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Up-regulation of glomerular extracellular matrix and transforming growth factor-β expression in RF/J mice

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Up-regulation of glomerular extracellular matrix and transforming growth factor-β expression in RF/J mice.

Background. RF/J mice were first reported as a murine model of spontaneous glomerulosclerosis by Gude and Lupton in 1960, but the precise histologic characteristics and immunopathological background of this mouse have not been investigated further.

Methods. Measurements of serum levels of immunoglobulins, anti-single strand DNA (anti-ss-DNA) antibody, complement (C_3), and circulating immune complex (IC) were performed. Analyses of glomerular histological and immunopathological lesions in association with the detection of mRNA expression of collagen IV, TGF-β, matrix protein turnover related enzymes, matrix metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinase-2 (TIMP-2) and platelet-derived growth factor (PDGF) were also performed in young (10-week-old) and elderly (60-week-old) RF/J mice with age-matched BALB/C mice as the controls.

Results. High levels of serum IgA and IgG from as early as 20 weeks of age were noted in the RF/J mice. Serum anti-ss-DNA antibody of aged RF/J mice increased up to 23% of that of aged MRL-*lpr/lpr* mice, and serum C_3 concentration significantly decreased with age, reaching lower levels than that of BALB/c mice. IgA-IC levels were significantly high compared to BALB/C mice both in the early and late stages of life, whereas IgG-IC levels were high only in mice younger than 20 weeks. Semiquantitative and quantitative analyzes of renal histopathological findings revealed significantly marked and age-related mesangial matrix expansion in RF/J mice, with increasing frequency of global glomerular sclerosis and tubulointerstitial damage. On the other hand, although precise measurements of glomerular cell numbers also showed an apparent augmentation in both young and old RF/J mice compared to BALB/C mice, glomerular cellularity decreased with age in RF/J mice. Immunohistochemical study revealed massive immunoglobulin deposition from a young age in association with significantly higher accumulation of matrix proteins, such as types I and IV collagen and laminin from the early stage of life. In addition, in these glomeruli, transforming growth factor-β1 (TGF-β1) was highly expressed both in young and old mice. The mRNA expression of MMP-2 was up-regulated only in the early stage of life. Although PDGF mRNA of RF/J mice was significantly up-regulated in the early stage of life, the differences between the mice disappeared in the late stage of life.

Conclusions. These findings suggest that in RF/J mice, an immunopathological background inducing high serum immunoglobulin and IC levels from the early stage of life is closely related to mesangioproliferative glomerular lesions mediated by PDGF, and that development of massive extracellular matrix accumulation in glomeruli was induced by up-regulated expression of TGF-β with inappropriate regulation of protein turnover-related enzyme production.

Key words: glomerulosclerosis, collagen type I and IV, laminin, transforming growth factor-β, extracellular matrix, protein turnover.

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Immune-complex-mediated glomerulonephritis is frequently observed not only in autoimmune diseases, including lupus nephritis, but also in primary glomerular diseases such as IgA nephropathy [1]. A substantial quantity of circulating immune complexes is often detected in autoimmune diseases, which evokes not only systemic symptoms (such as arthritis) or vasculitis, but also local lesions found especially in active glomerulonephritis. In immune-complex mediated glomerulonephritis, the glomerular lesions are characterized by the prominent proliferation both of resident and infiltrative cells and marked accumulation of extracellular matrices that sometimes progress to glomerular sclerosis. In these sclerotic lesions, in addition to the increased expression of extracellular matrix (ECM) components normally observed in glomeruli, such as collagen types IV and V, laminin and heparan sulfate proteoglycan (HSPG), altered components of ECM are also included, appearing as the expression of collagens I and III [2], which comprise the interstitial matrix under normal conditions. Although the precise mechanism of the pathogenic action of immune complexes (IC) leading to progressive glomerulosclerosis is not yet known, experimental trials using prepared IC have produced various types of glomerular lesions [3].

On the other hand, studies using cultured mesangial cells revealed a marked increase of ECM components in

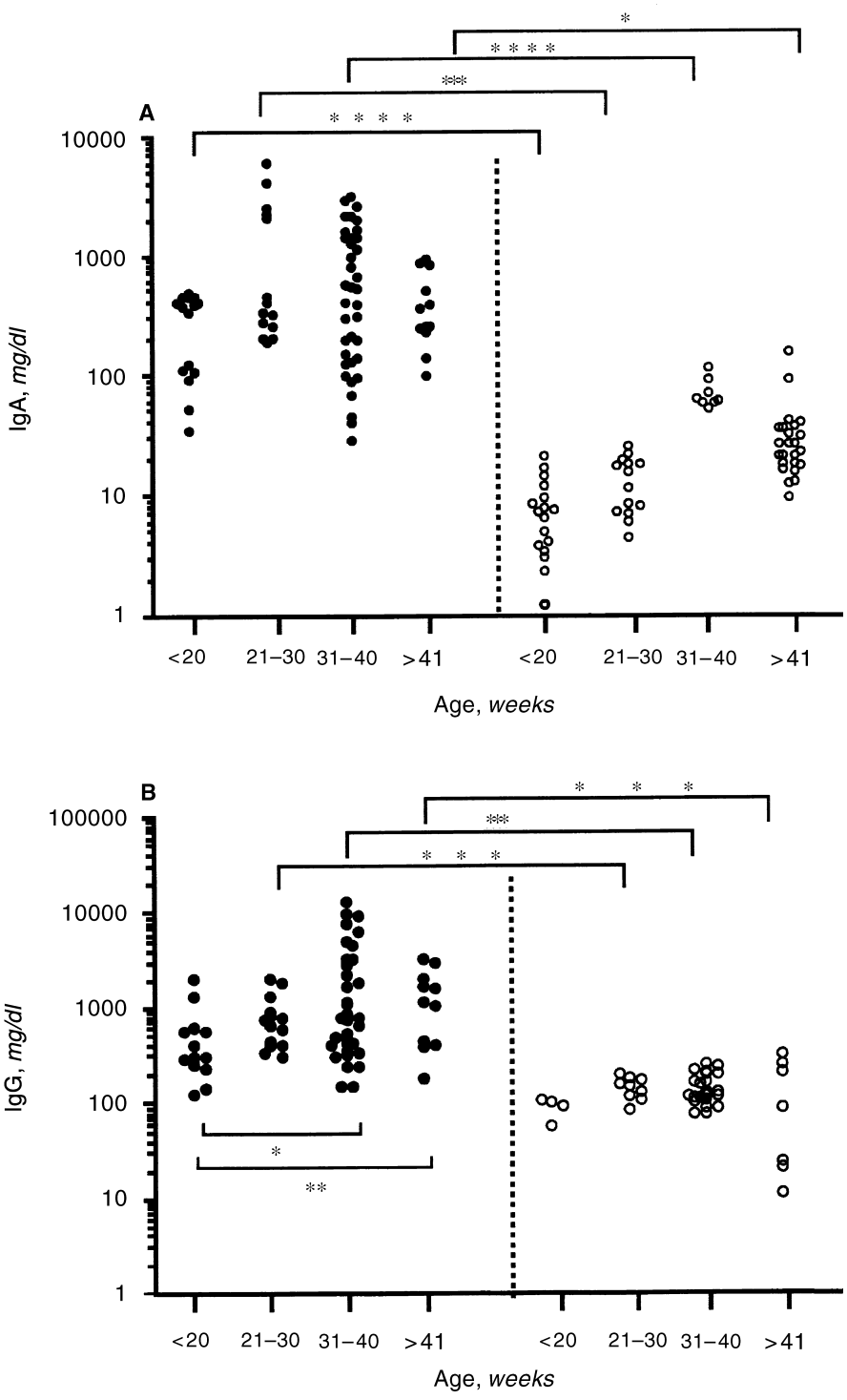

Fig. 1. Serum concentration of IgA (*A*) and IgG (*B*) in RF/J mice (●) and BALB/c mice (○). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.0001$.

culture supernatant and highly up-regulated transforming growth factor-β (TGF-β) mRNA expression of cells after stimulation with IC, which were neutralized by the addition of specific antibodies to TGF-β in the culture medium [4].

Although these studies using experimental models and cultured mesangial cells clearly proved the pathogenic effect of IC stimulation, studies evaluating chronic IC stimulation *in vivo*, which may frequently occur in human IC-mediated glomerulonephritis, are limited.

In this regard, animal models of spontaneously developing IC-mediated glomerulonephritis are expected to be very useful. Although there are several murine models of autoimmune diseases with both systemic and renal

Table 1. Serum levels of anti-single strand-DNA antibody

Mouse strain	Serum anti-ss-DNA antibody[a]
BALB/c 10 weeks old	1.3 ± 0.1%
BALB/c 60 weeks old	2.4 ± 0.3%
RF/J 10 weeks old	9.3 ± 0.8%
RF/J 60 weeks old	22.6 ± 4.1%[b]

[a] Serum anti-ss-DNA antibodies were measured by ELISA. Results were expressed as percentage to that of pooled sera of aged MRL-1*pr/1pr* mice. $N = 8$ in each group

[b] $P < 0.01$ vs. RF/J at 10 weeks old

Table 2. Serum levels of complement

Mice	Serum C_3[a]
RF/J 10 weeks old	117 ± 12%
RF/J 60 weeks old	80 ± 5%[b]

[a] Serum complement (C_3) of RF/J mice measured by single radial immunodiffusion assay. Results are expressed as percentage of that of pooled age matched BALB/c sera. $N = 8$ in each group

[b] $P < 0.01$ vs. RF/J 10 weeks old

lesions, it is relatively difficult to establish models showing high circulating IC levels and specific glomerular lesions that progress to sclerosis. Recently, by selective mating of ddY mice, we established an inbred strain as a murine model of IgA nephropathy that shows high serum IgA levels and progressive mesangial matrix accumulation with highly up-regulated expression of TGF-β mRNA in renal tissue with aging [5]. In this mouse, however, expressions of apparently altered ECM components, such as collagen I and III, which are sometimes seen in highly sclerotic lesions, were not observed.

RF/J mice were first reported as a murine model of spontaneous glomerulosclerosis by Gude and Lupton [6], who found that moderate to severe glomerulosclerosis was present in animals 8 to 12 months old. While analyzing renal lesions in this mouse, we detected massive immunoglobulin deposition in the glomeruli and high serum levels of both immunoglobulins and IC. In the present study, to evaluate this strain of mouse as a spontaneous murine model of IC-mediated glomerulonephritis progressing to glomerulosclerosis, we analyzed the immunopathological characteristics of sera and renal lesions in young and elderly RF/J mice, including serum levels of immunoglobulins, antinuclear factor, serum complement (C_3) and IC, and mRNA and protein expressions of the ECM components of the glomerulus and interstitium. The renal expression of TGF-β, profibrogenic cytokine, and ECM turnover-related enzyme proteins, such as metalloproteinase and tissue inhibitor of metalloproteinase, were also examined.

METHODS

Solid-phase immunoglobulin and antinuclear factor assay

Serum levels of IgA, IgG, and IgG isotype anti-single strand DNA (anti-ss-DNA) antibodies were measured by sandwich ELISA as described previously [7, 8].

Serum complement measurement

Serum levels of C_3 were measured by single radial immunodiffusion assay. Briefly, diluted sera of RF/J mice and serially diluted pooled sera of BALB/c mice were applied to wells made in gel containing 0.1% agarose-LGT (Nakalai, Kyoto, Japan), 26 mM barbital sodium (Nakalai), 50 mM sodium acetate, 10 mM EDTA-2Na (Dojin, Kumamoto, Japan) and 300 μg/ml goat-antimouse C_3 antiserum (Cappel Laboratories, Malvern, PA, USA). After a 24 hour incubation at room temperature in a moist chamber, the diameters of precipitin rings were measured. The concentration of the serum C_3 was calculated and expressed as a percentage using the standard curve obtained by age matched pooled BALB/c sera.

Immune complex measurement

Serum IgA and IgG class immune-complexes (IC) were measured by solid phase anti-C_3 assay by a modification of previous methods [9]. In brief, microtiter wells coated with rabbit antimouse C_3 (Cappel Laboratories) were incubated with serum samples diluted 1:10 with 1% BSA-PBS containing 0.01 M EDTA and 0.05% Tween 20 at 37°C for two hours. After washing, alkaliphosphatase labeled goat antimouse IgG and IgA F $(ab')_2$ was applied and incubated at 37°C for two hours, and after addition of the substrate solution (paranitrophenyl phosphate (Sigma Chemical Co., St. Louis., MO, USA), absorbance at 405 mm was measured.

Urinary protein examination

Urinary protein was examined when the mice were sacrificed, using Multisticks (AMES, Tokyo, Japan). The results were graded semiquantitatively from (−) to (++++), as follows for urinary protein: (−) negative, (±) trace, (+) 30 mg/dl, (++) 100 mg/dl, (+++) 300 mg/dl, (++++) 1000 mg/dl.

Histopathological analysis of renal tissues

Histopathological evaluation was performed as previously described [5]. Briefly, a portion of renal specimens from RF/J and BALB/c mice aged 10 and 60 weeks were fixed in Doubosque Brazil solution and embedded in paraffin. Sections (2 μm) were stained with hematoxylin and eosin (HE) and periodic acid Schiff (PAS). Semiquantitative microscopic analysis was performed with a grading of (−) normal, (+) mild, (++) moderate, and (+++) severe employed to denote the extent of mesangial matrix expansion. Tubulointerstitial damage was also graded in the areas where 100 glomeruli were counted as follows: (−) no lesions showing cell infiltration and

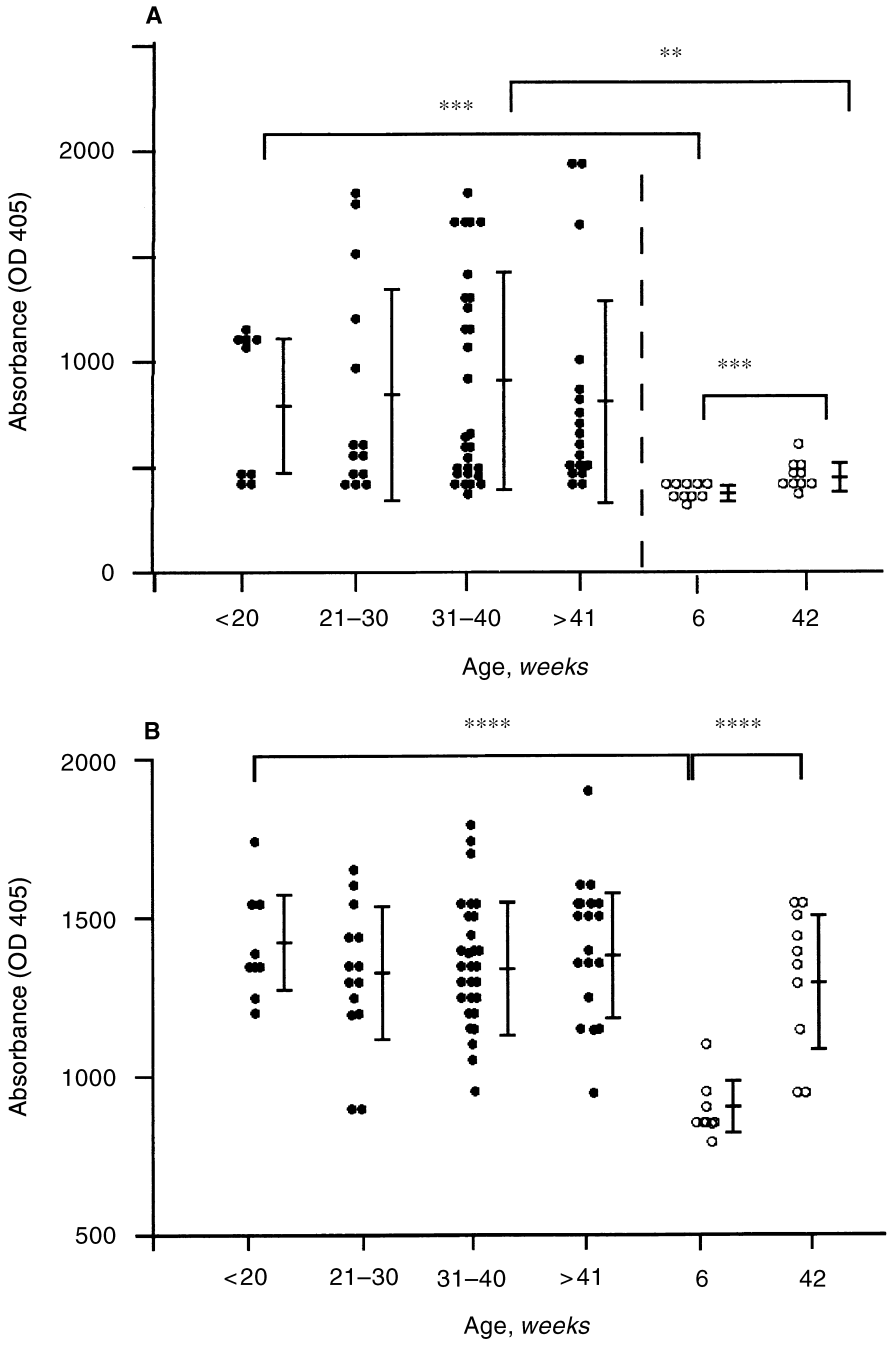

Fig. 2. Serum immune-complex levels in RF/J mice (●) and BALB/c mice (○). (*A*) IgA-C_3, (*B*) IgG-C_3. $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.0001$. Data are means ± SD.

fibrosis, (+) single focus of lesion, (++) more than two isolated foci, (+++) diffuse infiltration and fibrosis. Positivity for global glomerular sclerosis was defined by the presence of more than one sclerotic glomerulus in a total count of 100 glomeruli. The presence of cellular crescent formation was also examined in 100 glomeruli.

To quantitatively evaluate the number of glomerular cells per area, renal tissue sections obtained from 10 RF/J mice and 6 BALB/c mice at age 10 and 60 weeks were randomly selected. Ten selected glomerular sections containing both vascular and urinary poles were counted, and the cell numbers per area of the glomerular tuft expressed as the number per glomerular cross section were counted as described previously [5]. To evaluate age-related changes in glomerular size in RF/J mice, the areas of 10 randomly selected glomeruli in each section from the same mice as those used for the cell count were measured with an image analyzer (Luzex 3 U; Nikon Ltd, Tokyo, Japan); the mean area was compared in animals of different ages.

Immunopathological examination

Serial immunofluorescence studies of IgG, and IgA were performed as previously described [10]. Deposition of immunoglobulins was also graded semiquantitatively, from absent (−) to severe (+++). All the histological examinations were performed independently by two investigators who had no knowledge of the experimental data. Staining of ECM was performed in 10- and 60-week-old RF/J and BALB/c mice by an indirect method using specific antibodies: polyclonal rabbit antimouse collagen type I antibody (Chemicon International Inc., Temecula, CA, USA), polyclonal rabbit antimouse collagen type IV antibody (Becton Dickinson, Bedford, MA, USA), polyclonal rabbit antimouse laminin antibody (Collaborative Biomedical Products, Bedford, MA, USA). The second antibody was FITC-labeled polyclonal goat anti-rabbit IgG (Zymed Laboratories, San Francisco, CA, USA). The detection of TGF-β was performed by a direct method using polyclonal rabbit antibody raised against a peptide corresponding to amino acid residues 328−353 mapping within the carboxy terminal region of human TGF-β1 (Santa Cruz Biochemistry Inc., Santa Cruz, CA, USA) [11] that was labeled with FITC in our laboratory. This antibody was proven to react with both human and murine TGF-β1 [12].

Electron microscopic examination

Small blocks of the renal cortex were fixed in phosphate-buffered 2% glutaraldehyde for two hours and then in buffered 1% osmium tetroxide for two hours at room temperature, after which they were dehydrated in a graded series of ethanol and embedded in Epok 812 (Oken, Tokyo, Japan). Ultrathin sections cut 0.1 μm thick were then double stained with uranyl acetate and lead citrate and observed by electron microscopy (HITACHI H7100, Hitachi Ltd, Tokyo, Japan).

Northern analysis of extracellular matrix and cytokines

RNA from the kidneys of BALB/c and RF/J mice aged 10 and 60 weeks was extracted by the acid guanidium thiocyanate-phenol-chloroform method [13]. Ten micrograms of total RNA was fractionated in 0.66 M formaldehyde-1% agarose gel and transferred to a nylon membrane (Biodyne PALL, Glen Cove, NY, USA). The following cDNA probes were labeled with ^{32}P using a random primed DNA labeling kit (Boehringer Mannheim Biochemica, Mannheim, Germany): cDNA probe for type IV collagen α1 chain was a gift from Dr. Y. Yamada (NIH, Bethesda, MD, USA) [14]; cDNA for human MMP-2 was provided by Dr Barry. L. Marmer (Washington University School of Medicine, St. Louis, MO, USA) [15]; human TIMP-2 cDNA [16] and human GAPDH [17] were purchased from ATCC (Rockville, MD, USA); PDGF-B chain cDNA was provided by Dr. Daniel F. Bowen-Pope (University of Washington School of Medicine, Seattle, WA, USA). The radioactivity of each lane of the membranes was visualized and analyzed with a bio-imaging analyzer BAS2000 II (Fuji Photo Film Co., Ltd, Tokyo, Japan) The quantitative densitometric measurements of the Northern blots were normalized to the signal for GAPDH mRNA.

Table 3. Proteinuria of female RF/J and BALB/c mice

Age *weeks*	Mouse *strain*	*N*	Proteinuria[a] −	±	+	++	+++
10	RF/J	7	0	4	2	1	0
10	BALB/c	7	2	3	2	0	0
30	RF/J	7	0	1	4	2	0
30	BALB/c	7	0	1	5	1	0
50	RF/J	10	0	0	6	1	3
50	BALB/c	10	1	6	3	0	0

[a] Semiquantitative analysis: (−) negative; (±) trace; (+) < 30 mg/dl; (++) 100 mg/dl; (+++) 300 mg/dl

Statistical analysis

All values are expressed as means ± SE. Statistical significance was determined by Student's *t*-test for the comparison of two sets of unpaired data, except for semiquantitative data, which was analyzed by Mann–Whitney's *U*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Serial analysis of serum IgG and IgA and antinuclear factor measurement

As shown in Fig. 1, serial analyses of serum immunoglobulin in RF/J mice showed high levels of serum IgA and IgG from as early as 20 weeks of age, although relatively high individual differences were observed. The average concentration peaked at 31 to 40 weeks for both IgA and IgG. Serum anti-ss-DNA antibody levels were not high in young RF/J mice, but in the late stage of life, it substantially increased up to 23% of that of aged MRL-*lpr/lpr* mice (Table 1).

Serum complement measurement

Table 2 shows the serum C_3 levels of RF/J mice. Although the concentration was somewhat higher than that of BALB/c mice at 10 weeks of age, it significantly decreased with age and reached a lower level than that of control.

Immune-complex measurement

IgA immune complex (IC) levels were significantly higher than those of BALB/c mice both in the early and late stages of life (Fig. 2). It was noted that in contrast to the uniformly significant increase of serum IgA in

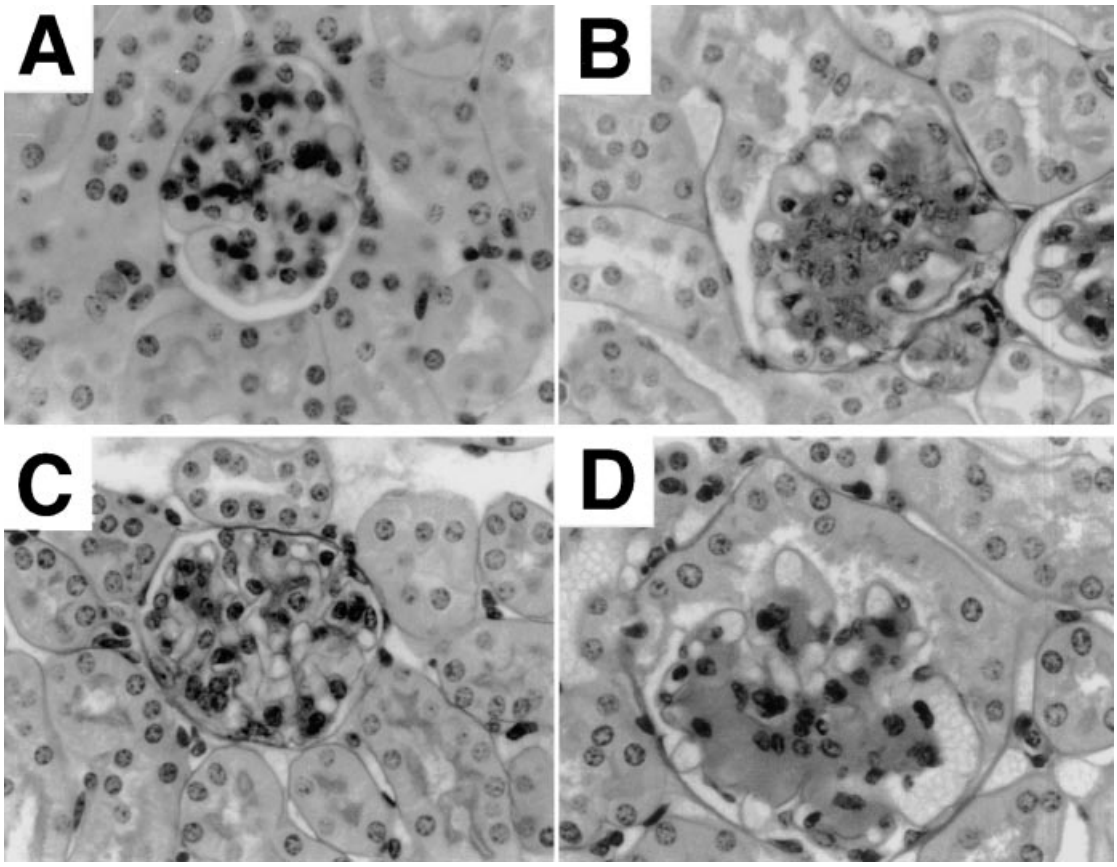

Fig. 3. Light micrographs of glomeruli. (*A*) A BALB/c mouse at 10 weeks and (*C*) at 60 weeks of age; (*B*) RF/J mouse at 10 weeks and (*D*) at 60 weeks of age. Panels A, B, C and D correspond to mesangial matrix expansion grades of (−), (++), (+) and (+++), respectively (PAS stain, ×600).

comparison with the control mice, the IgA-IC showed relatively greater individual differences. On the other hand, IgG-IC in RF/J mice were significantly higher at 10 weeks of age than BALB/c mice, but no significant difference was observed at more than 40 weeks.

Urinary findings

Table 3 shows the serial findings of proteinuria in female RF/J and BALB/c mice. The number of mice showing proteinuria of more than 30 mg/dl was increased in those older than 50 weeks. None of the mice showed severe proteinuria of 1000 mg/dl. There was no marked hematuria in RF/J mice at any time (data not shown).

Immunohistopathological findings

Microscopic findings. Serial microscopic findings were examined at the ages of 10 and 60 weeks in both BALB/c and RF/J mice. As shown in Figure 3, analysis of the mesangial lesion revealed a significant matrix expansion with aging, as previously reported [6]. The results of the semiquantitative analysis are summarized in Table 4. At 60 weeks of age, 90% of the RF/J mice showed moderate to marked mesangial matrix expansion, in contrast to only 25% of BALB/c mice of the same age. In addition to semiquantitative analysis, quantitative analysis using morphometry was performed (Fig. 4A). A marked increase in the number of glomerular cells per area was noted in both young and old RF/J mice compared with BALB/c mice, although this significantly decreased with aging. On the other hand, quantitative analysis of glomerular size revealed a significant increase with age in RF/J mice (Fig. 4B).

Although the incidence of mice showing crescent formation was not high, and there was no increase in this incidence with aging, about 20% of RF/J mice showed glomeruli with this lesion. In contrast, absolutely no BALB/c mice showed crescent formation. On the other hand, although both mice showed a relative increase in the incidence of glomerulosclerosis with aging, the increase in RF/J mice was remarkably high compared to that in BALB/c mice at 60 weeks of age. Tubulointerstitial lesions, namely, focal cellular infiltration and fibrosis, were frequently observed in aged RF/J mice.

Immunofluorescence findings

Immunoglobulin deposition. As shown in Figure 5, marked granular deposition of IgA and IgG, mainly in

Table 4. Serial semiquantitative analysis of microscopic findings in RF/J and BALB/c mice

Mouse strain	Age *weeks*	N[a]	Mesangial matrix expansion				Crescent formation	Global sclerosis	Tubulointerstitial lesion			
			−	+	++	+++	Positive mice[b]		−	+	++	+++
RF/J	10	18	3	13	2	0	4(22)	1(6)	17	1	0	0
	60	19	0	1	7	11	4(21)	11(58)	3	7	9	0
BALB/c	10	17	17	0	0	0	0(0)	1(6)	17	0	0	0
	60	12	2	7	3	0	0(0)	3(25)	10	2	0	0

[a] Total number of mice
[b] Number of mice with more than one glomerulus showing crescent formation or global sclerosis out of a total of 100 glomeruli; percentages of crescent formation shown in parentheses

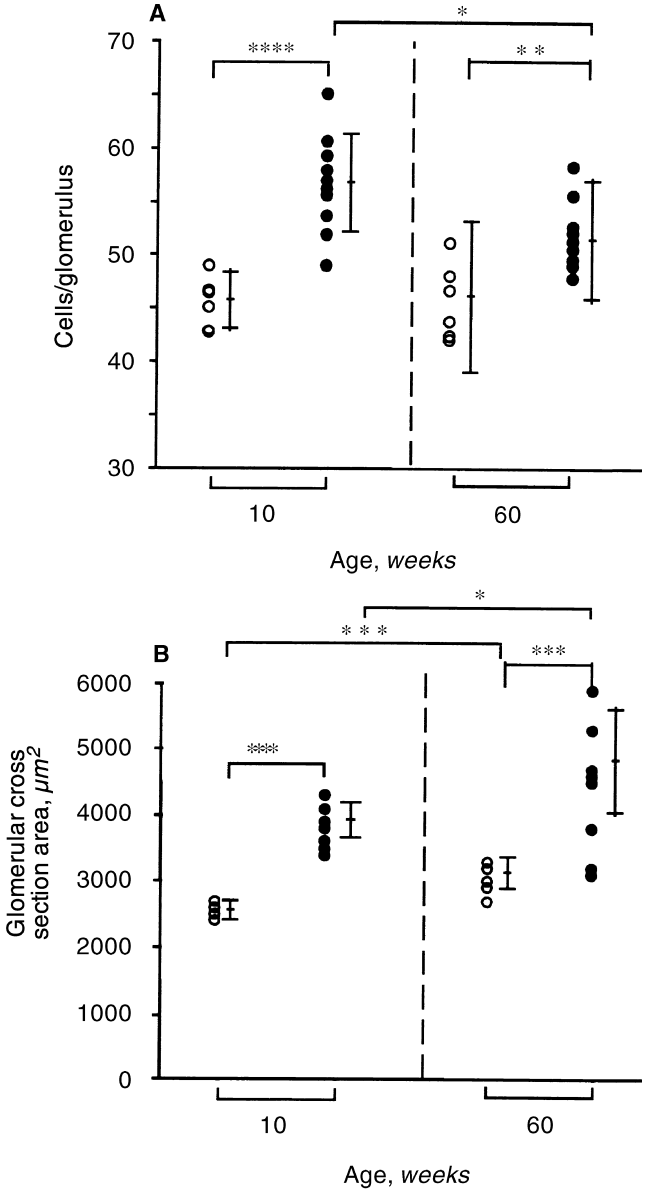

Fig. 4. Morphometric analysis of average cell number per glomerular cross section (*A*) and glomerular size (*B*) in the renal tissues of RF/J mice (●) and BALB/c mice (○) at 10 and 60 weeks of age. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$, $****P < 0.0001$. Data are means ± SD.

the mesangial area, was noted in RF/J mice. These depositions were apparent at as early as 10 weeks and increased with aging (Fig. 6). Although both IgA and IgG deposits were significantly marked at any age, in comparison with IgA deposition, IgG deposits were more intensely noted in both young and old mice.

Extracellular matrix staining. Immunofluorescent analysis of ECM in RF/J and BALB/c mice revealed a significant increase in collagen types IV and I and laminin staining in glomeruli of young and aged RF/J mice (Fig. 7). There were few individual differences in ECM staining between the two groups of mice.

Glomerular TGF-β staining. As shown in Figure 8, marked staining of TGF-β was detected in the glomeruli of RF/J mice. It was also noted that the localization of the TGF-β was exclusively limited to the glomeruli. As shown in the semiquantitative analysis (Fig. 9), staining was apparent at as early as 10 weeks of age and markedly increased with aging.

Electron microscopic findings

As shown in Figure 10, marked expansion of the mesangial area with numerous granular electron dense deposits was observed in 60-week-old RF/J mice. The number of mesangial cells was relatively low.

Northern blot analysis of ECM and cytokines

Figure 11 summarizes renal mRNA expressions of type IV collagen (A), MMP-2 (B), TIMP-2 (C), TGF-β (D), and PDGF-B chain (E) in RF/J and BALB/c mice aged 10 and 60 weeks. In contrast to the immunofluorescence findings, the expression of mRNA of type IV collagen and TGF-β showed no significant differences between the strains or between the two age groups of each strain. In contrast, mRNA expression of matrix protein turnover enzymes and PDGF revealed some difference: while TIMP-2 mRNA expression did not change significantly, MMP-2 mRNA in RF/J mice was significantly up-regulated compared with BALB/c at the early stage, and no change was observed in the late stage of life. Also, RF/J mice had significantly increased PDGF mRNA levels at a young age, which attenuated after aging.

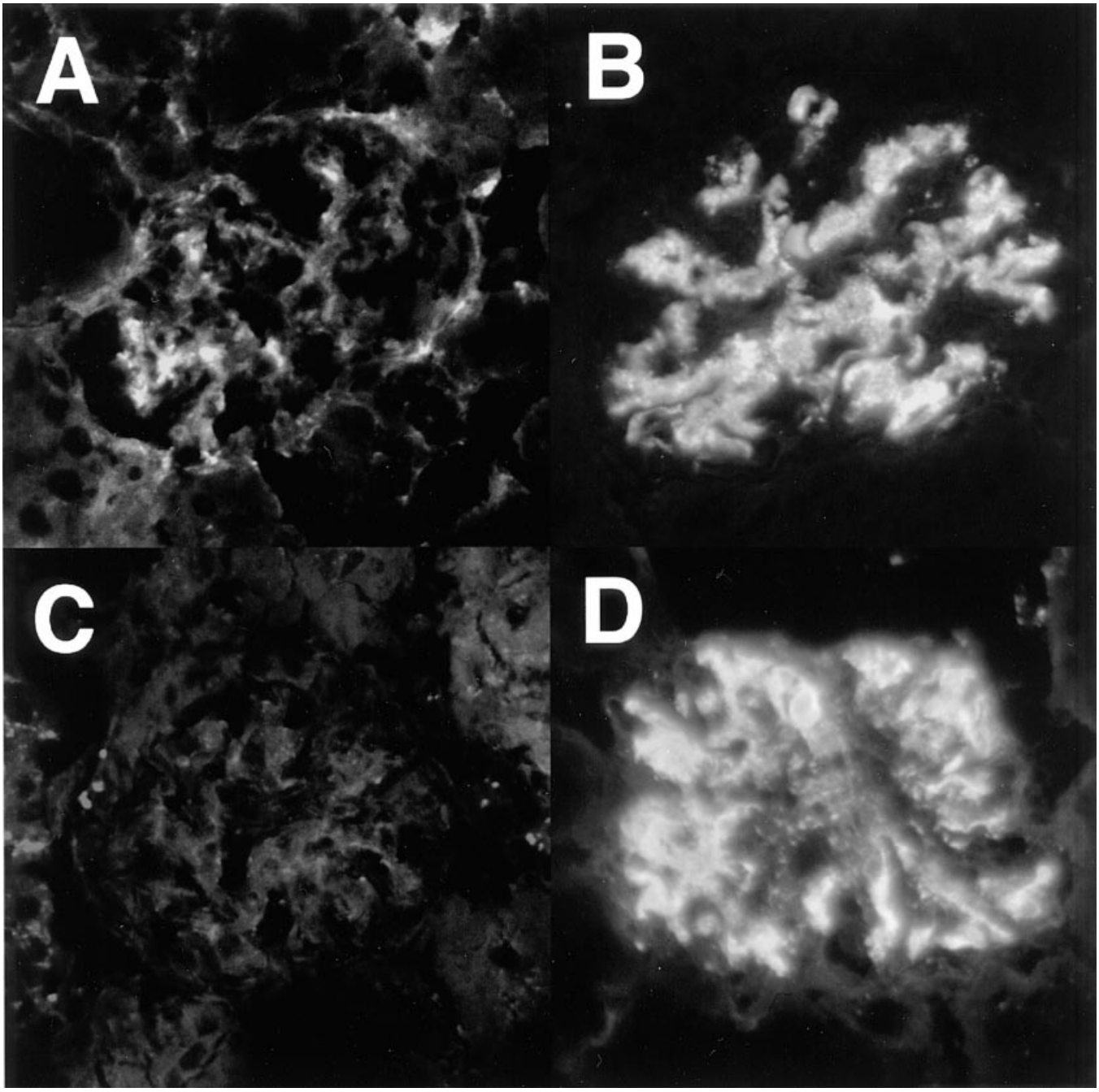

Fig. 5. Immunofluorescent micrographs of IgA staining of BALB/c mouse (*A*) and RF/J mouse (*B*) glomeruli, and IgG staining of BALB/c mouse (*C*) and RF/J mouse (*D*) glomeruli at 60 weeks of age (×800).

DISCUSSION

In the present study, we analyzed the immunopathological background of renal lesions in RF/J mice, a strain originally reported by Gude and Lupton as a murine model of spontaneously developing glomerulosclerosis with age. Serial analyzes of the sera in these mice revealed significantly high serum IgA and IgG concentrations and immune complex levels from a young age. Quantitative evaluation of the microscopic exams revealed not only a significant enlargement of glomerular size with matrix expansion, but also a marked increase in cellularity, especially in young mice. Immunohistological analysis revealed mesangial depositions of IgA and IgG in association with increased matrix components of collagen types I and IV and laminin, with colocalization of TGF-β, which was associated with high mRNA expres-

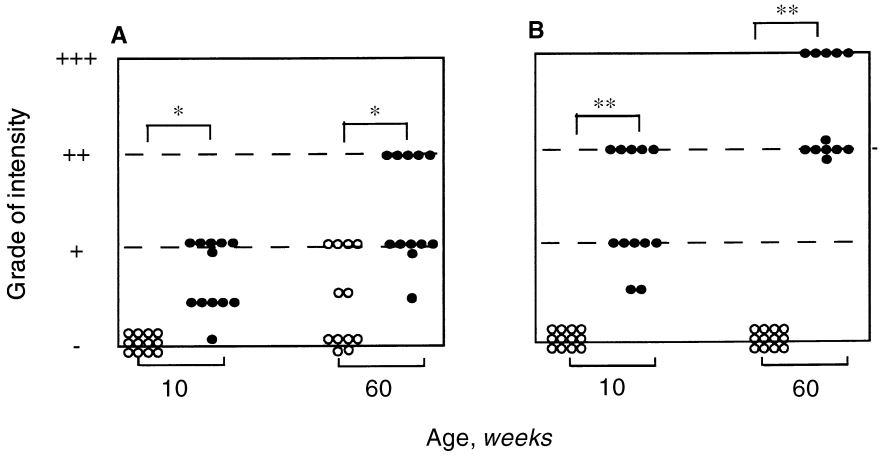

Fig. 6. Semiquantitative analysis by immunofluorescent staining of IgA (*A*) and IgG (*B*) in the glomeruli of RF/J mice (●) and BALB/c mice (○) at 10 and 60 weeks of age (*$P < 0.001$, **$P < 0.0001$).

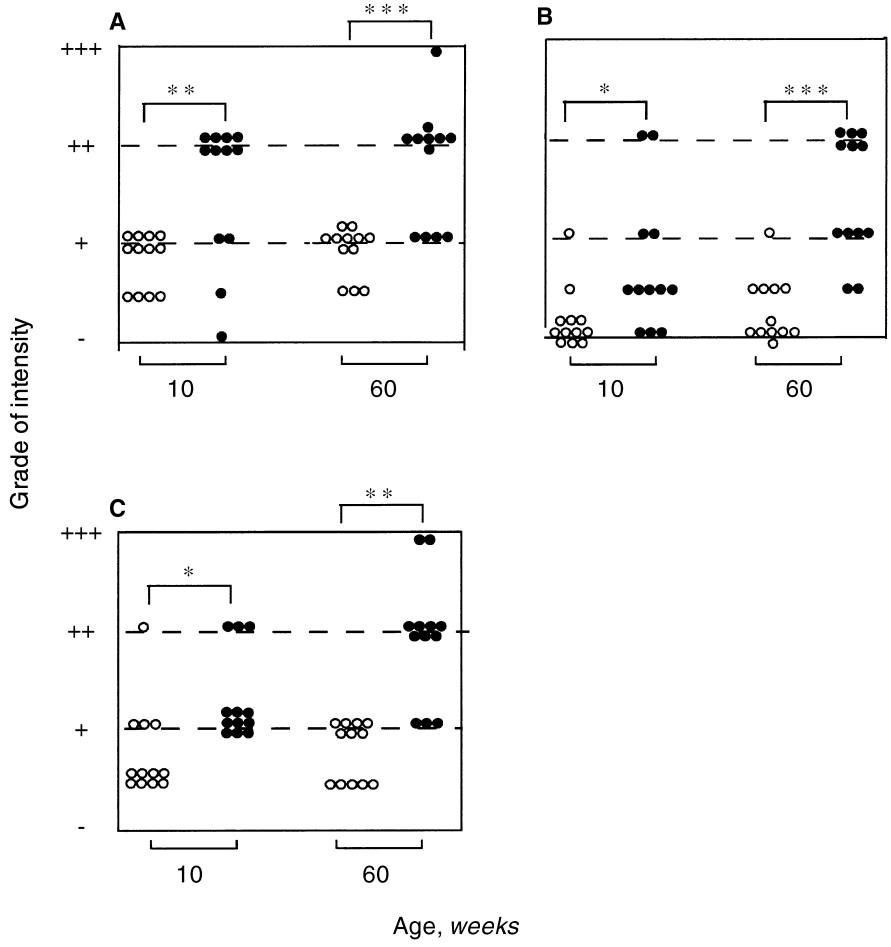

Fig. 7. Semiquantitative analysis by immunofluorescent staining of type IV collagen (*A*), type I collagen (*B*), and laminin (*C*) in the glomeruli of RF/J mice (●) and BALB/c mice (○) at 10 and 60 weeks of age.

sion of PDGF and MMP-2 in the early stage of life. The mRNA levels of collagen IV and TGF-β did not show significant change. Regarding the discrepancy between the results of the Northern blot and immunohistochemical analyses, the following possibilities were postulated: (*1*) Local accumulation of matrix protein depends not only on the degree of production, but also on that of degradation. (*2*) In addition to locally produced TGF-β, circulating TGF-β may be trapped in the mesangial matrix. Our findings indicated that the renal lesions in this strain of mice are mediated by immune complexes that may stimulate renal, and possibly glomerular, sequential

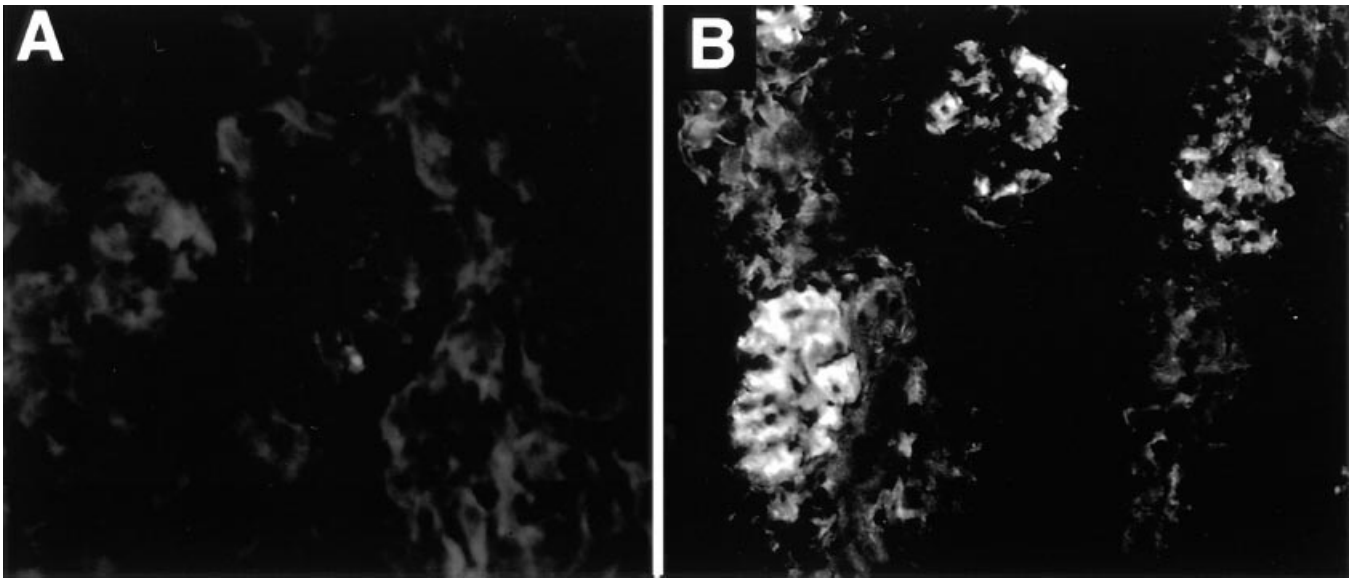

Fig. 8. Immunofluorescent micrograph of a BALB/c mouse (*A*) and an RF/J mouse (*B*) glomeruli at 60 weeks of age (stained with anti-TGF-β1 antibody; ×400).

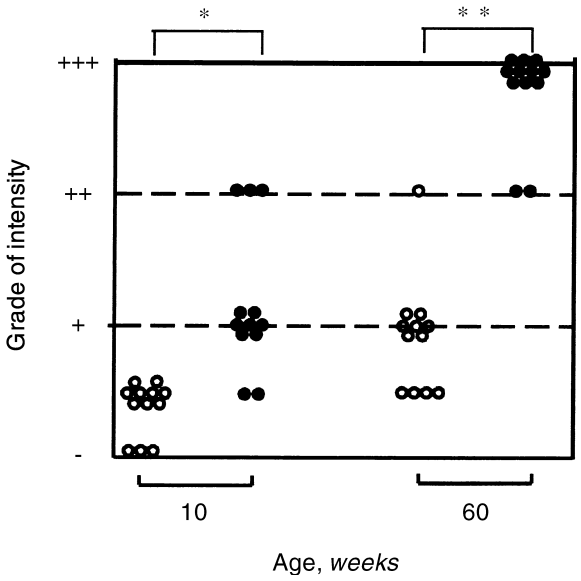

Fig. 9. Semiquantitative analysis by immunofluorescent staining of TGF-β1 in the glomeruli of RF/J mice (●) and BALB/c mice (○) at 10 and 60 weeks of age (*$P = 0.0001$, **$P < 0.0001$).

production of TGF-β and MMP-2. These findings also lead to at least two possibilities as to the cause of renal lesions in RF/J mice: (*1*) the activation of systemic humoral immunity, which induced high serum levels of immunoglobulins and sequential immune-complex formation, and (*2*) specific response of local glomerular resident cells, especially of mesangial cells, to these serological abnormalities.

The immunopathological background of this mouse has been examined in some previous studies. In the 1980s, several studies on cellular immunity in this mouse were published. Nara et al disclosed that among several different stains of inbred mice, a specific susceptibility to infection by Candida Albicans was noted in a subgroup of strains, including RF/J mice. Low or nondetectable amounts of migration inhibitory factor (MIF) and interferon (IFN)-γ are released into the circulation with an insufficient elicitation of delayed hypersensitivity [18]. They reported that this high susceptibility to Candida Albicans infection was improved by the up-regulation of MIF [19]. These findings indicated that cellular immunity is suppressed in this mouse. On the other hand, there have been no reports on the humoral immunity in this mouse. The significant hypergammaglobulinemia and massive formation of circulating immune complexes evident from a young age in our present study are the first data indicating the specific deviation to the activated humoral immunity of this mouse. Although the antigens of these immune complexes are unknown, some endogenous antigens may be involved, in light of the genetic uniformity of this mouse. Substantial increase of serum anti-DNA antibody accompanied by the decrease in serum C_3 with age indicate a predisposition to lupus-like pathology in this strain, and suggest the endogenous DNA as a candidate for the pathogenic antigen.

In the analysis of glomerular cellularity of this mouse, a highly increased number of glomerular cells was noted, especially in young mice, in association with increased PDGF mRNA expression. On the other hand, in old mice, the decrease in number of cells was noted in contrast to the enlargement of glomerular size. These find-

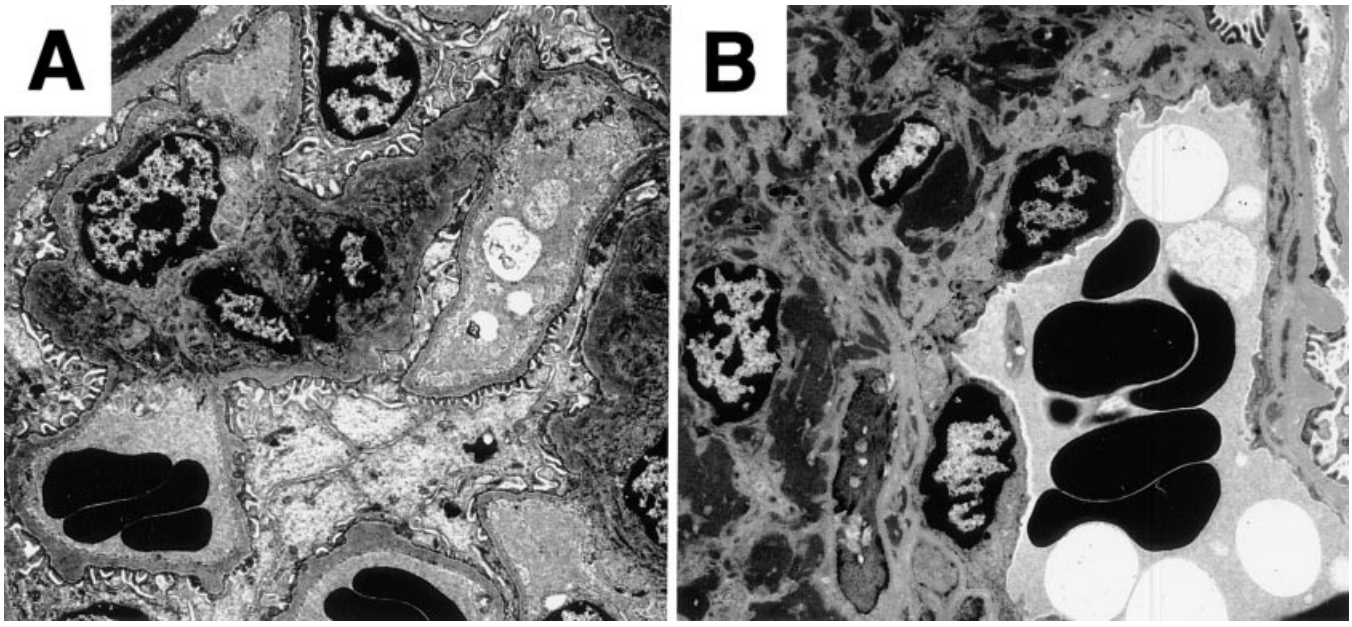

Fig. 10. Electron microscopic photograph of the glomerulus of a BALB/c mouse (*A*) and an RF/J mouse (*B*) at 60 weeks of age (×5000).

ings were also associated with a decrease of PDGF mRNA expression. There have been several reports, not only in human glomerulonephritis, but also in animal models, showing the combined findings of up-regulated PDGF expression and marked glomerular immunoglobulin deposition with proliferative increase of glomerular cells [20]. Recently, in experimental immune-complex mediated glomerulonephritis, a high level of intraglomerular PDGF mRNA was detected in association with interleukin (IL)-1 mRNA [21]. Although there was no correlation between the histologic grade of renal damage and the expression of PDGF mRNA, the authors suggested that PDGF played the role of the competence factor, rather than a progressive factor in the pathogenesis of their model of immune-complex induced glomerulonephritis. In RF/J mice, however, an association between the grade of proliferative glomerular lesion with high cellularity and the expression of PDGF mRNA was observed. Although the precise analysis was limited in the present study, since the detection of mRNA was not performed in isolated glomeruli, we highly suspect that the up-regulated PDGF mRNA expression was induced by augmented circulating immune complexes that were markedly deposited in glomeruli from the early stage of life. Considering the significant attenuation of both glomerular cellularity and PDGF mRNA after aging and progressive glomerulosclerosis, PDGF might play a pathogenic role in initiating glomerular lesions in this mouse.

In the present study, we observed a marked accumulation of glomerular ECM as well as TGF-β1 in RF/J mice compared with BALB/c mice. This may be not only the consequence of proliferative glomerular injury due to immune-complex deposition, but also the result of direct stimulation of glomerular cells by immune-complexes. Recently, Lopez, Gomez and Egido reported that the exposure of cultured human mesangial cells to IgA and IgG complexes increased extracellular matrix components at both mRNA and protein levels [4]. They also showed that both IgA and IgG complexes caused an augmentation in TGF-β1 mRNA expression and the conversion of latent TGF-β to the biologically active form in mesangial cells, possibly mediated by the linking of immunoglobulin to the Fc receptors, although they only examined the involvement of the Fc alpha receptor. In our study, although the glomerular staining of IgG was more intense than that of IgA, simultaneous onset of immunoglobulin deposition and glomerulopathy was observed. This suggests that the high serum levels of immunoglobulins and the resultant increased formation of immune-complexes of both IgA and IgG in the circulation are inevitable in the progression of ECM accumulation in this strain of mice.

TGF-β belongs to a family of polypeptides that regulates various biological activities such as embryogenesis tissue repair, class switching of immunoglobulins, and ECM formation [22] as further supported by its suppression by antisense oligonucleotides for TGF-β1 in an anti-Thy 1.1 glomerulonephritis model [23]. TGF-β is also reported to mediate ECM accumulation by decreasing synthesis of MMPs and increasing synthesis of TIMPs and plasminogen activator inhibitor [24]. On the other hand, in an experimental model of acute immune-complex mediated glomerulonephritis, a significant increase of mRNA expressions of TGF-β and MMP-2 was ob-

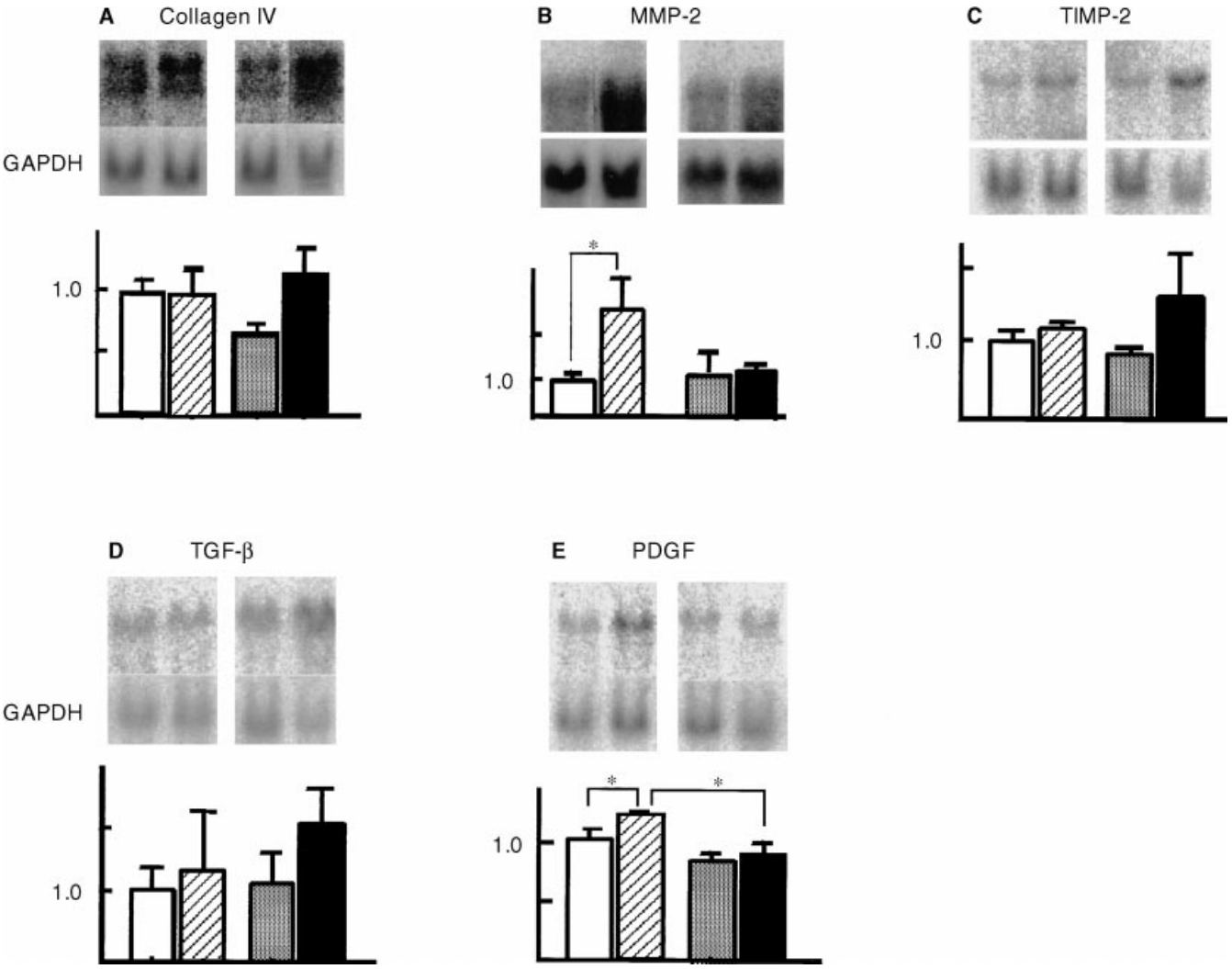

Fig. 11. Northern blot analysis of type IV collagen (*A*), MMP-2 (*B*), TIMP-2 (*C*), TGF-β (*D*), and PDGF (*E*) mRNA levels in renal tissues from RF/J mice (▨, 10 weeks; ■, 60 weeks old) and BALB/c mice (□, 10 weeks; ▩, 60 weeks old). The numbers on the *y*-axis represent a relative scale ($*P < 0.05$; $N = 4$ in each group).

served in the expanded mesangial hypercellular lesion [25]. In the present study, we demonstrated the distinct glomerular TGF-β1 staining and an early surge of renal MMP-2 mRNA with no significant change in TIMP-2 expression in RF/J mice that showed marked hypercellular lesions with immune complex deposition. As for the TIMP expression in the murine kidney, using standard polymerase chain reaction, Carome et al reported the complete absence of glomerular TIMP-1 gene expression *in vivo* both in normal and sclerotic transgenic mice for bovine growth hormone, in contrast to the markedly upregulated expression of the MMP-2 gene specifically in sclerotic glomeruli [26]. They suggested the possibility of the glomerular expression of another type of TIMP, such as TIMP-2 or TIMP-3, whose DNA probes were not available at that time. Our finding of TIMP-2 expression in the kidney was consistent with their suggestion, and the cDNA probe that we used was for human TIMP-2, which showed 92% homology for murine TIMP-2 [27]. It is interesting that after aging, in contrast to the more apparent TGF-β expression at the protein level, MMP-2 mRNA decreased in renal tissue. These findings indicated that in this specific strain of mice the mesangial depositions of overproduced immune complexes might promote TGF-β production, which alter the balance of matrix protein turnover enzymes and lead to subsequent ECM accumulation.

In summary, to our knowledge this study is the first report on the immunopathological findings in renal lesions in young and old RF/J mice, a possible murine model of spontaneous immune-complex mediated glomerulonephritis because it shows severe mesangial matrix expansion with accumulation of ECM proteins and TGF-β in the glomeruli accompanied by an increase in serum and

glomerular IgA and IgG, and immune-complex deposition with age. The possibility of a local specific response, especially by mesangial cells, to activated humoral immunity remains to be studied.

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