Immune Complex Type Glomerulonephritis in Cirrhosis of the Liver

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Glomerular lesions were detected in 9 of 10 patients with liver cirrhosis: these lesions consisted of a) thickening of basement-membrane-like material, b) electron-dense deposits in mesangial areas and in capillary walls, c) round areas of rarefaction in the membrane-like material and in some deposits, and d) presence of IgA, with IgG and/or IgM and /or C3, in the deposits. The association of these four abnormalities seems to be characteristic of "cirrhotic glomerulonephritis." The deposits could be the result of precipitation in the glomeruli of either aggregated immunoglobulins or circulating immune complexes. (Am J Pathol 80:329–340, 1975)

COMPLETE systematic immunomorphologic investigations have not been applied to the study of glomerular lesions in patients with cirrhosis: therefore, the immunomorphologic features of these lesions and their incidence are not well established. By light microscopy, Baldus *et al.*[1] and Goreski and Kumar[2] did not observe any obvious glomerular changes. Jones *et al.*[3] found unspecific alterations and could not demonstrate any difference between a group of patients with cirrhosis and a group of controls of same age; Horn and Smetana,[4] Raphael and Lynch,[5] Baxter and Ashworth,[6] and Patek *et al.*[7] described glomerular lesions in those patients; Bloodworth and Sommers[8] pointed out that cirrhosis might induce a characteristic glomerular lesion and proposed the designation of "cirrhotic glomerulosclerosis." By electron microscopy, Sakaguchi *et al.*,[9] Salomon *et al.*,[10] Fisher and Perez-Stable [11] described the ultrastructural changes in this type of glomerulonephritis. By immunofluorescence microscopy, early investigators [9-11] observed γ-globulin glomerular fixation, but did not use specific sera against IgG, IgA, and IgM; Manigand *et al.*[12] demonstrated the presence of IgG, IgA, and C3 in glomerular tufts from 3 of the 4 patients with cirrhosis they studied.

The aforementioned studies were based on a small number of cases and/or were made in cirrhotic patients selected for renal manifestations and/or did not include a control group; moreover, in none of these studies were

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complete examination of renal parenchyma by light and electron and immunofluorescence microscopy performed together.

The present investigation was undertaken to describe the glomerular lesions detected by light and electron and immunofluorescence microscopy in a group of unselected patients with cirrhosis and in a control group of subjects without liver disease.

Materials and Methods

Ten patients (6 men and 4 women between 31 and 55 years of age) with histologically proven cirrhosis (alcoholic cirrhosis, 8 patients; posthepatic cirrhosis. HBsAg positive, 1 patient; cryptogenic cirrhosis, 1 patient) and portal hypertension underwent surgical biopsies of kidney and liver during portacaval shunt procedures. Ten subjects (6 men and 4 women between 24 and 87 years of age) with histologically proven normal liver and without any clinical manifestation of renal disease underwent the same biopsies during surgical interventions involving organs other than the liver or the kidney.

Morphologic Studies

The kidney specimens were divided into three fragments for light, electron, and immunofluorescence microscopy.

Light Microscopy

The fragments were fixed for 12 to 24 hours in Bouin's fixative. Sections, 3 μ thick, were stained with hematoxylin–phloxin–saffron, Masson's trichrome, periodic acid–Schiff (PAS), silver impregnation after periodic oxidation according to Marinozzi.

Electron Microscopy

Portions of renal cortex were fixed by immersion for 90 minutes in 1.55% glutaraldehyde at 4 C and were postfixed for 1 hour in 2% osmium tetroxide. Both solutions were buffered at pH 7.35 with Millonig's buffer. Dehydration was carried out through a graded series of alcohols and propylene oxide. Specimens were embedded in epoxy resin, stained with aqueous uranyl acetate and lead citrate, and examined with a Zeiss EM 9 B electron microscope.

Immunofluorescence

The fragments were frozen in liquid nitrogen and sectioned with a cryostat. The sections, 3 to 5 μ thick, were stained without any fixation with anti-IgG, anti-IgA, anti-IgM, anti-C3, and antifibrinogen fluoresceinated sera (Hyland Laboratories).

Laboratory Investigations

In all patients and controls, investigation for proteinuria and Addis count were performed, and serum creatinine, blood urea, and blood glucose levels were measured. Eight patients with cirrhosis had a determination of serum C3 level and 7 had a determination of serum IgG, IgA, and IgM levels by radial immunodiffusion[13]; serum cryoglobulin[14] was looked for in 7 patients. In all patients and controls, serum was tested for HBsAg by counterimmunoelectrophoresis.

Results

Patients with Cirrhosis of the Liver

Light Microscopy

Glomerular lesions (Table 1) were detected in 9 of the 10 patients studied: a) Glomerulosclerosis was marked in 6 and mild in 3 and consisted of a widening of the mesangial matrix often with a thickening of the capillary loops. b) The cellular proliferation was absent in 8 and moderate in 2; in 1 of these 2 patients, numerous capillary loops had a double-contour appearance. c) "Fibrinoid" deposits that were PAS positive, stained red by Masson's trichrome, and were nonargyrophilic were observed in 4 patients; these deposits were usually located in the mesangial area; they were less often observed along the capillary loops in a subendothelial position.

In 3 patients, interstitial sclerosis was observed. In 2 patients, sclerosis of the arterial intima and arteriolar fibrinoid deposits were present.

Electron Microscopy

The following lesions were observed (Table 1): a) In 8 patients, the mesangial matrix, which sometimes contained mature collagen, was enlarged (Figure 1); the basement membranes of the glomerular capillaries were irregularly thickened and sometimes showed a folded appearance. b) In all patients, finely granular, osmiophilic, electron-dense deposits were observed. These deposits were located in the mesangium (Figure 1) and occasionally in the walls of the capillary loops; in 1 patient (Patient 10, Table 1), they were scattered and tiny. These deposits correspond to the fibrinoid deposits seen by light microscopy. c) Peculiar formations were observed in 9 patients; these formations were found especially in the mesangial matrix (Figure 1), sometimes in the basement membrane in a subendothelial position and/or in the finely granular dense deposits (Figure 2). These formations appeared as round, lucent areas not limited by a membrane, 500 to 1100 Å wide, isolated or gathered in clusters. These lucent areas appeared empty or contained a material having a density equal to, or greater than, that of the basement membrane or the deposits. d) Some mesangial cells surrounded by the mesangial matrix extended along the inner surface of the capillary loops. In 8 patients, this mesangial interposition, which accounted for the double-contour loops observed by light microscopy, was found in few capillary loops. In 1 patient, the proliferation and interposition of the mesangial cells, associated with endomembranous deposits, gave an ultrastructural appearance similar to that of membranoproliferative glomerulonephritis.

Table 1—Glomerular Immunomorphologic Findings in Patients With Cirrhosis

Patient	Age (yrs)	Light microscopy		Electron microscopy			Immunofluorescence				
		Glomerulo-sclerosis	Deposits	Glomerulo-sclerosis	Deposits	Rarefaction areas	IgA	IgG	IgM	C3	Fibrin-ogen
1	46	+	+	+	+	0	±	+	±	+	±
2	42	±	+	+	+	+	+	+	+	+	0
3	51	+	+	+	+	+	+	±	+	+	0
4	52	+	±	+	+	+	+	±	±	0	0
5	52	+	0	+	+	+	+	+	±	±	0
6	31	+	0	+	+	+	+	±	+	+	0
7	52	±	0	0	+	+	+	+	+	0	0
8	36	±	0	+	+	+	+	0	+	±	0
9	32	0	0	0	+	+	+	±	0	+	+
10	55	+	0	+	±	+	0	0	±	0	0

0 = Absent, ± = mild, + = important.

Immunofluorescence Microscopy

Massive (Figure 3) or limited (Figure 4) mesangial and, occasionally, capillary wall fixation of anti-IgA serum was observed in 9 patients. The mesangial and, occasionally, capillary wall staining also observed with anti-IgG, anti-IgM, and anti-C3 sera was frequent but, generally, less intense. Fixation of antifibrinogen serum was observed in only 2 patients. In 1 patient (Patient 10, Table 1) moderate fixation of only anti-IgM serum was seen.

Laboratory Investigations

None of the patients had proteinuria, renal insufficiency (serum creatinine $\leq$ 1.2 mg/100 ml), or arterial hypertension. Only 1 patient had microscopic hematuria (Patient 5, Table 1). Serum C3 levels were low in 3 patients and at the lower limit of the normal range in 1 patient (Table 2). In 7 patients, serum IgA was high (between two and five times the normal value). Serum IgG was increased in 5 patients. Serum IgM was normal. In 4 patients, a cryoglobulin was detected; the cryoglobulin was analyzed in 2 and contained IgG, IgA, and IgM. Serum HBsAg was detected in only 1 patient (Patient 9, Table 1).

Control Group

Light Microscopy

Four subjects out of 10 had a glomerulosclerosis. Scarce mesangial fibrinoid deposits were observed in only 1 patient (Table 3).

Electron Microscopy

Electron microscopy confirmed the presence of glomerulosclerosis in the 4 subjects in whom this lesion was detected by light microscopy. In a fifth

Table 2—Serum Immunoglobulins, C3, and Cryoglobulin in Patients With Cirrhosis

Patient	IgG* (mg/100 ml)	IgA* (mg/100 ml)	IgM* (mg/100 ml)	C3* (mg/100 ml)	Cryoglobulin
1	4000	1400	130	100	Absent
2	1040	800	160	28	—
3	1360	1120	94	170	Present
4	1800	600	128	58	Absent
5	—	—	—	74	Present
6	4000	960	160	90	Absent
7	1680	1520	120	36	Present
8	910	210	130	—	Absent
9	—	—	—	—	—
10	2000	1120	220	100	Present

* Normal values (mean ± 2 SD) in this laboratory: IgG, 934 ± 380 mg/100 ml; IgA, 185 ± 160 mg/100 ml; IgM, 90 ± 70 mg/100 ml; C3, 132 ± 66 mg/100 ml.

Table 3—Glomerular Immunomorphologic Findings in Control Subjects

Subjects	Age (yrs)	Light microscopy		Electron microscopy			Immunofluorescence				
		Glomerulosclerosis	Deposits	Glomerulosclerosis	Deposits	Rarefaction areas	IgA	IgG	IgM	C3	Fibrinogen
1	50	0	0	0	0	0	0	0	0	0	0
2	40	0	0	0	0	0	0	0	0	0	0
3	67	+	±	+	±	0	0	0	0	0	0
4	24	0	0	0	0	0	0	0	0	±	0
5	87	+	0	+	0	0	0	0	0	0	0
6	36	0	0	0	±	+	0	0	0	±	0
7	31	±	0	+	0	±	0	0	0	0	0
8	72	0	0	+	0	0	0	0	0	0	0
9	35	±	0	±	0	0	0	0	0	0	0
10	73	0	0	0	0	0	0	0	0	0	0

0 = Absent, ± = mild, + = important.

subjects, glomerulosclerosis, although not detected by light microscopy, was demonstrated by electron microscopy. Scattered mesangial deposits were found in 2 patients. A few areas of rarefaction were seen in 2 patients (Table 3).

Immunofluorescence Microscopy

Immunofluorescence was negative in all subjects except 2, in whom mild fixation of only anti-C3 serum on few glomerular tufts was observed.

Laboratory Investigations

None of the control subjects had proteinuria, microscopic hematuria, arterial hypertension, or impairment of renal function. The serum C3, IgG, IgA, and IgM levels were normal; cryoglobulinemia was not found. Serum HBsAg was absent in all controls.

Discussion

Glomerular lesions were present in 9 of the 10 cirrhotic patients of the present series. This incidence is greater than that observed in several published series.[4,5,7,8,11] This discrepancy might be explained by either a bias in our material, since all our cirrhotic patients have been selected for portacaval shunt or, more likely, by the fact that systematic immunomorphologic investigations were performed in all our patients.

The glomerulonephritis observed in our patients was characterized by four immunomorphologic features: a) presence of electron-dense granular deposits usually mesangial in location; b) thickening of basement-membrane-like material resulting in glomerulosclerosis (often those two features were demonstrated only by electron microscopy); c) presence of round areas of rarefaction in the basement-membrane-like material and in some deposits on electron microscopy; d) presence of IgA often associated with other immunoglobulins and C3.

Although several of these changes have already been described in patients with various types of cirrhosis[9,15] or with alcoholic hepatitis,[9] this association of these 4 histologic features has not been pointed out as being characteristic. The mesangial and subendothelial deposits have been observed by electron microscopy by Sakaguchi *et al.*,[9] Salomon *et al.*,[10] and Fisher and Perez-Stable.[11] These authors have also found areas of rarefaction in the basement membrane and mesangial matrix, but not within the deposits. The presence of γ-globulins without C3 has been reported,[9] but Manigand *et al.*[12] were the first to note the presence of deposits containing IgA, IgG, and C3 in the kidneys of 3 of 4 patients with cirrhosis.

The association of these immunomorphologic findings is very suggestive of cirrhotic glomerulosclerosis, but each of them may be found in other diseases. a) The occurence of diffuse mesangial deposits staining for IgA is observed in some primary glomerulonephritides,[16,17] and in some glomerulonephritides secondary to rheumatoid purpura or systemic lupus erythematosus.[18] b) Cirrhotic glomerulosclerosis with mesangial interposition and large amounts of diffuse capillary wall deposits resembles primary membranoproliferative glomerulonephritis.[19] c) Glomerulosclerosis has been also observed in patients with diabetes, in patients with nephrosclerosis, and even in some apparently normal elderly subjects (as in 3 subjects of our control group); but, in these types of glomerulosclerosis, there are usually not any IgA deposits.[20] d) The round areas of rarefaction present in the membrane-like material[9-11] and in some deposits are not specific of cirrhosis since they were found in 2 subjects of our control group; however, their abundance is suggestive of cirrhotic glomerulonephritis.

In patients with acute or chronic viral hepatitis, Type B, glomerular immune deposits containing HBsAg had been observed[21]; in our only patient with HBsAg present in serum, the part of either cirrhosis or HBsAg itself in the development of the glomerular changes cannot be ascertained because HBsAg was not looked for in renal tissue.

Glomerulonephritis associated with cirrhosis may induce urinary or renal function abnormalities.[11,12] In this series, only 1 patient was symptomatic, which suggests that cirrhotic glomerulosclerosis is usually infraclinical.

A low level of serum complement in cirrhosis was observed by several authors.[22-24] The low concentration of C3, associated with a low level of other complement fractions [23,24] results from an impairment in hepatic synthesis.[23,24] The following hypotheses are proposed to explain the relations between the decrease in serum complement and cirrhotic glomerulonephritis: a) the decrease in serum complement might be also caused in part by the activation of the complement system such as was observed in patients with poststreptococcal acute glomerulonephritis, lupus glomerulonephritis, and hypocomplementemic membranoproliferative glomerulonephritis[25]; b) the decrease in the synthesis of some complement fractions due to hepatic disease might be responsible for cirrhotic glomerulonephritis by increasing the risk of infections and, therefore, by inducing the formation of immune complexes [26]; c) the low level of serum complement and cirrhotic glomerulonephritis might be independent disorders.

To explain glomerulonephritis in patients with cirrhosis, it is tempting

to relate the high levels of serum immunoglobulins, particularly IgA,[27] to the glomerular deposits rich in IgA. Serum IgA could be aggregated and deposited in the mesangium, as is observed in mice after intravenous injection of aggregated IgG.[28] Alternatively the deposits could be the result of the deposition of circulating immune complexes,[29] the antibodies of the complexes being directed against antigens originating from the digestive tract, especially bacterial, viral, or dietary antigens.[30-32] In fact, in the rat with CCl_4-induced cirrhosis, the response to oral immunization is more intense than in the normal rat.[33] Moreover, in noncirrhotic rats with portacaval shunt, an increase in the level of γ-globulins [34,35] and antibodies against *E. coli* [34] has been described. Thus, cirrhosis or portacaval shunt might result in sustained hyperimmunization of digestive origin.

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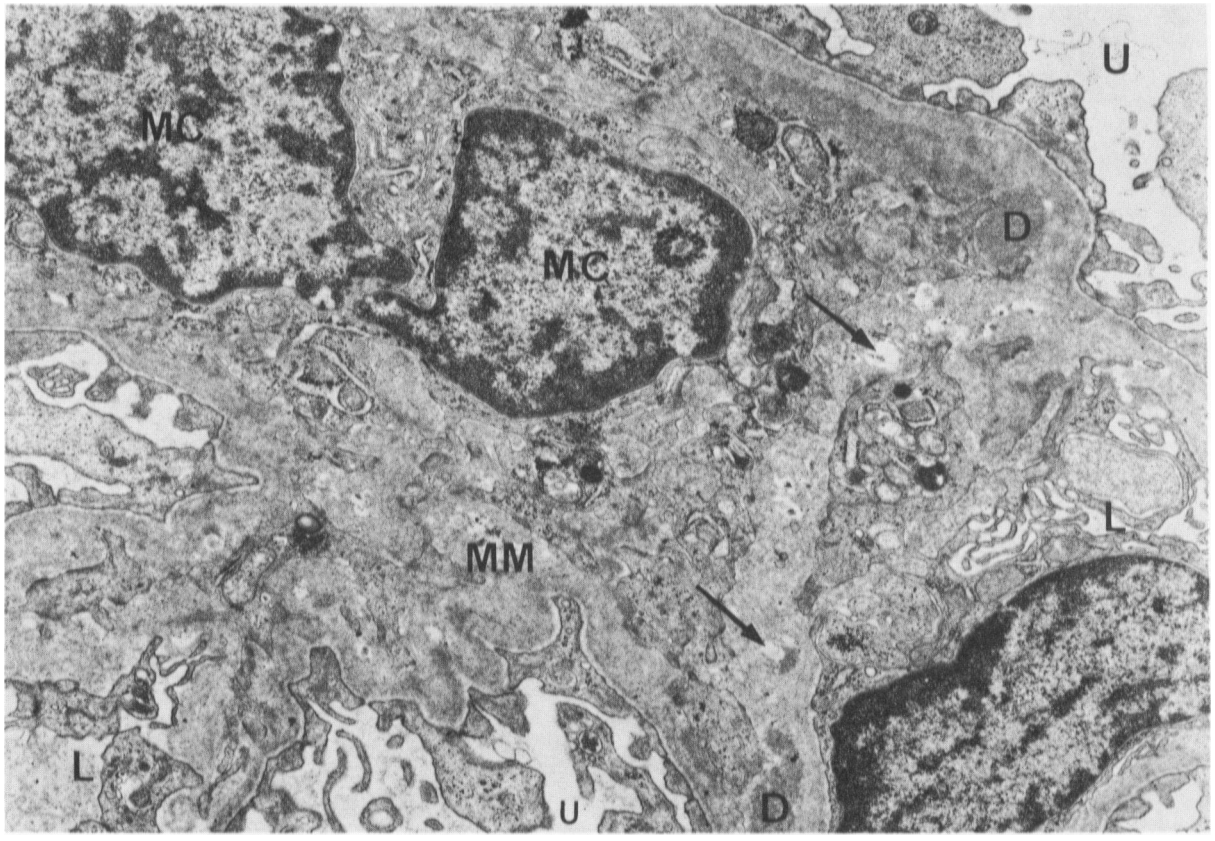
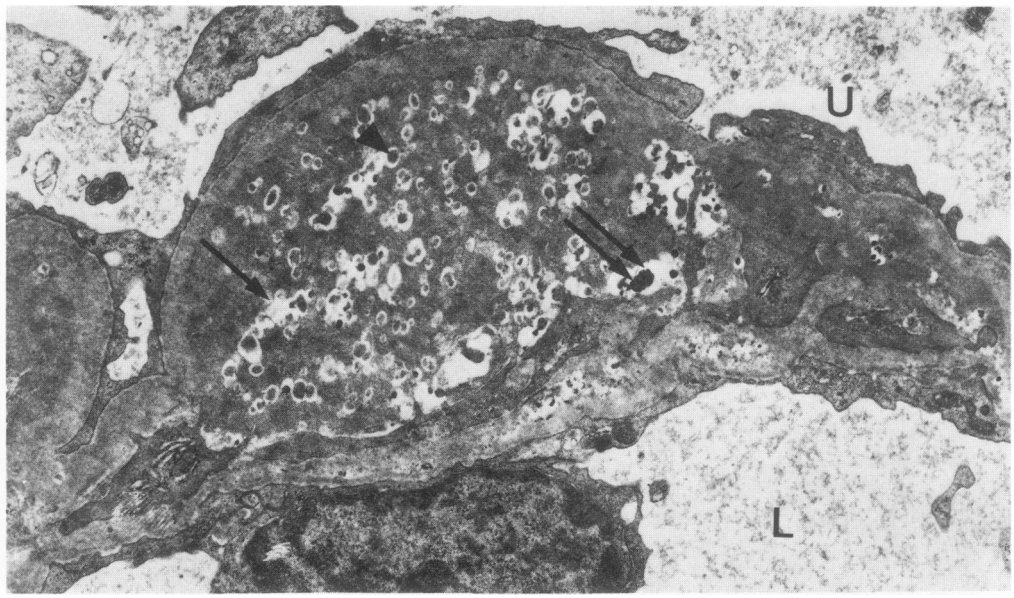


Figure 1—Electron micrograph of the kidney from a patient with cirrhosis. In the mesangial area one can see endomembranous, dense deposits (*D*), enlargement of the mesangial matrix, (*MM*) and round lucent areas (*arrow*). *U* = urinary space, *L* = lumen, *MC* = mesangial cell. (× 11,900)



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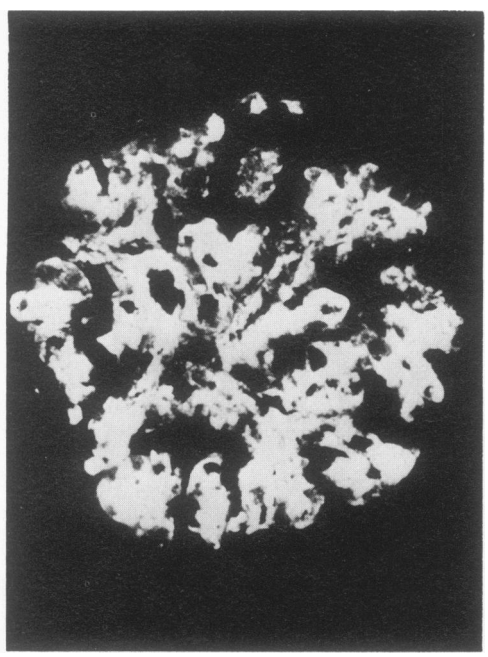

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Figure 2—Electron micrograph of a kidney specimen from a patient with cirrhosis. In the mesangial deposit, a cluster of round lucent areas can be seen. These areas appeared empty (*arrow*) or contained a material as dense as (*arrowhead*) or denser than (*double arrow*) the deposits. *L* = lumen, *U* = urinary space. (× 9500) **Figure 3**—Glomerular tuft from a patient with cirrhosis. Heavy fixation of anti-IgA serum can be seen throughout the mesangium and in some capillary loops. (× 270) **Figure 4**—Glomerular tuft from a patient with cirrhosis. A scattered fixation of anti-IgA serum can be seen. (× 270)