Immunofluorescent analysis of extracellular matrix (ECM) components in glomeruli of the hepatic glomerulosclerosis

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Key words: ECM components, immunofluorescence, hepatic glomerulosclerosis

Abstract

An immunofluorescence study was carried out to evaluate alterations in the distribution and/or intensity of extracellular matrix (ECM) components, immunoglobulins and complement (C3) in the glomeruli of 7 autopsy patients with hepatic glomerulosclerosis. As compared with the findings in normal renal tissues, an increase in type IV collagen, laminin and fibronectin was observed in expanded glomerular mesangial areas and along glomerular capillary walls. Depositions of IgA, mainly IgA1, and C3 in glomeruli were in parallel with those of the ECM components. These ECM components were markedly decreased in glomeruli showing global sclerosis. In contrast, type I collagen was observed at central portions of the sclerotic glomeruli. There was an increase in the type I collagen along Bowman’s capsules, especially at the sites of capsular adhesion and crescent formation. It appears that hyperproduction and/or infiltration of glomerular ECM components and interstitial collagen is closely linked to the progression of glomerular sclerosis in patients with liver diseases.

Introduction

It has long been recognized that renal glomerular injuries occur in association with chronic liver diseases of various etiologies and have been generally called hepatic glomerulosclerosis [1, 2]. The WHO Committee for the Classification of Renal Diseases defined the nephropathy of liver disease as structural abnormalities of glomeruli observed in patients with hepatitis and cirrhosis, consisting of mesangial proliferation and sclerosis, or in more severe cases, mesangiocapillary or membranous nephropathy [3]. In 1970, Manigand et al. [4] reported mesangial depositions of IgA, IgG and C3 in the glomeruli of patients with liver cirrhosis and with fatty liver. In the following studies, Berger et al. [5] reported that glomerular deposits of IgA were the main immunofluorescent findings in patients with alcoholic liver cirrhosis.

The extracellular matrix (ECM) of the glomerular mesangium and capillary walls was found to consist of type IV collagen, laminin (LN), fibronectin (FN) and other proteins based on biochemical and immunochemical analyses [6]. Type I and III collagens are the predominant collagens in interstitial tissues of the kidney.

In the present study, we evaluated alterations in renal glomerular distribution and/or intensity of ECM components by immunofluorescence in autopsy patients with hepatic glomerulosclerosis to determine whether such changes are associated with progression of the disease.

Materials and Methods

Autopsy cases: Renal tissues from 7 autopsy patients (4 males and 3 females, aged 39 to 65 years) with liver diseases and 5 normal renal tissues were
examined in this study. Laboratory data of all these patients at death are summarized in Table 1. Five patients of these had liver cirrhosis and 2 had hepatic cell carcinoma. Patients associated with HB antigenemia were excluded from this study. The autopsy was performed within 12 hrs of death. Histological diagnosis of renal tissues was defined according to the WHO classification [3]. Glomerular findings in such patients revealed diffuse mesangial proliferative change (DMP) in 5 patients and membranoproliferative change (MP) in 2 patients (Fig. 1a, b).

**Table 1.** Laboratory data of patients with hepatic glomerulosclerosis at death.

<table>
<thead>
<tr>
<th>Case</th>
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<td>7</td>
<td>58 M</td>
<td>LC</td>
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</table>

M; male, F; female, LC; liver cirrhosis HCC; hepatic cell carcinoma RBC; red blood cell

**Immunofluorescence:** Renal specimens were embedded and rapidly frozen in liquid nitrogen, and sectioned to a thickness of 3 μm with a rotary microtome at about −25°C. Immediately before staining, cryostat sections were washed three times in 0.02M phosphate-buffered isotonic saline (PBS, pH 7.4) for 9 min. Immunofluorescence was performed using 1) polyclonal rabbit anti-human type IV collagen (05/AB 748, Chemicon Int. INC., CA, USA, 1:80 in PBS), 2) polyclonal rabbit anti-human fibronectin (FN) (90-0251, Biomedical Products, MA, USA, 1:80 in PBS), 3) polyclonal rabbit anti-human laminin (LN) (04/AB949, Chemicon Int. INC., CA, USA, 1:80 in PBS), 4) polyclonal rabbit anti-human type I (05-91/AB745, Chemicon Int. INC., CA, USA, 1:40 in PBS) antisera and 5) mouse monoclonal anti-human IgA1 and IgA2 antibodies (L1142 and M0359, Becton Dickinson, CA, USA, 1:40 in PBS, respectively). FITC-labeled goat anti-rabbit IgG antiserum (1:40 in PBS) and FITC-labeled goat anti-mouse IgG antiserum (1:20 in PBS) were obtained from Cappel Laboratory PA, USA and absorbed with human normal plasma prior to use. The renal sections were incubated with these antisera or antibodies in a moist chamber at room temperature for 30 min. After washing with PBS, the sections were stained with the

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**Fig. 1.** Light microscopic findings of a glomerulus (PAS stain). a. Diffuse mesangial proliferative change (DMP) (x400); b. Memanoproliferative change (MP) (x400).
FITC-labeled IgG antiserum at room temperature for 45 min. The optimum incubation periods were determined by preliminary experiments. The sections were examined with a Zeiss Universal microscope (Carl Zeiss Inc., New York, USA) at a wavelength of 495 nm. The intensity of fluorescence was graded as none (--), trace (±), 1 (+), 2 (+) or 3 (+), and then semiquantitatively evaluated by scoring as follows: (--) 0 point, (±) 0.5 point, (+) 1.0 point, (++) 2.0 points, and (+++) 3.0 points [7].

Results

1) Immunofluorescence of Immunoglobulins and C3
Deposition of IgA in the glomerular mesangial areas was observed in all 7 patients examined (Fig. 2). Segmental deposition of IgA in the glomerular capillary walls was observed in 6 out of the 7 patients (85.7%) (Table 2). The intensity of IgA, IgM, IgG and C3 deposition in the glomerular capillary walls was higher than that in the glomerular mesangial areas as shown in Table 2. The intensity of C3 deposition in glomeruli of patients with MP was higher than that in cases of DMP (Table 2).

Deposition of IgA1 in the glomerular mesangial areas was observed in 4 out of 5 patients (80%) and along capillary walls in all patients (100%) examined. The intensity of IgA1 deposition was marked in the glomerular capillary walls and less intense in the mesangial areas (Fig. 3). Deposition of IgA2 was observed in the glomerular mesangial areas and/or capillary walls in 2 out of 5 patients (40%). The intensity of IgA2 deposition in the glomeruli was significantly less than that of IgA1 deposition.

![Fig. 2. Glomerular deposition of IgA in a patient with diffuse mesangial proliferative change (DMP) (×400).](image1)

![Fig. 3. Glomerular deposition of IgA1 in a patient with diffuse mesangial proliferative change (DMP) (×400).](image2)

<table>
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M: male; F: female
Mes: glomerular mesangial areas, Gc: glomerular capillary walls
±: not done
DMP: diffuse mesangial proliferative change
MP: membranoproliferative change

Table 2. Immunofluorescent findings of immunoglobulins and C3 in glomeruli of patients with hepatic glomerulosclerosis.
2) Immunofluorescence of ECM components

Distribution of type IV collagen and LN was observed linearly in the glomerular capillary walls in all renal tissues from the 5 autopsy patients without renal disease. Although FN was weakly positive for the glomerular mesangial areas in all normal renal tissues, it was virtually negative or very weakly positive for the glomerular capillary walls. Type I collagen was observed in the interstitium, but not in the glomeruli of renal tissues (Fig. 4a, b, c).

The results of immunofluorescence on ECM components in patients with hepatic glomerulosclerosis are summarized in Table 3. Type IV collagen and LN were observed in the glomerular mesangial areas and capillary walls of all patients examined (Fig. 5a). Distribution of type IV collagen in glomeruli was similar to that of LN as seen by immunofluorescence. Intensity of type IV collagen was markedly decreased in the global glomerulosclerosis (Fig. 5b). FN was observed in the glomerular mesangial areas in all patients examined (Fig. 6). Type I collagen was restricted to the areas of glomerular adhesion to Bowman’s capsules and the outer portions of the sclerotic glomeruli. Type I collagen was also distributed at the central portion of the sclerotic glomeruli (Fig. 7 and Table 3).

3) Correlations between the intensities of immunoglobulins, C3 and ECM staining in glomeruli

The mean intensity score of IgA deposits in the

Fig. 4. ECM staining in normal renal tissues. a. type IV collagen; b. fibronectin (FN); c. type I collagen (×400).

<table>
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<tr>
<th>Case</th>
<th>Type IV collagen</th>
<th>Laminin</th>
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Mes: glomerular mesangial areas
Gc: glomerular capillary walls
nd: not done
Fig. 5a. Type IV collagen is markedly extended in glomerular capillary walls and mesangial areas in a patient with membranoproliferative change (MP) (×400). b. Intensity of type IV collagen was decreased markedly in the global sclerotic glomerulus in the same patient (×200).

Fig. 6. Glomerular findings of fibronectin (FN) in a patient with diffuse mesangial proliferative change (DMP) (×400).

Fig. 7. Type I collagen was observed around the glomerular sclerotic lesions and in the central parts of the sclerotic glomerulus in a patient with membranoproliferative change (MP) (×400).

Fig. 8. Mean intensity score of immunoglobulins, C3, type IV collagen, laminin (LN), fibronectin (FN) and type I collagen in glomeruli.
glomerular mesangial areas was parallel with that of type IV collagen, LN and FN and less than that of type I collagen. The mean intensity score of IgA and IgM in the glomerular capillary walls was parallel with that of LN. The intensity of C3 deposits in the glomerular capillary walls was higher than that of IgA, IgM, IgG, type IV collagen, LN, FN and type I collagen (Fig. 8).

Discussion

Hepatic glomerulosclerosis is characterized by the deposition of IgA and C3 in the glomerular mesangial areas and capillary walls. In electron microscopy, electron dense deposits were observed in the glomerular mesangial areas and capillary walls. These depositions were thought to be either IgA-dominant immune-complexes or aggregated immunoglobulins. Since the coexistence of IgA (IgA1) and C3 in the glomeruli was observed, the depositions were thought to be immune-complexes.

The subclass of glomerular IgA deposits was found to consist mainly of IgA1 in this study, in agreement with the previous report [8]. Conley and Delacroix [9] reported that while IgA-producing plasma cells in bone marrow consisted of 88% IgA1 and 12% IgA2, those in intestinal mucosal membrane consisted of 65% IgA1 and 35% IgA2. It remains to be determined whether the IgA1 deposited in glomeruli is produced by plasma cells in the mucosal membrane or in bone marrow in patients with hepatic glomerulosclerosis. On the other hand, Andre et al. [10] found IgA2 to be the major component of glomerular mesangial deposits in cirrhotic patients.

The development of glomerular changes and mechanisms of glomerulosclerosis in patients with liver diseases remain obscure. Recently, glomerular basement membrane (GBM) and/or mesangium have been shown to consist of type IV collagen, heparan sulfate proteoglycan, fibronectin, laminin and other proteins [6]. Weiss et al. [11] detected an increase in fibronectin in the expanded glomerular mesangium and capillary walls of various glomerular diseases. Type IV collagen, laminin and fibronectin were increased in the expanded glomerular mesangial areas of the patients in the present study. Type IV collagen, laminin or fibronectin also extended into the glomerular capillary walls. These ECM components were markedly decreased in global sclerosis of the glomeruli. Alterations of the extracellular matrix in the glomeruli of patients with hepatic glomerulosclerosis are basically similar to those in cases of primary glomerulonephritides. However, alterations of glomerular capillary walls, such as deposits of IgA (IgA1) and C3 and expression of ECM components in patients with hepatic glomerulosclerosis were significantly greater than those in patients with primary IgA nephropathy or membranoproliferative glomerulonephritis. Thickening of the glomerular capillary walls and/or circumferential interposition of the glomerular mesangial matrix were also observed in such patients by electron microscopy (unpublished data). Thus, IgA (IgA1)-dominant immune-complexes may stimulate the production of ECM components in this disease.

Several cytokines and/or growth factors such as IL-1, IL-2, IL-6, tumor necrosis factor-alpha (TNF-α), platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) have been shown to be involved in glomerular mesangial cell proliferation and the hyperproduction of ECM components [12-16]. Thus, these factors may be involved in the mechanisms of progression of hepatic glomerulosclerosis. We previously reported that the hyperproduction and/or invasion of interstitial collagens, i.e. types I and III, are closely linked to the progression of glomerulosclerosis in patients with various types of glomerulonephritis [17]. In this study, type I collagen was distributed adjacent to the Bowman’s capsules, especially at the adhesion sites or crescents, and in the central parts of the sclerotic glomeruli. The presence of interstitial collagens in such glomeruli may be due to their invasion through the Bowman’s capsules during the progression of glomerulosclerosis. Alternatively, glomerular cells may produce type I collagen because of an altered gene expression induced by various growth factors and/or cytokines. Thus, it is necessary to clarify the type of factors that stimulates synthesis of the extracellular matrix in the glomeruli of patients with hepatic glomerulosclerosis.

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