Changes of Renal Lesion-Related Parameters in FGS/Nga and the Parental Mouse Strains, CBA/N and RFM/Nga

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Abstract: The FGS/Nga mouse strain, established from an outcross between CBA/N and RFM/Nga mice strains, has previously been reported as a spontaneous mouse model for focal glomerular sclerosis (FGS) and is considered to have two pairs of autosomal recessive genes associated with FGS. In this study, we examined the changes of seven renal lesion-related parameters, blood urea nitrogen (BUN), creatinine, albumin and total protein in plasma, urinary protein, systolic blood pressure, and a glomerulosclerosis index on histological observation, in 20-week-old FGS/Nga mice and their age-matched two parental strains, CBA/N and RFM/Nga. The levels of plasma BUN and creatinine, urinary protein and systolic blood pressure were significantly increased in FGS/Nga, compared with those of the parental strains. RFM/Nga mice showed slightly elevated levels of all biochemical makers. In histological analysis, a higher glomerulosclerosis index was observed in FGS/Nga than the two parental strains. RFM/Nga mice appeared to have slight sclerotic lesions of glomeruli, but no renal failure was observed in CBA/N mice. These results suggest that at least one mutant gene that causes the progression of renal lesion in FGS/Nga mice is derived from RFM/Nga.

Key words: animal model, focal glomerular sclerosis (FGS), mouse, proteinuria

Introduction

Focal glomerular sclerosis (FGS) is one of the most common and nonspecific patterns of glomerular injury, and is usually associated with proteinuria, steroid resistance, hypertension and progressive loss of renal function in human renal diseases [9, 17]. Although it has been demonstrated by previous studies that the development of FGS is involved in immunologic injury, hemodynamic injury and metabolic abnormalities [1, 6, 7, 10], the precise pathogenesis of FGS is still unclear.

The FGS/Nga strain of mouse was introduced to

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KRBIB (Korea Research Institute of Bioscience and Biotechnology) as an animal model with manifesting spontaneous high proteinuria and glomerulosclerosis. This mouse strain was established from offspring at the F5 generation of an outcross between CBA/N and RFM/Nga strains, and FGS/Nga mice have been reported as three types: mice with high proteinuria and wasting syndrome, mice with high proteinuria only, and normal mice [11]. In previous studies, general features such as genetic profile, immunohistochemical and electron microscopical findings for the renal lesion were investigated, and bone marrow stem cell disorder has been suggested as a causative factor for the glomerulosclerosis development of FGS/Nga mice [13, 23]. Recently, it has also been reported that recombinant interleukin-10 gene injection to the kidneys of this mouse reduced the development of glomerulocscerosis [3]. These studies suggest that the renal injury mechanism of FGS/Nga mice might be closely involved with immunologic factors. However, physiological and biochemical observations are insufficient in detail in FGS/Nga mice and the parental strains, CBA/N and RFM/Nga. Therefore, this study was performed to examine the changes of seven renal lesion-related parameters, blood urea nitrogen (BUN), creatinine, albumin, and total protein in plasma, urinary protein, systolic blood pressure, and plasma biochemical markers were measured. For the determination of urinary protein, urine samples collected in metabolic cages for 24 h were directly assessed with an uropaper strip (Eiken chemical, Japan), and a 2+ (100 mg/dl) or higher value was considered as proteinuria. The number of mice with proteinuria was divided by the total number of tested mice, and the obtained values (%) were used for proteinuria incidence. Systolic blood pressure was measured by the tail-cuff method using a SOFTRON (BP-98A, Japan) blood pressure measuring system after pretreatment in a 37°C incubator for 10 min. Blood was collected from the retro-orbital sinus with heparinized capillary tubes, and then plasma was obtained by centrifugation. Using the plasma samples, blood urea nitrogen (BUN), creatinine, total protein and albumin were measured with a blood chemistry analyzer (550 Express, Ciba Corning, USA).

### Materials and Methods

#### Animals

At 20 weeks of age, male FGS/Nga mice without wasting syndrome and its parental strains, CBA/N and RFM/Nga strains, were used for this study (n=20 in each group). FGS/Nga and PFM/Nga mice were introduced from Nagoya University, Japan. The CBA/N were introduced from Central Institute for Experimental Animals, Japan. These mice strains were maintained under a barrier system with controlled temperature (22 ± 2°C), humidity (55 ± 5%), and a 12/12-h light/dark cycle at KRBIB. They were fed a commercial diet (PMI, USA) with tap water ad libitum. Body weight, urinary protein, systolic blood pressure and plasma biochemical markers were measured. For the determination of urinary protein, urine samples collected in metabolic cages for 24 h were directly assessed with an uropaper strip (Eiken chemical, Japan), and a 2+ (100 mg/dl) or higher value was considered as proteinuria. The number of mice with proteinuria was divided by the total number of tested mice, and the obtained values (%) were used for proteinuria incidence. Systolic blood pressure was measured by the tail-cuff method using a SOFTRON (BP-98A, Japan) blood pressure measuring system after pretreatment in a 37°C incubator for 10 min. Blood was collected from the retro-orbital sinus with heparinized capillary tubes, and then plasma was obtained by centrifugation. Using the plasma samples, blood urea nitrogen (BUN), creatinine, total protein and albumin were measured with a blood chemistry analyzer (550 Express, Ciba Corning, USA).

#### Statistical analysis

The data obtained from different groups were analyzed by unpaired Student’s t-test, and a p value of < 0.01 was considered to be statistically significant.

#### Histology

For light microscopic observation, kidneys were removed under ether anesthesia, fixed in 10% neutral-buffered formalin (pH 7.2), embedded in paraffin, cut into 2 µm thick sections, and then these samples were stained with periodic acid-Schiff (PAS). In order to evaluate the glomerular sclerotic changes, a glomerulosclerosis index was calculated by a semiquantitative method using the following criteria. In each specimen, at least 50 glomeruli were graded from 1 to 4 according to the PAS-positive area (1 = 5–15%, 2 = 16–35%, 3 = 36–60%, 4 = 61–100%), then the mean score of the graded glomeruli was obtained by dividing the sum of the graded numbers with the total number of the observed glomeruli. Also, the percentage of affected glomeruli with more than 5% of PAS-positive area (more than 5%) per section was scored from 1 to 4 (1 = 0–10%, 2 = 11–25%, 3 = 26–40%, 4 = >41%). Then, the glomerulosclerosis index was calculated by multiplying the percentage score of affected glomeruli with the mean score of the graded glomeruli.
Results

Body weight

Body weight of FGS/Nga mice was significantly lower than that of the age-matched parental strains, CBA/N and RFM/Nga (p<0.001) (Fig. 1). There was no significant difference between body weights of CBA/N and RFM/Nga mice.

Proteinuria incidence

The incidence of proteinuria, determined with a 2+ or higher value in the uropaper strip test for 24-h urine samples, was significantly increased in FGS/Nga mice compared to the parental strains (p<0.001) (Fig. 2). However, although it was not significant, proteinuria incidence of RFM/Nga mice was slightly higher in RFM/Nga mice than in the CBA/N strain.

Blood pressure

The levels of systolic blood pressure were 110 ± 4.6 mmHg in FGS/Nga, 96 ± 4.6 mmHg in CBA/N and 101 ± 5.2 mmHg in RFM/Nga mice. Although these levels seemed to be normotensive in all groups, the systolic blood pressure of FGS/Nga mice was higher than that of its parental strains. No significant difference was found between CBA/N and RFM/Nga mice.

Biochemical markers

Plasma BUN and creatinine levels indicating renal function were significantly increased in FGS/Nga mice, compared to those of the parental strains (p<0.01) (Fig. 3A and B). However, plasma total protein and albumin were slightly lower in FGS/Nga than in RFM/Nga and CBA/N mice (Fig. 3C and D). Although the difference was not significant, BUN level was higher in RFM/Nga than in CBA/N mice.

Histological findings

In kidney microscopy on PAS-stained specimens, glomerular lesions characterized by focal and segmental collapse of capillaries associated with expansion of mesangial matrix and tubular atrophic changes were easily observed in FGS/Nga mice (Fig. 4A). Also, the semiquantitative score for glomerulosclerosis was significantly higher in FGS/Nga than those of age-matched CBA/N and RFM/Nga mice (p<0.001) (Table 1) (Fig. 4B). However, no severe glomerular sclerotic changes were found in either CBA/N or RFM/Nga mice.

Discussion

In this study we examined the changes in the biochemical markers of kidney glomerular sclerosis in urine and plasma, and blood pressure and histological findings in FGS/Nga mice and the parental strains, CBA/N and RFM/Nga, at 20 weeks of age. By these examinations, we confirmed that the FGS/Nga mouse strain had serious renal lesion and related biochemical and physi-
It is well known that massive proteinuria is related with glomerular lesions including capillary walls, swelling and hypertrophy of visceral epithelial cells, extensive fusion or effacement of foot processes and hyaline granular changes [8, 12, 19]. Also, high levels of plasma BUN and creatinine have been commonly used for the evaluation of renal function in previous studies [21, 22]. Moreover, glomerular sclerosis is often accompanied with hypoproteinemia and hypoalbuminemia in animal models and humans [16]. In this study, we observed a high incidence of proteinuria, increased plasma creatinine and BUN, and decreased total protein and albumin in FGS/Nga. These results strongly suggest that the FGS/Nga mouse strain has severe kidney glomerular defects.

Clinically, persistent hypertension is known to be a deleterious factor that may often predispose to renal diseases with irreversible fatal destruction [2, 14]. In the previous experimental studies in different hypertensive models, it was found that increased glomerular pressure might be involved in the development of glomerulosclerosis which was associated with a compensatory glomerular hyperfiltration [4, 5, 18]. In this study, we observed that the systolic blood pressure of FGS/Nga was slightly higher than CBA/N and RFM/Nga mice. However, blood pressure is an unlikely causative factor for renal problems in FGS/Nga mice because the level of systolic blood pressure is normotensive. Moreover, the systolic blood pressure measured in FGS/Nga at 4 weeks of age was similar to that of age-matched CBA/N and RFM/Nga mice (data not shown). Based on these examinations, the slightly higher blood pressure observed in FGS/Nga mice compared to the parental strains at 20 weeks of age, is likely to be one of the physiological changes due to the progression of renal lesion. However, intraglomerular blood pressure should be measured to examine if the aforementioned
Physiologic Changes of FGS Mouse Strain

Renvascular hemodynamic factors affect on renal lesion progression in FGS/Nga mice. Histologically, FGS/Nga mice showed glomerular sclerotic changes such as focal and segmental collapse of capillaries associated with expansion of mesangial matrix and tubular atrophy when stained with PAS. Semiquantitative analyses for glomerular sclerotic changes gave an index score that was significantly higher than those of the parental strains. Morphologically, these histological findings are very similar to those of human FGS lesions reported in previous studies [15, 20]. On the other hand, one of the parental strains, RFM/Nga, showed glomerular sclerosis and proteinuria, even though their severity and incidence were weaker and lower than FGS/Nga, whereas there were no significant changes in the other of the parental strains, CBA/N. These results suggest that the FGS/Nga mouse might have at least one recessive allele that causes the progression of renal lesion derived from RFM/Nga. Also, the FGS/Nga mouse strain was confirmed as a desirable animal model for studying human FGS and its related pathophysiological changes.

Table 1. Glomerular Sclerotic Changes in FGS/Nga, RFM/Nga and CBA/N Mice

<table>
<thead>
<tr>
<th>Strains</th>
<th>No. of mice</th>
<th>Number of affected glomeruli (%)</th>
<th>Mean score of sclerotic area</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGS/Nga</td>
<td>20</td>
<td>46.9 ± 3.8*</td>
<td>2.70 ± 0.09*</td>
</tr>
<tr>
<td>RFM/Nga</td>
<td>20</td>
<td>11.7 ± 2.1</td>
<td>1.52 ± 0.02</td>
</tr>
<tr>
<td>CBA/N</td>
<td>20</td>
<td>7.2 ± 1.7</td>
<td>1.00 ± 0.03</td>
</tr>
</tbody>
</table>

* All values are represented as mean ± SE. * significantly different from RFM/Nga and CBA/N (p<0.001).

Fig. 4. A representative glomerular micrograph showing mesangial matrix expansion stained with PAS in 20 week-old FGS/Nga mice × 200, Bar = 45 µm (A); and glomerulosclerosis index obtained by semiquantitative method in FGS/Nga, RFM/Nga, and CBA/N mice (B). Values are the mean ± SE. * significantly different from RFM/Nga and CBA/N (p<0.001).

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References
