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Localization of Extracellular Matrix Receptors in ICGN Mice, a Strain of Mice with Hereditary Nephrotic Syndrome

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ABSTRACT. Fibrotic degeneration was examined in the kidneys of ICR-derived glomerulonephritis (ICGN) mice, a novel inbred mouse line with a hereditary nephrotic syndrome of unknown etiology considered to be a good model of human idiopathic nephrotic syndrome. In the present study, we histochemically revealed changes in accumulation of extracellular matrix (ECM) components and in localization of integrins, cellular receptors for ECM, in the kidneys of ICGN mice with the progression of renal failure. Excessive accumulation of basement membrane (laminin and collagen IV) and interstitial (type III collagen) ECM components were demonstrated in the glomeruli and tubulointerstitum of ICGN mice. Marked deposition of type I collagen and tenascin was seen only in the glomeruli of ICGN mice but not in those of ICR mice as normal controls. Increased expression of integrin α1-, α2-, α5- and β1-subunits in glomeruli with fibrotic degeneration and abnormal distribution of α6-subunit were noted in the kidneys of ICGN mice. Excessive laminin, a ligand of $\alpha 6\beta 1$-integrin, was demonstrated on the tubular basement membrane, but α6-subunit diffusely disappeared on the basal side of the tubular epithelial cells. We presumed that abnormal integrin expression in renal tubules causes epithelial cell detachment, and consequently tubular nephropathy, and results in disorder of ECM metabolism causing excessive accumulation of ECM components in the kidneys of ICGN mice.

KEY WORDS: extracellular matrix (ECM), hereditary nephrotic mouse (ICGN), immunohistochemistry, integrin, kidney.

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ICR-derived glomerulonephritis (ICGN) mice develop a spontaneous nephrotic syndrome and are thus considered a good model of human idiopathic nephrotic syndrome. The nephrotic syndrome in ICGN mice is characterized by thickening of the glomerular capillary basement membrane, mesangial expansion without noticeable cellular proliferation and tubulointerstitial fibrotic degeneration [12–17, 28–31]. Adult ICGN mice show progressed nephrotic syndrome, that is, development of proteinuria, hypoproteinemia, anemia and systemic edema [12–17]. Recently, we histochemically demonstrated that levels of extracellular matrix (ECM) components are increased in glomeruli and the tubulointerstitum of ICGN mice with progressed nephrotic syndrome [29, 30], and suggested that the abnormal ECM accumulation was due to overexpression of active-TGF-β1, which was detected only in kidneys of ICGN mice but not in those of ICR mice used as normal controls [29]. Then, we biochemically measured the activities of matrix metalloproteinases (MMPs), which degrade ECM components, in the kidney tissues of ICGN mice to elucidate the mechanisms responsible for the abnormal accumulation of ECM components. Lower activities of MMP-1 and MMP-2 and -9, which dominantly degrade type I and type IV collagens, respectively, were demonstrated in the kidneys of ICGN mice as compared with those of ICR mice, but there were no significant differences in the activity of MMP-3, which has a broader substrate specificity and degrades type IV and V collagens, proteoglycans and laminin, between these strains [30]. Thus, degradation of ECM components is reduced in the kidneys of ICGN mice, and the excess accumulation of ECM components may be caused by this reduction. However, the mechanisms responsible for the pathogenic correlation between the abnormal accumulation of ECM components and cellular degeneration in the kidneys of ICGN mice have not yet been determined.

Previously, using lectin histochemistry we demonstrated the changes in cell-surface carbohydrate constitution in the kidneys of ICGN mice [28]. Positive staining of *Bandeiraea simplicifoli*a lectin-I (BSL-I), which specifically binds to α-D-galactopyranosyl groups, was observed only in distal tubules of ICGN mice but not in those of ICR mice. The levels of three characteristic cell-surface glycoproteins (45, 58 and 64 kD), that were identified by lectin blotting for BSL-I in the ICGN kidney, were augmented in kidneys of ICGN mice with the progression of renal failure. These characteristic cell-surface molecules are considered to be pathogenic factors that cause renal disease in ICGN mice. Thus, determination of the interaction between cell-surface molecules and ECM components is necessary to investigate the molecular mechanism of renal disorders in ICGN mice. Cell to ECM interaction, i.e. the interaction between integrin (cell-surface adhesion receptor for ECM) and ECM (ligand), plays an important role not only in maintaining tissue integrity but also in pathogenic mechanisms of disease [1, 2, 5, 21, 22]. Integrins constitute a family of heterodimeric transmembrane glycoproteins consisting of non-covalently associated α- and β-subunits, which extra-

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cellularly bind to matrix components and are intracellularly linked with cytoskeletal elements. To date, at least 13 α-subunits and 10 β-subunits have been identified, and each α-subunit is coupled with a corresponding β-subunit. Most of the integrin α-subunits are usually coupled with the β1-subunit and are exceptionally coupled with other β-subunits. At least nine potential α-subunits coupled with β1-subunit (heterodimers associated with α1- to α8- and αv-subunits, named VLA-1 to -8 and -αv, respectively) have been reported, most of which, with exception for VLA-4 (α4β1-integrin) which is a ligand for vascular cell adhesion molecule-1 (VCAM-1), are cellular receptors for ECM components, work as cell adhesion molecules, and regulate the cell functions such as proliferation, death, differentiation, transformation and degeneration. In addition, α4-, α6- and αv-subunits couple with different β-subunits, leading to alteration of cell functions [10–14]. We hypothesized that the cellular degeneration, i.e. deciduation of tubular epithelial cells, in the kidneys of ICGN mice with progressed nephrotic symptom is caused by abnormal interactions between ECM components and integrins. Therefore, it is essential to determine the changes in the localization of excessive ECM components and its cellular receptors for elucidation of the pathogenic mechanisms responsible for cellular degeneration in the kidneys of ICGN mice. We have reported that ECM distributions in the kidneys of ICGN mice were altered with the development of pathema, but the changes in the localizations of integrins have not yet been investigated. In the present study, we histochemically examined differences in the localization of integrins and their related ECM components, which predominantly regulate renal cell functions, in the kidney tissues of ICGN mice with the progression of renal failure and of ICR mice as healthy controls.

MATERIALS AND METHODS

Animals and sample preparation: ICGN mice were prepared by mating between homozygous males (nep/nep) and heterozygous females (nep/-) at the laboratory of the National Institute of Infectious Diseases (NIID, Tokyo, Japan) [12–17, 28–31]. Five-, 10-, 15- and 30-week-old homozygous male ICGN mice from a specific-pathogen-free colony in NIID and age- and sex-matched ICR mice purchased from Clea Japan (Tokyo, Japan) were used. All animals were housed in autoclaved metal cages and were given a standard diet (CM, Oriental Yeast Co., Tokyo, Japan) and tap water *ad libitum* in an air-conditioned room (23 ± 1°C), under controlled lighting conditions (12L/12D). They received humane care as outlined in the "Guide for the Care and Use of Laboratory Animals" (Kyoto University Animal Care Committee according to NIH (#86–23; revised 1999). For clinical biochemical analyses, urine samples were collected during the 24 hr before sacrifice (24-hr urine samples), and blood samples were obtained from the cervical vein under ether anesthesia. Serum was prepared by centrifugation at 3,000 rpm for 15 min. The animals were killed under deep ether anesthesia, and then the kidneys were rapidly removed. For conventional histopathological evaluation, a half of each kidney was immediately fixed in 10% phosphate buffered formalin, pH 7.4. For histochemical measurement of total and type I collagens and immunohistochemical detection of integrin-subunits and ECM components in the kidney tissues, the remainder of each kidney was put on filter paper, mounted in OCT compound (Ames Co., Elkhart, IN, U.S.A.), and then rapidly frozen in dry ice-cooled isopentane [29, 30]. The frozen tissue samples were kept at –80°C until use.

Clinical biochemistry: As previously reported [31], blood and 24-hr urine samples were examined to evaluate nephrotic state and loss of renal function as follows: serum and urinary albumin (sAlb and uAlb, respectively), serum creatinine (sCr), blood urea nitrogen (BUN) and total cholesterol (sTC) levels were measured using an automatic analyzer (Fuji Dri-Chem 3500V; Fuji Film Co., Tokyo, Japan). All procedures were performed according to the manufacturers' protocols.

Renal histopathology: As previously reported [28–31], renal histopathological evaluation was performed. Briefly, formalin-fixed paraffin-embedded sections (3 μm thick) were stained with hematoxylin-eosin and with Sirius red F3B (BDH Chemicals Ltd., Poole, U.K.). Sirius red staining detects interstitial collagens, and the extent of glomerulosclerosis was expressed as the degree of ECM deposition [10, 11]. The morphological changes in the glomeruli (capillary aneurysm and hypercellularity), and tubular (cystic tubular dilation, epithelial cellular atrophy and intraluminal cast formation) and tubulointerstitial (tubulointerstitial expansion and mononuclear cell filtration around arterioles) lesions were evaluated, and 100 glomeruli/kidney selected at random were evaluated for mesangial expansion in the glomeruli and scored.

Histochemical quantification of total and type I collagens: As described in our previous report [10, 11, 31], total and type I collagen levels in each kidney section were measured by a microquantitative methods for evaluation of the ECM deposition in kidney sections. Briefly, frozen sections (5 μm thick) mounted on glass slides were incubated with rabbit anti-mouse tail type I collagen antibody (LSL Co., Tokyo, Japan) and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (American Qualex, La Mirada, CA, U.S.A.). After washing, chromogenic substrate solution (30 mM phenol, 3 mM 4-aminoantipyrine and 2 mM H_2O_2 in 50 mM Tris-HCl, pH 7.2) was applied to each section, and the optical density of the solution at 450 nm was determined with a spectrophotometer (Ultrospec 3000, Pharmacia Biotech, Uppsala, Sweden), and then the type I collagen content in each section was calculated. After measurement, the total protein and total collagen levels in each section were measured colorimetrically.

Immunohistochemistry for ECM components and integrin-subunits: To elucidate the changes in localization of integrins and ECM components in the kidney sections of ICGN mice, immunofluorescence analyses were performed.

Table 1. Antibodies used in this study and their dilutions

First antibodies	Dilution
Anti-α1 integrin subunit Goat IgG[a)]	1/100
Anti-α2 integrin subunit Goat IgG[a)]	1/100
Anti-α3 integrin subunit Rabbit IgG[b)]	1/150
Anti-α4 integrin subunit Rabbit IgG[b)]	1/200
Anti-α5 integrin subunit Rabbit IgG[b)]	1/150
Anti-α6 integrin subunit Rat IgG[b)]	1/200
Anti-β1 integrin subunit Rabbit IgG[b)]	1/150
Anti-β2 integrin subunit Rat IgG[b)]	1/100
Anti-laminin Rabbit IgG[c)]	1/100
Anti-tenascin Rat IgG[d)]	1/200
Anti-fibronectin Rabbit IgG[d)]	1/200
Anti-type I collagen Rabbit IgG[d)]	1/100
Anti-type III collagen Rabbit IgG[e)]	1/150
Anti-type IV collagen Rabbit IgG[d)]	1/100

a) Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.; b) Chemicon, CA, U.S.A.; c) ICN Pharmaceuticals, Aurora, OH, U.S.A.; d) Sigma; and e) Rockland, Girbertville, PA, U.S.A.

Briefly, fresh frozen sections (5 μm thickness) cut on a cryostat were mounted on glass slides precoated with 3-aminopropyltrimethoxysilane (Aldrich, Tokyo, Japan) and fixed with cold acetone (–80°C). After washing with phosphate buffered saline (PBS, pH 7.4) for 10 min at room temperature (RT, 20–25°C), the slides were incubated with each specific antibody against integrin subunits (see Table 1) diluted to the optimum concentration with PBS-bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, Mo, U.S.A) for 18 hr at 4°C. They were washed with PBS and incubated with each appropriate fluorescein isothiocyanate (FITC)-conjugated antibody (American Qualex) for 90 min at RT. When double staining was performed to compare the localization of integrin subunits and integrin ligands, second antibodies conjugated with FITC and Fluoro-Li nk™Cy™3 (Amersham Pharmacia, Little Chalfont, U.K.) or Texas Red (ICN Pharmaceuticals, Aurora, OH, U.S.A.) were used. After washing with PBS, the slides were mounted with glycerol and examined with a conventional fluorescence microscope (BHS-RFC, Olympus, Tokyo, Japan) and/or with a confocal laser scanning microscope (Fluoview FV300, Olympus). Intensity of immunohistochemical reaction was graded as strongly positive (+++), moderately positive (++), weakly positive (+) and negative (-).

Statistical analysis: ANOVA with Fisher's least significant differences test for comparison of biochemical data, and Wilcoxon's signed rank test for morphological estimation were carried out with the StatView IV program using a Macintosh computer. Differences at a probability of $p<0.05$ were considered significant. All data are expressed as mean values ± SD (n=10).

RESULTS

Biochemistry, histopathology and renal collagen levels: Adult ICGN mice (30 weeks old) manifested clinical abnormalities such as exercise intolerance, pale ears and weight loss. Table 2 shows biochemical parameters measured to evaluate the nephrotic state and loss of renal function. ICGN mice showed severe hypoalbuminemia (1.49-fold decreases as compared with age-matched ICR mice) and albuminuria (no urinary albumin was detected in ICR mice). Marked increases were observed in sCr (3.00-fold increases as compared with ICR mice) and BUN (2.22-fold increase as compared with ICR mice) in ICGN mice, indicating the loss of renal function in these animals. Increased sTC (1.70-fold increases as compared with ICR mice) suggested severe hypercholesterolemia in ICGN mice. Thus, ICGN mice were in the nephrotic state.

Table 2. Serum and urine biochemical features in control (ICR) and ICGN mice

	ICR mice	ICGN mice
sAlb[a)], (g/d*l*)	3.18 ± 0.13	2.13 ± 0.16***
uAlb[a)], (mg/m*l*)	not detected	13.15 ± 3.56
sCr[a)], (mg/d*l*)	0.30 ± 0.03	0.90 ± 0.10***
BUN[a)], (mg/d*l*)	31.8 ± 1.9	70.7 ± 5.4***
sTC[a)], (mg/d*l*)	120.2 ± 5.1	204.5 ± 15.1***

a) For details, see Materials and Methods.
* $p<0.05$; $p<0.001$ versus each ICR group.

Histopathological examination revealed that adult ICGN mice had abnormal kidneys; 54.8 ± 3.8 and 12.5 ± 2.3% of kidney sections showed expanded mesangial area and capillary aneurysm, respectively (Table 3). In contrast, most age-matched ICR mice as healthy controls (97.8%) had normal kidneys. As previously reported [8], when compared with ICR mice, enlargement of the mesangial region without cellular proliferation, tubulointerstitial expansion, appearance of cysts, expansion of renal tubules, and infiltration of inflammatory cells were observed in the kidneys of 30-week-old ICGN mice. Moreover, excessive accumulation of interstitial collagen in glomeruli and the tubulointerstitum of ICGN kidneys, but no progressed fibrotic degeneration was seen in the renal medulla. Thus, glomerulonephritic, and glomerular- and tubulointerstitial-fibrotic degeneration became severe only in ICGN mice.

Total and type I collagen levels in renal cortex sections of ICR and ICGN mice assessed by a microquantification method are summarized in Table 3. ICGN mice showed 1.73-fold increases in total collagen levels, and 1.75-fold increases in type I collagen levels, as compared to ICR mice, indicating renal fibrotic degeneration in ICGN mice.

Immunohistochemical localization of ECMs and integrin subunits: As same as our previous report [29], immunohistochemical localization of ECM components in the kidney sections of ICR and ICGN mice were seen (data were not shown). In brief, type I collagen was not detected immunohistochemically in normal glomeruli or the tubulointerstitum of ICR mice. In the kidney sections of ICGN mice, however, marked deposition of type I collagen was seen in expanded glomeruli and tubulointerstitial lesions. Trace

Table 3. Histopathological changes of glomeruli, and total and type I collagen levels in the renal cortex of control (ICR) and ICGN mice

	ICR mice	ICGN mice
Normal[a)], (%)	97.8 ± 1.2	32.7 ± 3.1***
Expansion of mesangial areas[a)], (%)	2.2 ± 1.2	54.8 ± 3.8***
Capillary aneurysm[a)], (%)	not observed	12.5 ± 2.3
Total collagen[b)], (mg/g protein)	4.35 ± 0.47	7.52 ± 0.43***
Type I collagen[b)], (mg/g protein)	2.00 ± 0.20	3.50 ± 0.20***

a) These histopathological changes are described in detail in Materials and Methods.
b) For details, see Materials and Methods.
*** $p<0.001$; versus each ICR group.

Table 4. Histochemical localization and immunohistochemical staining intensities of each integrin subunit in kidney sections of control (ICR) and ICGN mice

Integrin subunits	Glomerulus		Proximal tubules		Distal tubules	
	ICR	ICGN	ICR	ICGN	ICR	ICGN
α1	+	++	–	+	–	+
α2	–	+	–	+	–	+
α3	+++	++/+++	–	+	–	+
α4	–	+	–	–	–	–
α5	+	++	–	–	–	–
α6	–	–	+	-/+/++	+	-/+/++
β1	++	+++	++	++	++	++
β2	–	–	–	–	–	–

Intensities of immunohistochemical reactions: +++: strongly positive, ++: moderately positive ,+: weakly positive and -: negative.

deposits of fibronectin and type III collagen were observed in glomeruli and the interstitium of the kidneys of ICR mice, but increased deposition of both fibronectin and type III collagen were noted in glomeruli and tubulointerstitial lesions in those of ICGN mice. Trace staining for tenascin was seen in normal ICR kidneys, but strong positive staining for tenascin was detected in the expanded ICGN glomeruli but not in tubulointerstitial regions. In specimens stained for type IV collagen and laminin, major components of the basement membrane, both the glomerular and tubular basement membranes of ICGN mouse kidneys showed stronger positive staining than those of normal ICR mouse kidneys. Thus, basal membrane components (type IV collagen and laminin) and interstitial components (type I and III collagens, fibronectin and tenascin) accumulated in expanded glomeruli and tubulointerstitial lesions of the kidneys of ICGN mice.

In the normal kidneys of ICR mice, β1 subunit was observed in all glomerulus cells, with especially strong reactions seen in glomerular epithelial cells. β1 subunit was also seen in the basal surface of both proximal and distal tubular epithelial cells and their interstitium cells. In the kidneys of ICGN mice, β1 subunit was markedly increased in the glomeruli. When compared with the kidneys of ICR mice (Fig. 1A), markedly increased expression of α1 subunit (potential ligands for α1β1 integrin are laminin and type I and IV collagens) was observed in the glomeruli with fibrotic degeneration in ICGN kidneys (Fig. 1B). Outside the glomeruli, α2 subunit (potential ligands for α2β1 integrin are laminin and type I and IV collagens) was expressed on the basal surface of some proximal and distal tubules in the kidneys of ICGN mice (Fig. 1D), but α2 subunit was not detected in the kidney sections of ICR mice (Fig. 1C). α3 subunit (potential ligands for α3β1 integrin are laminin, type I collagen, fibrinectin and epiligrin) was observed throughout the glomeruli of ICR mice (Fig. 1E), and strong staining was seen only in the glomerular wall of ICGN mice (Fig. 1F). Such α3 subunit-positive cells in ICR mice were glomerular epithelial cells. Small numbers of glomerular and interstitial cells were immunohistochemically positive for α4 subunit (potential ligands for α4β1 integrin are fibronectin and VCAM-1) in both ICR and ICGN mice. When compared with ICR mice, larger numbers of glomerular cells were positive for α4 subunit in ICGN mice. In the kidney sections of ICGN mice, increased expression of α5 subunit (potential ligand for α5β1 integrin is fibronectin) was seen in the glomeruli with fibrotic degeneration, and these α5 subunit-positive cells were glomerular epithelial cells. The most striking alterations were found for the laminin receptor, integrin α6 subunit (potential ligand for α6β1 integrin is laminin) as follows. In normal ICR kidneys, α6 subunit was observed on the basal side of proximal and distal tubular epithelial cells (Fig. 2A). In the kidney sections of ICGN mice, α6 subunit-positive reaction products disap-

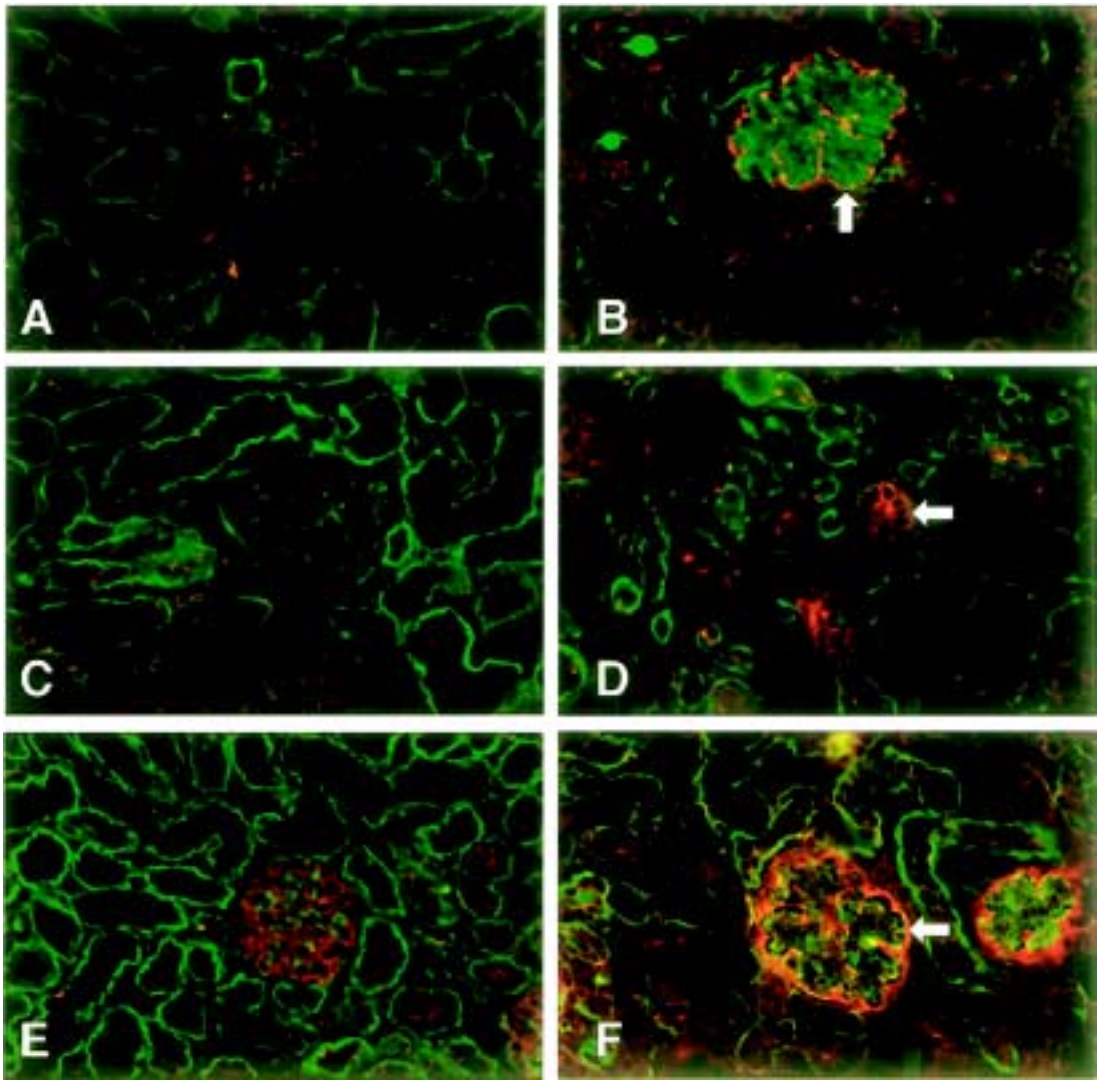

Fig. 1. Double staining for integrin α1 subunit and laminin (A and B), integrin α2 subunit and laminin (C and D), and integrin α3 subunit and laminin (E and F). α1, α2 and α3 subunits were visualized using Cy™3-conjugated second antibodies (red), and laminin was visualized using FITC-conjugated second antibody (green). When compared with the normal kidney of ICR mouse (A), markedly increased expression of α1 subunit was observed in the glomeruli (arrow) of ICGN kidney (B). α2 subunit was not detected in the kidney of ICR mouse (C), but it was expressed on the basal surface of some proximal and distal tubules (arrow) in the kidney of ICGN mouse (D). α3 subunit was observed throughout the glomeruli (glomerular epithelial cells) of ICR mouse (E), and strong staining was seen only in the glomerular wall (arrow) of ICGN mouse (F).

peared in injured proximal tubular epithelial cells, but strong reaction was seen in distal tubular epithelial cells (Fig. 2B).

DISCUSSION

Excessive accumulation and abnormal localization of ECM components cause many types of chronic diseases. In the kidneys, ECM accumulation collapses renal functions, filtration, absorption, secretion and so on. ICGN mice show progressed nephrotic syndrome, that is, developed proteinuria, hypoproteinemia, anemia and systemic edema [12–17, 28–31]. Previously, we revealed marked thickening of the glomerular basement membrane and fusion of the epithelial foot processes by electron microscopic observation of glomerular lesions of ICGN mice [14]. Then, we histochemically demonstrated excessive accumulation of ECM components, type I, III and IV collagens, laminin, fibronectin and tenastin, and abnormal localization of these ECMs in glomeruli and the tubulointerstitum of ICGN mice [29]. We presumed that this abnormal accumulation and localization of ECMs caused the progression of renal disorders in ICGN

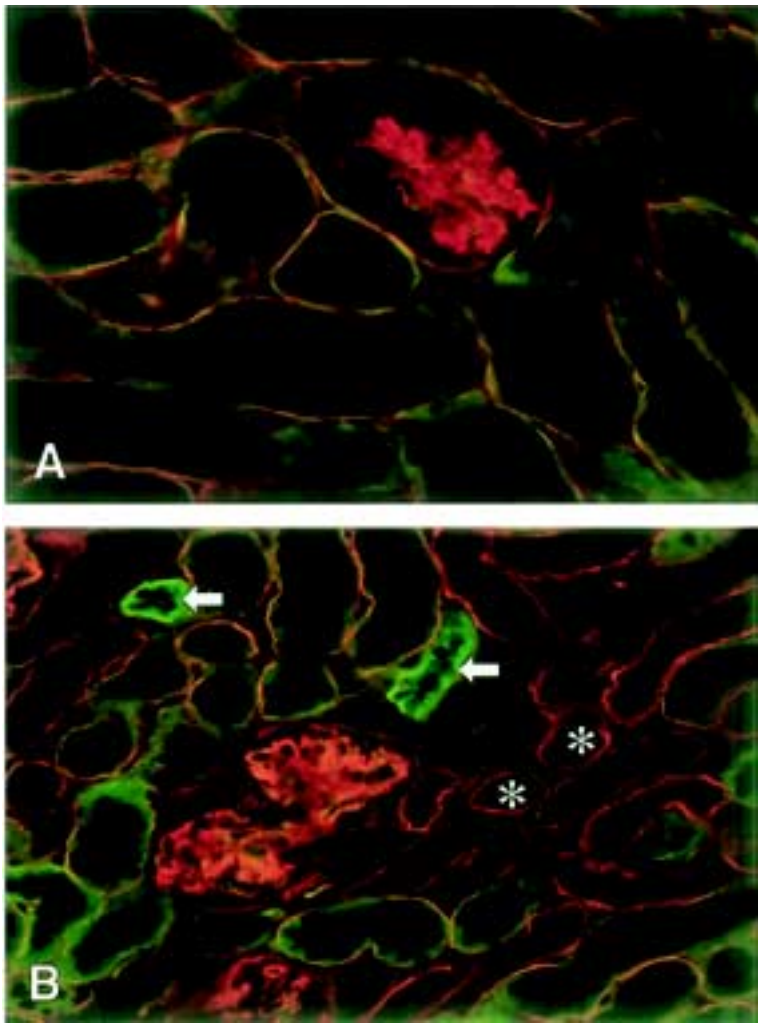

Fig. 2. Double staining for integrin α6 subunit and laminin. FITC-conjugated second antibody was used to detect α6 subunit (green), and Texas Red-conjugated second antibody was used to visualize laminin (red). In the kidney section of ICR mouse (A), α6 subunit was observed on the basal side of proximal and distal tubular epithelial cells. In the kidney section of ICGN mouse (B), α6 subunit-positive reaction products disappeared in injured proximal tubular epithelial cells (asterisks), but strong reaction was seen in distal tubular epithelial cells (arrows).

mice. Therefore, we measured the activities of matrix metalloproteinases (MMPs), that is interstitial collagenase (MMP-1), gelatinase (MMP-2 and MMP-9) and stromelysin (MMP-3), in the kidney tissues of ICGN mice [30]. Lower activities of MMP-1 and MMP-2 and MMP-9 were demonstrated in the kidneys of ICGN mice as compared with those of ICR mice, but there were no significant differences in the MMP-3 activities between these strains. These observations indicated that the abnormal ECM accumulation in the kidneys of ICGN mice is, in most cases, caused by a decline of ECM degradation.

ECMs are closely related to their receptors, the integrins. It has been suggested that abnormal localization of ECM components leads to alterative localization of integrins, and/or that abnormal localization of integrins leads to alterative localization of ECMs [3, 4, 26, 27]. Thus, abnormal localization of integrins causes abnormal formation of basement membrane structure, and the deficiency of integrins causes the lack of the basement membrane. Recent studies on cellular physiological roles of integrins indicated that the $\beta 1$ integrin family, major members of the laminin-binding integrins, are involved in basement membrane assembly [23]. Furthermore, the alternative localization and abnormal expression of integrins cause various forms of nephrosis both clinically and in experimental models [6–8, 18–20, 25]. In the present study, therefore, we immunohistochemically

investigated the localization of integrin subunits (α1, α2, α3, α4, α5, α6 and β1 subunits) in the kidneys of ICGN mice. Particularly, alterative localization of laminin receptors, that is $\alpha1\beta1$, $\alpha2\beta1$, $\alpha3\beta1$ and $\alpha6\beta1$ integrins, was carefully observed in the frozen sections of ICGN mouse kidneys using a confocal laser scanning microscope. Laminin is a major essential component of basement membranes and plays critical roles in the physiological functions of epithelial cells, and the basement membranes play very important roles in kidneys. For instance, the basement membrane in the glomeruli controls filtration ability and the basement membranes in the renal tubules are involved in secretion and absorption. Thus, abnormalities of basement membranes in glomeruli and renal tubules are lethal lesions. The results of the present study demonstrated immunohistochemically the changes in the localization of integrin subunits in the kidneys of ICGN mice, and showed that α1, α3 and α5 subunits were increased in glomeruli of ICGN mice with severe renal disorder estimated by conventional biochemistry and histopathology. Excessive accumulation of ECM components and abnormal localization of integrin subunits in glomeruli may block the filtration ability of glomeruli in ICGN mice. It has been reported that α3 subunit is related to epithelial foot processes in the glomeruli and is localized adjacent to the glomerular basement membrane on the basal face of podocytes [9, 24], and that the physiological functions of podocytes are maintained by their proper polarity determined by the localization of integrin subunits, especially integrin α3 subunit in the glomeruli. By electron microscopic observation of glomerular lesions in the kidneys of ICGN mice, fusion of the epithelial foot processes in glomeruli was demonstrated [14]. In this study, we demonstrated strong immunohistochemical reactivity for α3 subunit in glomerular epithelial cells of normal ICR mice, but weaker or trace staining for α3 subunit was seen in those of ICGN mice. These observations indicated a decrease in α3 subunit expression in the glomeruli of ICGN mice due to fusion of the epithelial foot processes. Basement membranes of renal tubules are important to maintain their physiological functions, that is the reabsorption of water, proteins and minerals, secretion of ions and so on. Degeneration of renal tubules leads to abnormal values of serum and urine albumin as estimated by biochemical methods. Detachment of the tubular epithelial cells from the tubular basement membrane according to the progression of renal injury is considered to be as a consequence of adhesion molecule dysfunction leading to loss of cell-to-cell and cell-to-ECM interactions [18]. Formation of casts, that is, detached cells and cellular debris, in the tubular lumen may be abetted by the omission or reorganization of cell-to-cell interactions accomplished through cell adhesion molecules. Previous observations indicated that integrin antagonists inhibited renal epithelial cell adhesion not only to ECMs but also to adjacent renal epithelial cells. Figure 2 shows the abnormal morphology of tubular basement membrane (immunohistochemical localization for laminin) and loss of α6 subunit (a component of laminin receptor) in kidney sections of ICGN mice, and such abnormalities may lead to dysfunction of tubular epithelial cells. Loss of α6 subunit in ICGN mouse kidneys may cause disturbance of tubular epithelial cell adhesion to the basement membrane, and then detachment of the cells into tubular lumen, and finally destruction of physiological functions of the renal tubules. Furthermore, the integrin α6 subunit has an important role in kidney morphogenesis during embryonic development, and mutation of α6 subunit results in the dysfunction of kidney morphogenesis in mouse embryos [4, 21]. The α6 subunit is thought to critically control basement membrane formation in the kidneys of mouse embryos, and α6 subunit mutation causes abnormalities of basement membrane, resulting in unusual development of the kidneys. Moreover, it has been reported that TGF-β1 increases β1 subunit expression [6]. We previously demonstrated that immunohistochemical expression of active form TGF-β1 increased in the glomeruli of ICGN mice as compared to those of normal ICR mice [29]. We hypothesized that the active form of TGF-β1 contributes to the increase in expression of β1 subunit in glomeruli of ICGN kidneys. The relationships between active form TGF-β1 and other integrin subunits have not yet been determined, and further work is in progress to reveal such relationships in our laboratory.

In conclusion, the present results revealed abnormal expression and alternative localization of integrins in the kidneys of ICGN mice with severe renal disorder. We presume that integrins play critical roles in kidneys to conserve renal construction and to maintain the physiological functions, and that abnormalities of integrins cause the progression of kidney disease in ICGN mice.

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