly insoluble (Class III) or poorly soluble (Class II) IC are produced, which are phagocytozed by the reticuloendothelial system [Germuth and Rodriguez 1973]. It is probable that in the presence of HBsAg such complexes are formed since deposits of HBsAg are found less often in the glomeruli.

In most cases the fluorescence of the HBsAg in the renal biopsies was of lower intensity and in 22 cases of smaller quantity than that of immunoglobulins and C3 (see Table 1). It is possible that, as a result of their spatial distribution, immunoglobulins and the activated complement components partially or completely cover the antigen, which would explain why in some of the HBs antigenemia cases the tissue antigen could not be demonstrated at all or only in trace amounts.

On the basis of the presence of HBsAg alone it cannot be decided with absolute certainity whether the antigen forms part of a nephritogenic IC even if immunoglobulins and C3 are detected in identical positions. In a case of GN caused by other IC, the circulating HBsAg may, together with other serum components, be trapped in the glomerular basement membrane as a result of the increase in permeability.

From Table 2 it may be seen that in some of our patients a long time elapsed between the onset of disease and the examination for HBsAg. In such cases it seems likely that frequent blood tests were responsible for the infection with HBV. In these cases either because of the immunedeficiency responsible for the primary renal disease or because of immunosuppression therapy, the IC containing HBsAg might have

contributed to the progression of the disease. However, on the basis of the frequent occurrence of HBsAg in the sera and renal tissue specimens in localizations characteristic of IC we believe that in some cases HBsAg-antibody IC might play a primary role in the pathogenesis of some varieties of GN. We recommend examination of the sera and kidney biopsies in all nephritic patients in order to detect HBsAg components of IC GN.

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Reprint request to Dr. Judit Nagy
Second Department of Medicine, University Medical School
Pécs, Squ. Széchenyi 5. 7621. Hungary

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