A Clinicopathologic Study of Focal Segmental Glomerulosclerosis: Comparison between Nephrotic and Non-nephrotic Focal Segmental Glomerulosclerosis

Akihiko TAKEUCHI, Nobuyuki YOSHIZAWA, Takao KUBOTA and Hirohumi NIWA

Seven out of 16 patients with primary focal segmental glomerulosclerosis (FGS) did not show the nephrotic syndrome throughout their clinical courses, and then patients with FGS could be divided into the two groups, nephrotic FGS group (NS-G) and non-nephrotic one (NO-G). The clinicopathologic findings of NS-G and NO-G were compared retrospectively to define the pathogenesis of the glomerular disease in FGS. No significant differences were found between the groups except for proteinuria and serum total protein or albumin, and it was impossible to distinguish the two groups only histologically. In addition, electron microscopic study revealed that the glomerular epithelial cell was altered more than the endothelial and mesangial cells in both groups, resulting in vacuolization, foot process fusion, and detachment, and the striking fact that the glomerular epithelial vacuoles consisted mainly of the dilated rough endoplasmic reticulums (RERs). The speculation is drawn that in FGS the glomerular epithelial cell is firstly damaged by unknown factor(s), manifested functionally proteinuria and/or hematuria, morphologically dilatation of RER, foot process fusion, detachment, and eventually segmental sclerosis and hyalinosis.

Key words: Focal segmental glomerulosclerosis, Nephrotic syndrome, Clinicopathologic study, Epithelial vacuolization, Rough endoplasmic reticulum

Focal Segmental Glomerulosclerosis (FGS), originally described by Rich (1), is characterized morphologically by glomerular segmental sclerosis and/or hyalinosis superimposed on minor glomerular abnormality to mild mesangial proliferative glomerulonephritis, and clinically by the corticosteroid-resistant nephrotic syndrome and the frequent progression to end-stage renal disease (2–5). However the etiology and pathogenesis remain uncertain.

In this study, patients with FGS, which was diagnosed only by the histologic methods, light and immunofluorescence microscopy, could be divided into the two groups, nephrotic FGS group (NS-G) and non-nephrotic one (NO-G). The clinicopathologic findings of the two groups were compared retrospectively to define the pathogenesis of the glomerular disease in FGS.

MATERIALS AND METHODS

Eight hundred cases underwent renal biopsies at National Defense Medical College between 1978–1988. Out of them, twenty patients were diagnosed FGS only morphologically by the existence of segmental sclerosis and/or hyalinosis with minor to mild glomerular changes, and by the exclusion of other pathologic conditions which exhibit the similar morphologic change to FGS. Four patients were excluded from the analysis because of the coexistence of systemic diseases. The remaining sixteen patients, primary FGS, were divided into the two groups, NS-G and NO-G. Once a patient showed the nephrotic syndrome (NS) in his clinical course, he was grouped into NS-G.
The onset of disease was defined as the first laboratory evidence of renal disease. The first clinical sign was the main laboratory finding at onset as follows: asymptomatic proteinuria (AP), non-nephrotic range proteinuria; asymptomatic hematuria (AH), microscopic hematuria; NS. NS was diagnosed when 24-h urinary protein exceeded 3.5 g/day and serum total protein or albumin concentrations were less than 6.0 g/dl or 3.0 g/dl, respectively, more than the duration of 1 month. Hypertension was diagnosed when the diastolic blood pressure was persistently > 90 mmHg or the systolic blood pressure > 160 mmHg. Hematuria was considered present when there were > 5 red blood cells per high-power field of centrifuged urine. In every patient serum and urine samples were obtained and examined at least every 3 and 1 month, respectively. And when evaluating serum total protein and 24-h urinary protein excretion on each patient, all data were used to the calculations and expressed as a mean ± 1 SD, and the values as a group were calculated from the mean values of each patient. For the evaluation of clinical course of renal function, the reciprocal of serum creatinine concentration was plotted against time for each patient and the data were then analysed by least-squares linear regression as previously reported (6, 7). And only the patients who exhibited the linear relation were evaluated by the determination of their slopes of the regression line.

Renal tissue obtained at renal biopsy was divided and processed for light, immunofluorescence, and electron microscopy. Tissue for light microscopy was fixed by 20% formalin, and paraffin sections cut at 3 μm were stained with hematoxylin and eosin, periodic acid-Schiff, periodic acid-silver methenamine and Azan. The analysis was defined to the specimens containing glomeruli more than 5. For immunofluorescence study, tissue was snap-frozen in dry ice and acetone immediately after biopsy. Five μm-thick sections were stained with fluorescein-conjugated antisera to human IgG, IgA, IgM, C3c, C1q, C4, and fibrinogen (Hoechst, Darmstadt, West Germany). Tissue for electron microscopy was fixed in Karnovsky’s solution, postfixed in 2% osmium tetroxide, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate. Glomeruli which seemed to be damaged artifactually during tissue processing or progressed to global sclerosis and/or hyalinosis were excluded from the observation, and it was limited to non-sclerotic portions and/or non-sclerotic glomeruli. Two to three glomeruli were observed for each specimen.

Statistical evaluation was performed using the Student’s t test and Fisher’s exact probability test. Results are expressed as a mean ± 1 SD in the text and table.

RESULTS

In a series of 800 renal biopsied patients, there were 20 cases of FGS, and 16 cases were analysed in this study, excluding 4 cases from this analysis because of the coexistence of systemic diseases, that is, diabetes mellitus, gout, rheumatoid arthritis, and sarcoidosis & chronic thyroiditis. Nine patients showed NS during their clinical courses, grouped into NS-G, and 7 patients did not for 40 to 316 months (168 ± 112) after the onset of the disease, grouped into NO-G. It is noteworthy that 7 out of 16 patients with primary FGS (44%) did not show massive proteinuria enough to induce NS throughout their clinical courses.

Clinical features

There were no differences between the two groups with respect to male/female ratio, age at onset, clinical sign to be firstly discovered, time interval between the clinical onset and the first visit to our hospital, and the duration of follow-up. The patients in NS-G progressed to NS 0 to 160 months after the onset of the disease, the mean being 46.3 months. (Table 1). It is interesting whether the patients in NO-G will become nephrotic in future, and if so how long it will take afterward. Twenty-four-h urinary protein excretion was more in NS-G than in NO-G (3.6 ± 1.8 vs 1.3 ± 0.8 g/day, p<0.01), while the value of serum total protein was less in NS-G than in NO-G (6.0 ± 0.4 vs 6.9 ± 0.3 g/dl, p<0.01). The prevalences of hypertension and hematuria were similar between the two groups (Table 1).

In NS-G, 4 out of 9 patients were treated with prednisolone when suffering from NS, 2 patients responded completely 5 times in all including relapses and did not become corticosteroid-resistant, and in 2 patients proteinuria decreased but persisted.
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Table 1. Clinical findings of NS-G and NO-G.

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<th>age at first clinical sign</th>
<th>duration of follow-up (months)</th>
<th>onset to first non-G (months)</th>
<th>H</th>
<th>sCr (mg/dl) at first</th>
<th>latest</th>
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NO-G

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AP, asymptomatic proteinuria; AH, asymptomatic hematuria; NS, nephrotic syndrome; H, hematuria; HT, hypertension; sCr, serum creatinine.

Two NS-G patients became nephrotic transiently and urinary protein excretion decreased before corticosteroid therapy was started. The remaining 3 NS-G patients were not treated with corticosteroid because one had gastric ulcer, one was in renal insufficiency, and the third one dropped out before therapy. NO-G patients were treated mainly with anti-platelet agents because their proteinuria was not so heavy.

Clinical course of renal function

No significant differences could be demonstrated in the values of blood urea nitrogen and serum creatinine at both the first and latest visit (Table 1). Only two patients in each group showed the linear relation of a reciprocal serum creatinine against time, and it was possible to assess the clinical course of renal function objectively only in them. Both showed a linear decline of renal function during the observation (Table 1). The number of patients was, however, too small to compare the two groups about renal function.

Pathology

In NS-G renal biopsy was performed once in 3 patients, twice in 5 patients, and three times in 1 patient, while in NO-G it was done once in 5 patients, and twice in 2 patients. The numbers of the tissue containing glomeruli more than 5 were 12 in NS-G and 9 in NO-G, totally 21 specimens out of 25. One NS-G patient was excluded from the analysis because his specimen contained glomeruli less than 5. The time interval between the onset of the disease and biopsy ranged 1 to 228 months (75 ± 79 months) in NS-G, while that did 25 to 318 (154 ± 125) in NO-G, showing no difference (p > 0.05). There was also no difference in the renal function at biopsy between the groups.

Light microscopic findings are shown in Fig. 1. The percentage of global sclerotic glomeruli was not significantly different between the two groups (31 ± 31 vs 22 ± 28%, p > 0.25). Similarly the significant differences were not demonstrated in the prevalences of glomeruli with segmental sclerosis (23 ± 20 vs. 16 ± 11%, p > 0.25) and segmental hyalinosis (11 ± 17 vs 6 ± 8%, p > 0.25). All patients were confirmed to be FGS in the first biopsy, and the specimens of follow-up biopsy of two patients in each group were found not to contain glomeruli with the segmental lesion. Any glomeruli with hyalinosis could not be found in 7 specimens out of 12 and 3 patients out of 8 in NS-G, while in 5 specimens out of 9 and 3 patients out of 7 in NO-G. The segmental lesion always located in the neighboring region of a glomerulus to the Bowman’s capsule, and the adhesion of the segmental lesion to the adjacent Bowman’s capsule was

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almost always found, even in the earliest lesion as previously reported (2, 8). Four specimens (36%) and patients (57%) in NS-G and 1 specimen (11%) and patient (14%) in NO-G showed mild mesangial proliferation in the glomeruli without sclerotic lesions. In one NS-G patient all glomeruli had sclerotic lesions. The intensity of tubulo-interstitial lesions, that is, tubular atrophy, interstitial fibrosis and cellular infiltration, was assessed only in the cortex region and graded semiquantitatively as the percentages of involved area to the whole as follows: 0%, −; <25%, +; 25 to 50%, ++; >50%, ++++. There was no difference of the intensity of tubulo-interstitial lesions between the groups. The intensity of vascular lesions, that is, wall thickening and hyaline deposition, was evaluated except 2 NS-G specimens in which no arterioles could be found. The prevalence of vascular lesions was similar in both groups (p > 0.2).

Immunofluorescence study was done on all patients, 10 specimens in NS-G and 7 in NO-G. As previously reported in the literatures (2, 9–12), immunoglobulins and complements, mainly IgM, C3c, and IgG, were detected in the segmental sclerotic regions of glomeruli and sometimes interruptedly in glomerular capillary walls. Less frequent and intense depositions of IgA, Clq, C4, and fibrinogen were also found inconstantly. It was considered that immunoglobulins and complements, plasma proteins, accumulated secondarily into the pathologically-altered sites. Although the findings of light and immunofluorescence microscopy tended to be more intense in NS-G than in NO-G, there were no significant differences between NS-G and NO-G.

In electron microscopic study, 6 specimens in NS-G and 5 in NO-G were available. Vacuoles were sometimes noted in the glomerular epithelial cytoplasm in both groups, and the precise observation revealed that the vacuoles consisted mostly of the dilated rough endoplasmic reticulums (RERs) (Fig. 2) and partly of the lysosomal bodies containing proteinaceous materials and lipids in it (Fig. 3b). The dilated RER varied in size and number in each epithelial cell (Fig. 3). Sometimes the perinuclear space also was found to dilate. However the Golgi complex did not dilate. The dilated RER contained electron-dense flocculent material in it and the concentration of the material was various, diluted to concentrated. Some of those were empty. The RER
Fig. 2. Dilated RER (*) in the glomerular epithelial cell. The cytoplasmic surface of the membrane is studded with polyribosomes. The electron-dense flocculent material is diluted. (×13,200).

which wall ruptured to open to the urinary space was rarely found. Although the size of each dilated RER on the one section does not always express its real size in vivo, there was the tendency that the more dilated the RER, the more diluted the content. The degree of dilatation was comparable in the two groups. Moreover the intensities of epithelial foot process fusion, expansion of the lamina rara interna, epithelial detachment, endothelial swelling, glomerular basement membrane (GBM) abnormality, and electron dense deposit were also similar. There was not a specific finding in each group either. The pathologic changes of the endothelial and mesangial cells were not so prominent compared to that of the epithelial cell. The visceral epithelial cell proliferation was not observed in non-sclerotic portions of

Fig. 3. Grades of dilatation of the RER in the glomerular epithelial cell. In comparison to the RERs (*) with a normal appearance (−) (a) (×5,300), the RERs (*) are slightly dilated, in addition the lysosomal body (L) which contained proteinaceous materials and lipids can be seen (+) (b) (×6,500). The RERs (*) are moderately dilated without the distortion of the cellular shape (+ +) (c) (×6,700), and further dilatation causes the distortion (+ + +). The parietal epithelial cell (P) proliferates (d) (×4,100). The contents of the dilated RERs are condensed in contrast to Fig. 2. The Golgi complex is not dilated. Epithelial detachment (arrowheads) is also seen (c) (d). BM, basement membrane; BC, Bowman’s capsule.
glomeruli and it was rather hypocellular as previously reported (13). Conversely the proliferation of the parietal epithelial cell was sometimes seen along the Bowman’s capsule (Fig. 3d), which was impressive in contrast with the visceral one.

DISCUSSION

In this study, we noticed that beyond our anticipation much proportion of patients with FGS had been non-nephrotic throughout their clinical courses as previously reported (3, 10, 14–16), although in future they might also become nephrotic, and that no differences of clinicopathologic findings between NS-G and NO-G existed except proteinuria and serum total protein or albumin as noted by Beaufils et al. (3), and it was impossible to distinguish the two groups histologically. In addition, electron microscopic study revealed that the change of the glomerular epithelial cell was more prominent than the other cell components of a glomerulus, endothelial and mesangial cells, and moreover the striking fact that the epithelial cell vacuoles consisted mainly of the dilated RERs.

Previous reports have demonstrated that the glomerular epithelial cell synthesized the material for the GBM in normal status (17, 18). This means that the GBM may be maintained by the glomerular epithelial cell. Accordingly it is supposed that the epithelial degeneration found in this study, accompanied the metabolic disturbances of the GBM, that is, proteinuria, hematuria, and probably even morphologic abnormalities. The epithelial cell is demonstrated to be important to the regulation of the permeability of the glomerular capillary wall (19–22). In addition, it has been suggested that the glomerular epithelial cell might be concerned with the mesangial-cell growth regulation (23). We believe that the glomerular epithelial cell may be firstly damaged and altered in FGS as have been previously reported in both human FGS (24) and an animal model of this disease, aminonucleoside nephrosis (25–28), which may result in proteinuria and/or hematuria, and morphologic abnormalities of the GBM. However the etiologic insult itself and the clear relationships of the epithelial degeneration, proteinuria and hematuria, and the segmental lesion remains to be defined. We think that the previously-reported phenomenon that nephrotic FGS patients progress to renal failure more rapidly than non-nephrotic ones (3, 7, 12, 14) may merely reflects the severity of a damage to epithelial cells, although we could not evaluate about it in this study because of the small number of the patients who exhibited the linear relation of a reciprocal of serum creatinine against time.

It is evident that the epithelial vacuoles in this study are the dilated RERs from the following findings: the existence of membrane-bound polyribosome on vacuoles, dilatation of the perinuclear space connecting to the endoplasmic reticulum. Although dilatation of the RER occurs artifactually, there is not its possibility as we could find the normal RER in the same sections. Glomerular epithelial vacuolization has been previously described in both human renal diseases (10, 29–33) and animal experimental models (20, 25, 26, 34–36). Venkatachalam et al suggested the existence of a vacuolar pathway of leaked proteins across the epithelial layer (34), and Messina et al agreed with them (36). It is interpreted that they supposed that the vacuoles belonged to the digestive system in cell, in which the lysosome plays a central role. It is very important to discriminate the dilated RER from the lysosome as these are quite different in each function in cell, although it is unclear whether the vacuoles which we are discussing here are the same as that previously demonstrated. Nowadays, the main function of the RER is considered to be the production of secretory or export proteins, the products are transported to the Golgi complex and secreted to the extracellular space after the processing in the Golgi complex (37). As the RER showed dilatation but the Golgi complex did not, the disturbance of this transportation may partly contribute to this pathologic alteration. The dilatation of the RER in this study is thought to be one expression of the glomerular epithelial degeneration in FGS, since the other findings were consistent with the cell degeneration. And this finding affords the indirect evidence that the metabolism of the GBM maintained by the epithelial cell is out of order in FGS.

Preliminary results are obtained from the electron microscopic study in our laboratory that the epithelial degeneration, particularly epithelial detachment, was marked in FGS compared to in minimal
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change nephrotic syndrome and mild-form IgA nephropathy (unpublished data). And this study demonstrated that the patients in NO-G whose proteinuria was slight showed the same degree of the epithelial degeneration as those in NS-G. Therefore the epithelial degeneration may be not a result but cause of proteinuria as described above and by itself may be a significant factor in the formation of the segmental lesion in FGS, although some investigators have proposed that the increase of glomerular permeability, proteinuria, leads to the segmental lesion (38, 39). The same conclusion as our speculation has been presented (24, 26, 27, 29). In this study the segmental lesion was frequently found to accompany the involvement of the Bowman’s capsule, even in an early lesion. In addition the parietal epithelial cell sometimes proliferated reactively. Therefore, the process, the adhesion of a capillary tuft to the Bowman’s capsule, may be necessary to the formation of the segmental lesion probably by causing the hemodynamic change in the lumen of the connecting tuft, and moreover the parietal epithelial cell may play a certain role in the formation of the segmental lesion. Hiller et al have pointed out the pathologic importance of the adhesion between capillary tufts and the Bowman’s capsule (16).

The segmental lesion can be seen in other glomerular diseases, such as, IgA nephropathy, membranous nephropathy, diabetic nephropathy, Alport’s syndrome, reflux nephropathy. However it becomes specific as the hallmark of FGS only when it occurs solely without the other kinds of morphologic changes and pathologic conditions, and the fact that the segmental lesion is not limited to FGS does not necessarily lower the significance of it. Some of the segmental lesions seen in the other glomerulonephritides may also occur in the same pathogenetic process as that in FGS.

This study invites the speculation that the glomerular epithelial cell is firstly damaged by unknown factor(s), which is manifested functionally as proteinuria and/or hematuria, and morphologically as dilatation of RER, foot process fusion, detachment, and eventually segmental sclerosis and hyalinosis, probably involving the parietal epithelial cell and Bowman’s capsule.

REFERENCES


