Virchows Arch (1998) 433:183–188

CASE REPORT

K. Naruse · H. Ito · T. Moriki · E.Miyazaki
Y. Hayashi · H. Nakayama · H. Kiyoku · M. Hiroi
T. Kurashige · H. Enzan

Mesangial cell activation in the collagenofibrotic glomerulonephropathy

Case report and review of the literature

Received: 28 January 1998 / Accepted: 23 March 1998

Abstract Collagenofibrotic glomerulonephropathy is a new disease entity of unknown pathogenesis, which is characterized by the deposition of type III collagen within the mesangial matrix. We have investigated a case in which many mesangial cells in the type III collagen-deposited glomeruli were α-smooth muscle actin (ASMA) positive and showed an increase of subplasmalemmal filaments, indicating the activation and myofibroblastic transformation. It is suggested that the activated mesangial cells may synthesize the type III collagen deposited in the subendothelial space and mesangial matrix.

Key words Collagenofibrotic glomerulonephropathy · Immunohistochemistry · α-Smooth muscle actin · Type III collagen · Myofibroblast

Introduction

Renal diseases such as myeloma nephropathy [22], renal amyloidosis [17], light chain glomerulopathy [1], immunotactoid glomerulopathy [14], fibrillary glomerulonephritis [6], and the nail-patella syndrome [10] exhibited deposits of fibrillary materials within the mesangial matrix and/or subendothelial space. In addition, a new entity "collagenofibrotic glomerulonephropathy" has been reported recently [3]. In this disease the fibrillary material is type III collagen [25].

Despite 31 previously reported cases of the disease, the mechanism of the deposition of type III collagen is unknown. We describe a patient with collagenofibrotic glomerulonephropathy and have clarified the nature of deposited material and the type of cell synthesizing the material using immunohistochemistry and electron microscopy.

K. Naruse (✉) · E. Miyazaki · Y. Hayashi · H. Nakayama
H. Kiyoku · M. Hiroi · H. Enzan
First Department of Pathology, Kochi Medical School,
Kohasu Okohcho Nankoku Kochi 783-8505 Japan
Tel.: (+81)888-66-5811 (ext. 2632), Fax: (+81)888-80-2332

H. Ito
Second Department of Internal Medicine,
Kochi Medical School Hospital, Kochi Japan

T. Moriki
Department of Clinical Laboratory Medicine, Kochi Medical School Hospital, Kochi Japan

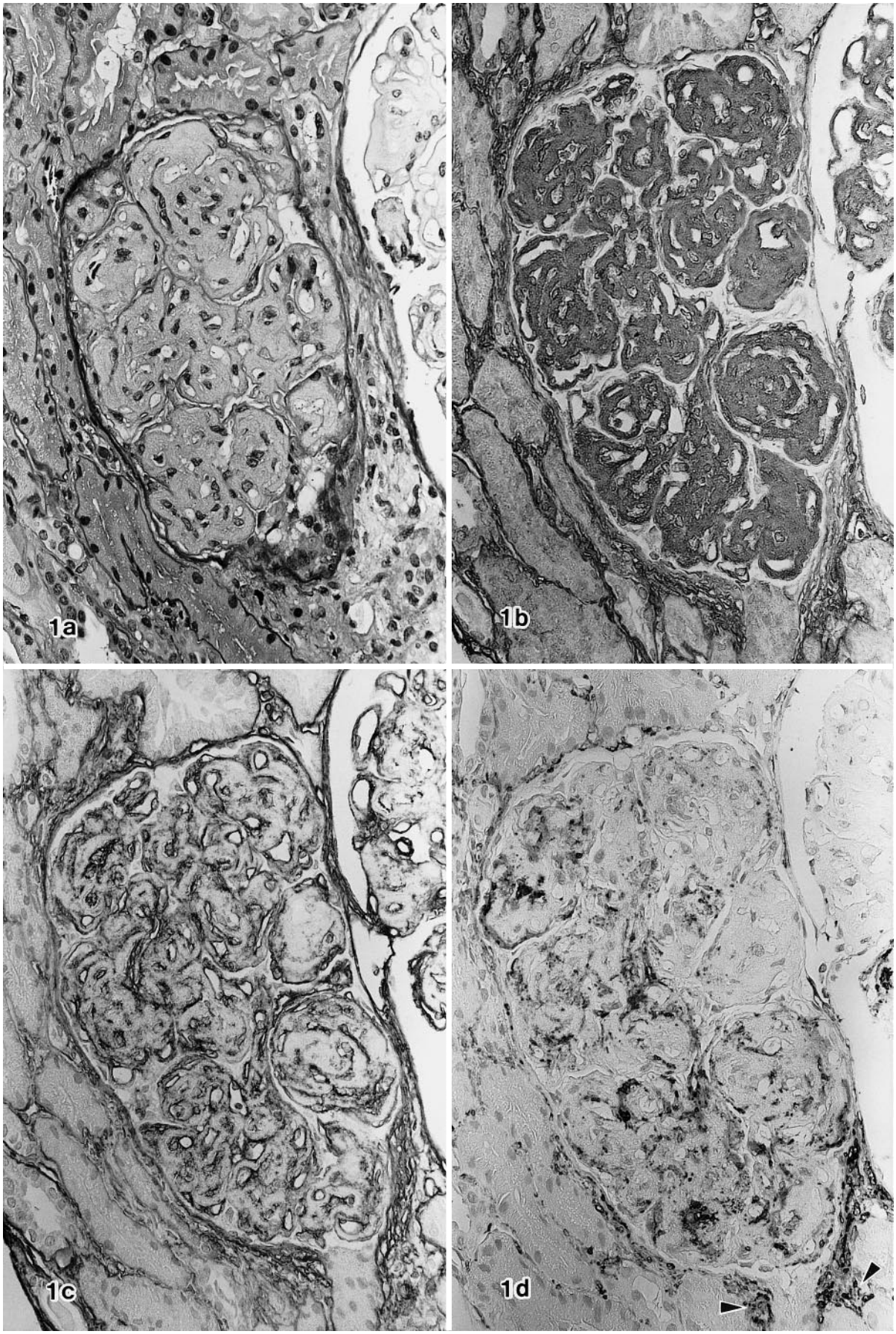
T.Kurashige
Department of Pediatrics, Kochi Medical School,
Kochi Japan

Clinical history

A 66-year-old Japanese woman was well except for hypercholesterolaemia until 1991, when proteinuria was detected during an annual physical examination. A renal biopsy was done in 1994, but the diagnosis was undetermined and the proteinuria continued. In August 1996 she was diagnosed with early gastric cancer by radiography, endoscopic examination and histology. A gastrectomy was performed. On admission, blood pressure was slightly raised (170/110 mm Hg). Urinalysis showed red blood cells (U-RBC), 1–4/HPF; white blood cells (U-WBC), 1–4/HPF; sugar 70 mg/dl; 24-h urinary protein 2.0 g without Bence-Jones protein. Antinuclear antibody, hepatitis B virus antigen and hepatitis C virus antibody were not detected. The serum level of procollagen III peptides was abnormally elevated (3.3 U/ml). To clarify the cause of the continuous proteinuria, renal biopsy was performed again in March 1997.

Materials and methods

The renal needle biopsy specimen was examined conventionally and immunohistochemically using polyclonal antibodies for anti-type I, III, IV collagen (MONOSAN, Netherlands) and monoclonal antibodies against α-smooth muscle actin (ASMA; 1A4, DAKO, Denmark) leucocyte common antigen (LCA; PD7/26 and 2B11, DAKO), CD68 (KP-1, DAKO), cellular fibronectin (DH1, BIOHIT, Finland) and CD31(JC/70A, DAKO). The immunohistochemical results were compared with those in 6 cases of minor glomerular abnormalities as controls. As a negative control for immunostaining, sections were incubated in normal serum at the same concentration as those of the primary antibodies. Snap-frozen biopsy material for immunofluorescent study was used, to demonstrate immunoglobulins (IgA, IgG, IgM), complement components (C3, C4) and fibrinogen, by direct immunofluorescence. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEM 100S electron microscope.


1a
1b
1c
1d

Pathological findings

The specimen contained 33 glomeruli. The glomeruli were found to be enlarged and lobulated, with focal and segmental mild mesangial cell proliferation. The glomerular architecture was altered by massive deposition of eosinophilic material. The material was weakly PAS positive (Fig. 1a) and fibrillary with PAM, showing prominently expanded mesangial area. Congo-red staining was completely negative. The glomerular basement membrane (GBM) was markedly thickened and frequently revealed a double contour appearance because of the material invading toward the peripheral capillary wall. The subendothelial and intermediate space of duplicated GBM were occupied with the material, resulting in the narrowness of capillary lumen and Bowman's space. However, adhesion was very rare. Neither crescent formation nor infiltration of inflammatory cells was observed in any of the glomeruli. The renal tubules were focally atrophic, with mild infiltration of lymphocytes and peritubular fibrosis.

The frozen specimen contained 6 glomeruli. They revealed weakly positive staining for IgM and C3 along the capillary walls with a fine granular pattern. However, no fluorescence of IgA, IgG, C4 and fibrinogen was observed.

The subendothelial homogeneous eosinophilic material corresponded to the diffuse deposition of type III collagen in the glomerular tufts (Fig. 1b). Type IV collagen was also detected along the capillary walls, as in the controls. In some tufts, it appeared to be double contour (Fig. 1c). Type I collagen staining was weakly positive at the peripheral capillary walls. Even though mesangial proliferation was mild, many mesangial cells were ASMA positive with segmental accentuation (Fig. 1d). The endothelial cells of glomerular capillaries were positive for CD31. Very few CD68- or LCA-positive cells were located in the capillary lumen. The tufts were negative for cellular fibronectin.

Electron microscopically, the intercellular space of the mesangium and the subendothelial space of the GBM was markedly expanded, giving a lucent or lytic appearance to these structures (Fig. 2a). In both spaces scattered fibrils were distributed diffusely, separately or partly matted. The fibrils, 40~100 nm in diameter, had a transverse band structure with a distinctive periodicity of 60 nm. They were twisted and frayed, forming irregularly arranged bundles in longitudinal sections. The characteristic ultrastructural features were similar to those of type III collagen (Fig. 2b). Mesangial cells had dilated cisternae of rough endoplasmic reticulum (rough ER) and straight bundles of microfilaments along the cell membrane (Fig. 2c). Unique microtubular structures such as are seen in immunotactoid glomerulopathy, fibrin and amyloid fibrils were not observed. The lamina densa was of normal thickness and did not show the "moth-eaten" appearance seen in the nail-patella syndrome. There were no electron-dense deposits or mesangial interposition. The endothelial cells were slightly swollen, and the foot processes of the epithelial cells were moderately effaced.

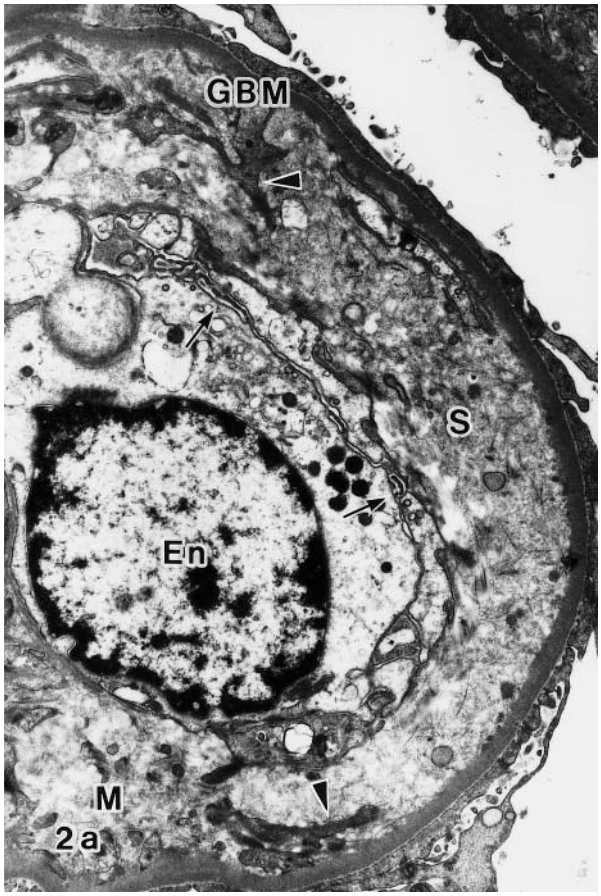
◀ **Fig. 1a–d** Light microscopy of a renal biopsy specimen showing an enlargement (about 300 μm in diameter) of the glomerulus. The following four figures are from the same glomerulus. **a** A massive accumulation of weakly PAS-positive homogenous material is seen in the mesangial area and glomerular capillary loop. PAS, ×300 **b** The material is positive for type III collagen. **c** Type IV collagen is positive along the capillary loop, partially showing double contour. **d** Many mesangial cells are positive for ASMA, focally and segmentally. As positive control smooth muscle cells of periglomerular arterioles are also stained (*arrowhead*). **b**, **c**, **d** Immunostaining for type III, IV collagens and ASMA, respectively, ×300

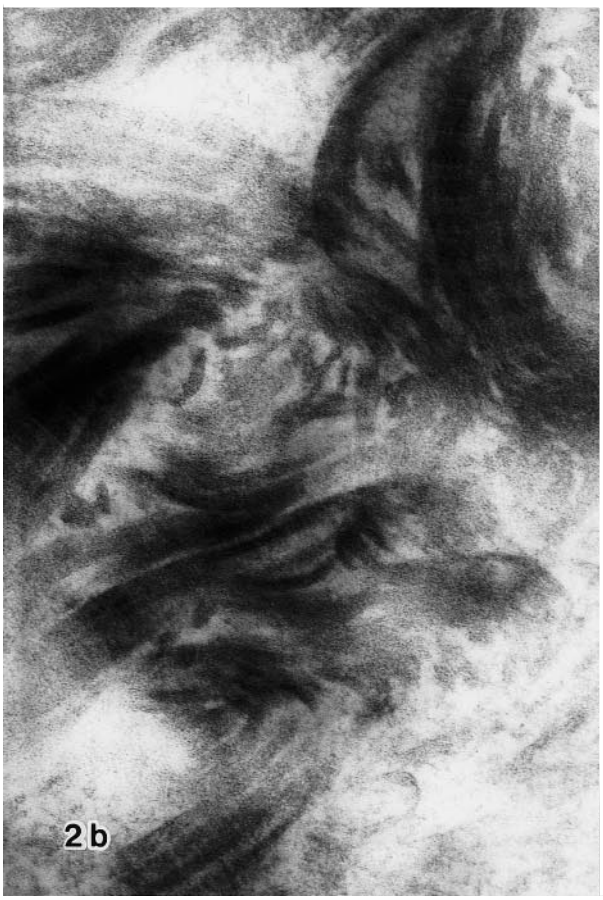
Discussion

Collagenofibrotic glomerulonephropathy is a new idiopathic glomerular disease. Two cases were first reported as "idiopathic mesangio-degenerative glomerulonephropathy" by Arakawa et al. in 1979 [4]. Since then, similar cases have been reported mainly in Japan under various names, such as "collagen fibers deposit nephropathy" [28], "primary glomerular fibrosis" [11] and "collagen type III glomerulonephropathy" [12]. However, in 1991 Arakawa and Yamanaka summarized 10 cases and proposed a new term, "collagenofibrotic glomerulonephropathy" [3]. Recently the term has been gradually accepted worldwide. After the first presentation, 31 cases have been reported (Table 1) [3–5, 7–9, 11, 12, 16, 18–21, 23–29].

The pathology of the disease is characterized by massive collagen fibre deposition, resulting in lucent expansion of the mesangial area and the subendothelial space of the GBM without mesangial cell proliferation [3]. Electron microscopy shows that the increased fibrils are curved and ragged, about 40~100 nm in diameter and have a distinct periodicity of about 60 nm. The fibrils were identified as type III collagen by Terashima et al. [25] using anti-collagen type III antibody. Ikeda et al. reported that in one patient the disease was associated with a high level of serum procollagen III peptide [11]. Our case has all the pathological and clinical features of collagenofibrotic glomerulonephropathy, and only the mild segmental proliferation of mesangial cells is not consistent with it. However, this may be an occasional finding, since only two previous reports have mentioned mesangial proliferation [18, 26]. As in other cases, type I collagen reacted strongly in the capillary wall or mesangium (in 1 case [29]) and weakly in parts of the capillary wall in others [19]. In our case, type I collagen was weakly positive in parts of the capillary wall. Type IV collagen was detected along the capillary wall, which had a double contour in parts [11]. This is the first reported case of ASMA immunoreactivity in collagenofibrotic glomerulonephropathy (Table 1) with diffuse and segmental staining. Many ASMA-positive cells were located in glomerular tufts, in contrast to controls. It is

Fig. 2a–c Electron microscopy of a renal biopsy specimen. **a** Mesangial area (*M*) and subendothelial space (*S*) reveal lucent or lytic appearance. In these regions there are large amounts of fibrillary materials which distributed irregularly. A few slender segments of mesangial cell processes are scattered (*arrowhead*). A capillary lumen is extremely narrowed (*arrow*; *En* endothelial cell, *GBM* glomerular basement membrane). ×6700 **b** Higher magnification of deposited fibrillary materials. They are 40~100 nm in diameter and show a periodicity of 60 nm, showing a type III collagen. ×80000 **c** A mesangial cell (Me) has expanded cisternae of rough ER (*arrow*) and actin filaments along cell membrane (*arrowhead*). Around the cell the fibrillary materials and irregularly shaped cytoplasmic processes of mesangial cells are seen (*asterisk*). ×17000





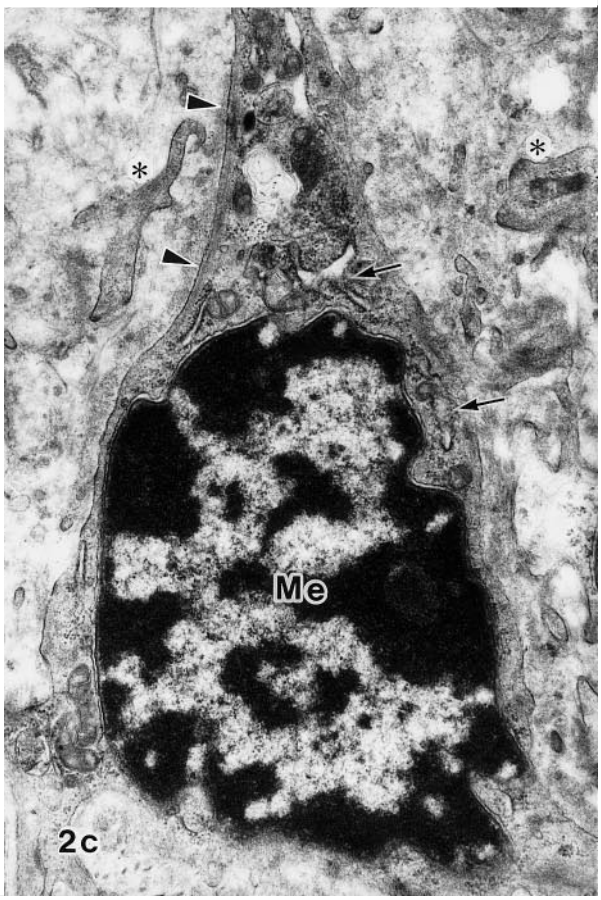


Table 1 Collagenofibrotic glomerulonephropathy, review of the literature (– not done or unknown, *ASMA* α-smooth muscle actin, *LCA* leucocyte common antigen, *U* urine)

Reference	Number of cases	Age	Sex	U-protein g/day)	U-RBC (/HPF)	Blood pressure (mm Hg)	Procollagen III peptide (ng/dl)	Immunohistochemistry of glomeruli	
								Type III collagen	Others examined
[4]	2	34	F	0.1–08.	0–5	130/70	–	–	–
		64	M	1.5–5.0	0–1	190/140	–	–	–
[6]	1	34	F	Neg.	4–5	–	–	–	–
[8]	2	48		0.1–0.2	Neg.	120/64	–	–	–
		54	F	1	Neg.	160/90	–	–	–
[28]	1	38	M	4.9	0–1	170/100	–	–	–
[16]	1	47	F	3.6	–	108/80	–	–	–
[25]	1	10	M	1.39	15	116/68	–	Positive	Type I, IV, VI collagen, laminin, fibronectin
[11]	1	38	M	3–5	–	146/80	221	Positive	Type IV collagen
[12]	1	49	F	0.76–1.19	Neg.	180/110	–	Positive	Type I, IV collagen
[21]	1	49	M	8.6	4–5	190/90	220	Positive	Type IV collagen
[23]	1	65	M	2.7	1–2	184/94	227	Positive	–
[20]	2	42	F	3.6	Neg.	108/80	–	Positive	Type I, IV, VI collagen
		65	M	1	Few	152/100	–	Positive	Type I, IV, VI collagen
[27]	1	30	F	0.5–13.	Neg.	Hypertension	114.2	Positive	–
[18]	1	49	F	0.6	Neg.	160/94	3.2 U/ml (0.3–0.8)	Positive	–
[7]	10	4 months to 14.8 years old 7 males and 3 females 10 cases (proteinuria), 6 cases (haematuria) and 5 cases (hypertension) type I, III, IV and VI collagen, laminin, fibronectoin (immunohistochemistry)							
[29]	1	54	F	3–5	–	180/92	–	Positive	Type I, IV collagen, laminin
[19]	1	49	F	8	Neg.	182/90	6.7 U/ml	Positive	Type I, II, IV, VI collagen
[26]	1	6	M	3–4	Pos.	166/128	–	Positive	–
[24]	1	33	F	6.5	Neg.	162/84	5.3 U/ml	Positive	Type I, IV collagen
[9]	1	66	M	2.5	–	160/90	2.4 U/ml	Positive	Type IV collagen
Present case (1998)	1	66	F	2.0	1–4	170/110	3.3 U/ml	Positive	Type I, IV collagen, ASMA, CD31 fibronectin, LCA, KP-1

known that mesangial cells become ASMA positive in experimental and other glomerulonephritides [2, 13], and that they can produce type I, III, and IV collagen [15].

Two pathogenesis for the disease have been proposed. According to one, collagen synthesis and/or degradation in the whole body is deranged, releasing precursors of type III collagen into the systemic circulation [10] as in the nail–patella syndrome. Subsequently, the serum level of procollagen III peptides is elevated and increased collagen precursors become entrapped in the GBM. The other hypothesis suggests that the deposited type III collagen in the mesangial matrix is produced by mesangial cells [11]. Collagen fibres in the glomeruli are often located near the mesangial cells, and cultured mesangial cells produce type III and IV collagen fibres [15]. In our case, we demonstrated the activation of mesangial cells by immunohistochemistry using the antibody against ASMA. The deposited material may have originated from an erroneous and disorganized production of type III collagen fibres by activated mesangial cells. The presence of very few LCA- or CD68-positive inflammatory cells suggested that the mesangial activation had occurred by way of a different mechanism than activation through the paracrine activity of cytokines. The pathogenesis of this disease may be a self-regulating disorder of type III collagen synthesis by the mesangial cells.

Acknowledgements The authors wish to thank Professor Kozo Hashimoto and Associate Professor Koji Nishiya, the Second Department of Internal Medicine, Kochi Medical School for providing us with laboratory data and a renal biopsy specimen.

References

1. Alpers CE, Hopper J, Biava CG (1984) Light-chain glomerulopathy with amyloid-like deposits. Hum Pathol 15:444–448
2. Alpers CE, Hudkins KL, Gown AM, Johnson RJ (1992) Enhanced expression of "muscle-specific" actin in glomerulonephritis. Kidney Int 41:1134–1142
3. Arakawa M, Yamanaka N (1991) Collagenofibrotic glomerulonephropathy: a new type of primary glomerulonephropathy revealing massive collagen deposition in the renal glomerulus. In: Arakawa M, Yamanaka N (eds) Collagenofibrotic glomerulonephropathy. Nishimura, Niigata Smith-Gordon, London, pp 3–8
4. Arakawa M, Hueki H, Hirano H, Sato M, Yamagishi T, Matsuda H, Kamimura S, Suzuki M, Yamagishi Y, Yamashita M, Nakagawa S (1979) Idiopathic mesangio-degenerative glomerulonephropathy (in Japanese). Jpn J Nephrol 21:914–915
5. Dombros N, Katz A (1982) Nail patella-like renal lesions in the absence of skeletal abnormalities. Am J Kidney Dis 1:237–240

6. Duffy JL, Khurana E, Susin M, Gomez-Leon G, Churg J (1983) Fibrillary renal deposits and nephritis. Am J Pathol 113:279–290
7. Gubler MC, Dommergues JP, Foulard M, Bensman A, Leroy JP, Broyer M, Habib R (1993) Collagen type III glomerulopathy: a new type of hereditary nephropathy. Pediatr Nephrol 7:354–360
8. Hirano H, Shindo T, Okamoto M, Shingai Y, Kiso N, Yamagishi T, Oosawa G (1982) Two cases with massive collagen fiber deposition in glomeruli (in Japanese). Jpn J Nephrol 24:1404–1405
9. Hisakawa N, Yasuoka N, Nishiya K, Kumon Y, Okamoto K, Itoh H, Hashimoto K, Moriki T (1998) Collagenofibrotic glomerulonephropathy associated with immune complex deposition. Am J Nephrol 18:134–141
10. Hoyer JR, Michael AF, Vernier RL, Sisson S (1972) Renal disease in nail-patella syndrome: clinical and morphologic studies. Kidney Int 2:231–238
11. Ikeda K, Yokoyama H, Tomosugi N, Kida H, Ooshima A, Kobayashi K (1990) Primary glomerular fibrosis: a new nephropathy caused by diffuse intra-glomerular increase in atypical type III collagen fibers. Clin Nephrol 33:155–159
12. Imbasciati E, Gherardi G, Morozumi K, Gudat F, Epper R, Basler V, Mihatsch MJ (1991) Collagen type III glomerulopathy: a new idiopathic glomerular disease. Am J Nephrol 11: 422–429
13. Johnson RJ, Iida H, Alpers CE, Majesky MW, Schwartz SM, Pritzl P, Gordon K, Gown AM (1991) Expression of smooth muscle cell phenotype by rat mesangial cells in immune complex nephritis. J Clin Invest 87:847–858
14. Korbet SM, Schwartz MM, Rosenberg BF, Sibley RK, Lewis EJ (1985) Immunotactoid glomerulopathy. Medicine 64:228–243
15. Kreisberg JI, Karnovsky MJ (1983) Glomerular cells in culture. Kidney Int 23:439–447
16. Kurosawa K, Kyogoku Y, Saito T, Yamakage K, Sato H, Furuyama T, Yoshinaga K (1984) A case of nephrotic syndrome with peculiar changes in the basement membrane characterized by abundant collagen formation-a new clinical entity? (in Japanese) Kidney Dial 16:241–249
17. Kyle RA, Greipp PR (1983) Amyloidosis (AL) Clinical and laboratory features in 229 cases. Mayo Clin Proc 58:665–683
18. Mizuiri S, Hasegawa A, Kikuchi A, Amagasaki Y, Nakamura N, Sakaguchi H (1993) A case of collagenofibrotic glomerulopathy associated with hepatic perisinusoidal fibrosis. Nephron 63:183–187
19. Ozu H, Nitta K, Yumura W, Horita S, Honda K, Nihei H (1994) A case of primary glomerular fibrosis associated with the accumulation of type I and type III collagen. Jpn J Nephrol 36:107–111
20. Saito T, Sato H, Akiu N, Kurosawa K, Soma J, Kyogoku Y, Shimizu K, Kamada M, Yamakage K, Furuyama T, Yoshinaga K (1991) Two cases of collagenofibrotic glomerulonephropathy with abundant type III collagen production. In: Arakawa M, Yamanaka N (eds) Collagenofibrotic glomerulonephropathy. Nishimura, Niigata/Smith-Gordon, London, pp 21–27
21. Sanaka T, Nakao H, Matsumura O, Sugino N (1991) A case of Nephrotic syndrome with collagenofibrinogen glomerulopathy. In: Arakawa M, Yamanaka N (eds) Collagenofibrotic glomerulonephropathy. Nishimura, Niigata/Smith-Gordon, London, pp 51–59
22. Schubert GE, Adam A (1974) Glomerular nodules and long-spacing collagen in kidneys of patients with multiple myeloma. J Clin Pathol 27:800–805
23. Sugiyama N, Shimizu J, Nakamura M, Matsuoka K, Fukuta K, Monden H (1991) A case of “collagenofibrotic glomerulonephropathy.” In: Arakawa M, Yamanaka N (eds) Collagenofibrotic glomerulonephropathy. Nishimura, Niigata/Smith-Gordon, London, pp 41–47
24. Tamura H, Matsuda A, Kidoguchi N, Matsumura O, Mitarai T, Isoda K (1996) A family with two sisters with collagenofibrotic glomerulonephropathy. Am J Kidney Dis 27:588–595
25. Terashima T, Hattori S, Ushijima T, Yoshioka H, Miyatake K, Kitaoka M, Murakami M, Ujuku G, Matsuda I (1988) Type I and type III collagen fibers observed in the renal glomeruli of membranoproliferative glomerulonephritis (in Japanese). Kidney Dial 25:527–530
26. Vogt BA, Wyatt RJ, Burke BA, Simonton SC, Kashtan CE (1995) Inherited factor H deficiency and collagen type III glomerulopathy. Pediatr Nephrol 9:11–15
27. Yamanaka N, Sugisaki Y, Wakamatsu R, Ono K, Naruse T (1991) A glomerular disease with a large amount of collagen deposition in the mesangium and the subendothelial space. In: Arakawa M, Yamanaka N (eds) Collagenofibrotic glomerulonephropathy. Nishimura, Niigata/Smith-Gordon, London, pp 69–81
28. Yasuda T, Miki K, Nakamoto Y, Miura R, Yamagishi T, Abe T, Kihara T (1984) A case of nephrotic syndrome with massive deposition of collagen fibers in glomeruli–collagen fibers deposit nephropathy (in Japanese). Kidney Dial 17:115–120
29. Yoshida F, Yuzawa Y, Shigematsu H, Ito A, Yamazaki C, Yoshioka K, Ooshima A, Matsuo S (1993) Nephrotic syndrome with massive accumulation of type I and type III collagen in the glomeruli. Intern Med 32:171–176