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Immunoreactivity of the JK-132 monoclonal antibody directed against basement membrane collagen in normal and diabetic glomeruli

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Abstract The possible involvement of basement membrane-associated collagen (recognized by the monoclonal antibody JK-132) in the evolution of diabetic nephropathy was studied in kidney specimens from seven patients with noninsulin-dependent diabetes mellitus, and its distribution was compared with those of antibodies against α1 to α4 chains of type IV collagen. JK-132, a monoclonal antibody against basement membrane-associated collagen, reacted immunohistochemically exclusively with the mesangial matrix of the glomerular capillary. In contrast, antibodies to the α1 and α2 chains (IV) reacted strongly with mesangial matrix, and less strongly with the glomerular basement membrane (GBM). Antibodies to the α3 and α4 chains (IV) reacted mainly with GBM. In diabetes, JK-132 reacted most extensively with the expanded mesangial matrix, its staining intensity increasing with progression of the diabetic glomerulosclerosis. Antibodies to the α1 and α2 chains (IV) reacted prominently with the expanded mesangial matrix but less strongly with the GBM. Antibodies to the α3 and α4 chains reacted intensely with the thickened GBM. These results suggest that basement membrane-associated collagen differs from α1 to α4 chains of type IV collagen and that basement membrane-associated collagen is a good marker of mesangial expansion in diabetic nephropathy.

Key words Diabetes mellitus · Type IV collagen
Glomerulus · Extracellular matrix
Monoclonal antibody

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Introduction

Diabetic nephropathy is characterized by expansion of the mesangial matrix and thickening of the glomerular basement membrane (GBM) (Hostetter 1991; Olson 1992). The GBM and mesangial matrix are collectively referred to as the glomerular extracellular matrix (ECM). This ECM is formed by a group of proteins that include type IV collagen (the major constituent), laminin, proteoglycans, fibronectin, entactin / nidogen (Fouser and Michael 1987; Kanwar 1984; Timpl and Aumailley 1989)

The protomer, or building block unit, of type IV collagen consists of three α (IV) chains and is characterized by three distinct structural domains: the noncollagenous 1 (NC1) domain at the carboxy-terminus; the triple-helical domain in the middle region; and the 7S domain at the amino-terminus (Hudson et al. 1989; Timpl and Aumailley 1989). The classical protomer is comprised of two α1 chains and one α2 chain (Hudson et al. 1989). New chains of type IV collagen have recently been proposed based on distinct NC1 amino acid sequences and designated as α3 (IV) and α4 (IV) chains (Gunwar et al. 1990; Saus et al. 1988). More recently, a novel chain α5 (IV) was discovered by cDNA cloning (Hostikka et al. 1990), and its distribution was reported to be restricted to a specific region in the kidney.

We recently characterized a novel chain of basement membrane-associated collagen (BAC) recognized by the monoclonal antibody JK-132, that was originally raised against human type IV collagen (Kino et al. 1991). Biochemical analysis of some peptides bound to an affinity column coupled with JK-132 antibody, showed an

amino acid composition similar to that of type IV collagen but with unique amino acid sequences distinct from those of other collagen chains reported (Brazel et al. 1988; Butokowski et al. 1987; Chu et al. 1985; Langeveld et al. 1988; Meyers et al. 1985; Muthkumaran et al. 1989; Ninomiya et al. 1985; Saou et al. 1989; Soininen et al. 1987; Van der Rest et al. 1985). These results suggested that BAC is a novel collagen chain.

To clarify the role of BAC in the progression of diabetic nephropathy, its distribution was compared with those of α1 to α4 chains (IV) in kidney specimens obtained from affected patients.

Materials and methods

Patients

Renal biopsy or autopsy specimens obtained from seven patients with noninsulin-dependent diabetes mellitus (NIDDM) were evaluated (Table 1). The patients were all middle-aged or elderly and none had ketoacidosis. For examination by light microscopy, specimens were stained with hematoxylin and eosin, periodic acid-Schiff and periodic acid-silver methenamine. The degree of glomerulosclerosis was graded 0–4 according to Gellman et al. (1959). Sections of uninvolved, normal renal parenchyma from three nephrectomy specimens from patients with renal tumors were used as controls.

Antibodies

The polyclonal and monoclonal antibodies used in this study are shown in Table 2. JK-132, a monoclonal antibody to type IV collagen isolated from human placenta was produced as previously described (Kino et al. 1991). By enzymelinked immunosorbent assay (ELISA), this antibody binds not only with native basement membrane collagen but also with thermally denatured material, indicating that it recognizes a sequential determinant present in human basement membrane collagen (Kino et al. 1991). The antibody JK-132 exhibits no detectable cross reactivity with native human collagen types I, III, V, and VI, human fibronectin, or mouse and human laminin (Kino et al. 1988).

Chain-specific antibodies against type IV collagen have been characterized (Butokowski et al. 1985, 1987; Johansson et al. 1991). For examination by immunoelectron microscopy, specimens were stained with JK-132 and polyclonal antibodies against α1 (IV) and α3 (IV). Anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC) and anti-mouse IgG conjugated with FITC were obtained from Cappel Co. (West Chester, Pa., USA). Anti-rabbit IgG and anti-mouse IgG, both conjugated with 15 nm colloidal gold, were obtained from Amersham (Buckinghamshire, UK).

Table 2 Specificity and source of antibodies

Antibody	Domain	References
Monoclonal antibody		
BAC, JK-132	Triple helical	Kino et al. 1991
α1 (IV)	NC1 domain M26	Johansson et al.
α3 (IV)	NC1 domain M28***	1991
Polyclonal antibody		
α1 (IV)	NC1 domain M1a	
α2 (IV)	NC1 domain M1b	Butokowski et al.
α3 (IV)	NC1 domain M2	1985 and 1987
α4 (IV)	NC1 domain M3	

BAC, basement membrane-associated collagen; NC1, non-collagenous 1

Immunohistological studies

The distributions of BAC and various chains of type IV collagen were first studied by indirect immunofluorescence techniques as described previously (Makino et al. 1986). In brief, the kidney specimen was immediately snap-frozen, and unfixed cryostat sections (4 μm thick) were prepared. Slides were stained with the primary antibodies, followed by the FITC-conjugated second antibodies. Immunofluorescence was independently evaluated for intensity and distribution by three observers without prior knowledge of the source of the sections. The intensity of immunofluorescence staining was graded on a (−) to (+ + +) scale using an Olympus immunofluorescent microscope (Tokyo, Japan).

Immunoelectron microscopy was performed in all five renal biopsy specimens from patients with diabetic nephropathy and in the three controls as previously described (Makino et al. 1993). In brief, part of the renal specimen was fixed in 0.1% paraformaldehyde and 0.05% glutaraldehyde for 30 min. It was then dehydrated in a graded ethanol series, and the temperature was gradually reduced to −20° C. A stepwise infiltration was carried out with Lowicryl K4 medium. The specimen was finally embedded in this low-temperature polymeric resin. Uniform polymerization was carried out under ultraviolet light at −20° C for 24 h and then at room temperature for 48 h. Thin sections picked up on nickel grids were pretreated with 20 mM Tris-buffered saline, pH 8.2, containing 0.25% bovine serum albumin. They were then incubated for 4 h at room temperature with the first antibody, i.e., JK-132. The grids were then washed with Tris-buffered saline and further incubated with anti-mouse IgG conjugated with 15 nm colloidal gold particles for 4 h at room temperature. The grids were then incubated with Tris-buffered saline, stained with uranyl acetate and lead citrate, and examined with an electron microscope at an accelerating voltage of 75 kv.

Results

The clinicopathological findings in the seven patients with diabetic nephropathy are summarized in Table 1.

Table 1 Clinicopathological findings in patients with NIDDM

Age years	Sex	Proteinuria (g/day)	Ccr (ml/min)	Sclerosis grade	Nodular lesion	Biopsy/ Autopsy
54	F	0.3	102	0	−	Biopsy
47	M	0.6	96	1	−	Biopsy
62	F	4.3	44	2	−	Biopsy
66	F	1.2	78	3	+	Biopsy
76	F	2.4	nd*	3	+	Autopsy
51	M	3.2	45	3	+	Biopsy
85	M	3.8	10	4	+	Autopsy

nd*, not done; Ccr, creatinine clearance; +, present; −, absent

They had various degrees of proteinuria, diminished renal function and glomerulosclerosis.

Normal controls

By immunofluorescence, JK-132 reacted with the mesangium of the glomerular capillaries. Bowman's capsule and the tubular basement membranes were also heavily stained (Fig. 1A).

Monoclonal and polyclonal antibodies to the same chains of type IV collagen, i.e. α1 (IV) and α3 (IV), showed the same distribution. With chain – specific antibodies to type IV collagen, there were two staining patterns. The α1 and α2 chains (IV) were seen predominantly in the mesangium, but also in the glomerular capillary wall, Bowman's capsule and tubular basement membranes (Fig. 1B). The α3 chain and α4 chains (IV) were present mainly on the glomerular capillary wall (Fig. 1C). Bowman's capsule was not stained but parts of tubular basement membranes were stained.

BAC was seen on immunoelectron microscopy to be localized in the mesangial matrix of the glomerular capillary (Fig. 2).

Diabetes

In the diabetic kidney, JK-132 reacted with the expanded mesangium (Fig. 3) and its staining intensity increased with progression of the diabetic glomerulosclerosis (Fig. 3A, B). However, less intense staining was seen in hyalinized glomeruli (Fig. 3C). Nodular lesions were also stained (Fig. 3D).

The α1 and α2 chains (IV) in mild to moderately sclerosed glomeruli reacted prominently with the expanded mesangial regions and weakly with the capillary walls (Fig. 4A). The α3 and α4 chains (IV) reacted strongly with the capillary walls (Fig. 4B). In advanced glomerulosclerosis with hyalinized glomeruli, the intensity of staining for the α1 and α 2 chains (IV) was decreased (Fig. 4C), in contrast with that for the α3 and α4 chains (IV) which was preserved (Fig. 4D). The immunofluorescent findings in both normal controls and in diabetic nephropathy are summarized in Table 3.

As observed by immunoelectron microscopy, JK-132 reacted almost exclusively with the expanded mesangial matrix of the glomerular capillary, only very few particles being observed in the thickened GBM (Fig. 5).

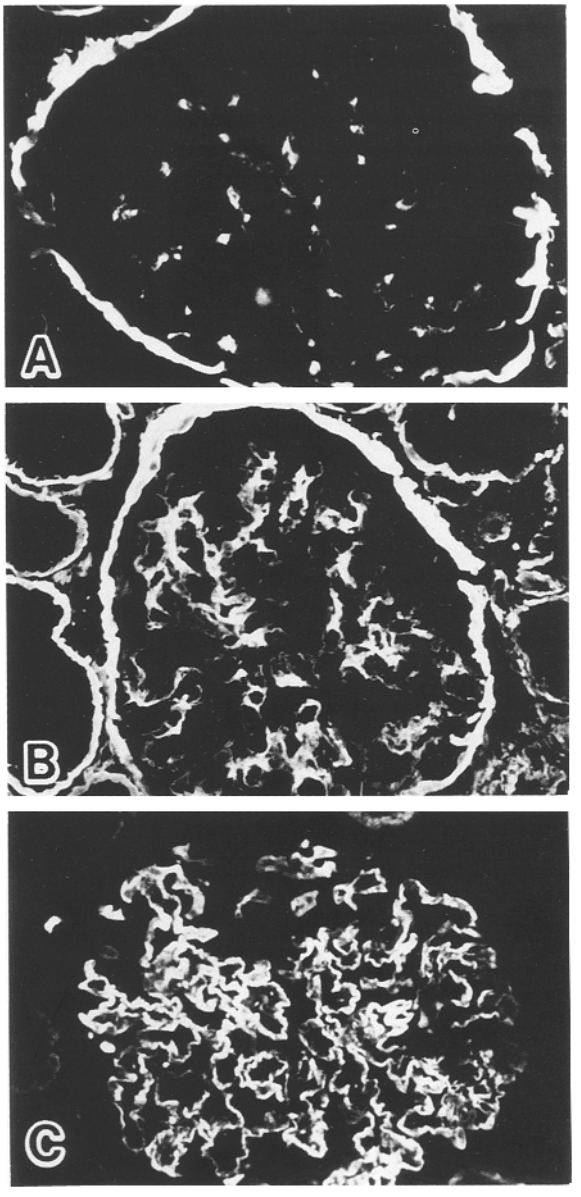

Fig. 1 Indirect immunofluorescence micrograph of normal control kidney. Staining with monoclonal antibodies to basement membrane-associated collagen (**A**), α1 (IV) (**B**) and α3 (IV) (**C**) is observed. Note the difference in the staining pattern. (**A–C**, × 400)

Discussion

In our previous study (Kino et al. 1991), the monoclonal antibody JK-132, produced against human basement membrane collagen fraction, recognized a characteristic epitope of basement membrane origin, in that the peptides with affinity for JK-132 were similar to type IV collagen in a triple-helical conformation in terms of amino acid composition and circular dichroism spectrum. However the epitope recognized by JK-132 contained amino acid sequences distinct from those recognized by a monoclonal antibody to α1 (IV) (Kino et al. 1988).

Immunohistochemically, JK-132 showed preferential staining of the basal laminae in blood vessels. It reacted strongly with the basement membranes of the capillaries in skeletal muscle but not with those of muscle fibers (Kino et al. 1991). JK-132 did not stain the basement membranes of the dermal-epidermal junction, of peripheral nerve, or of skeletal muscle fiber from the snow monkey, although all those specimens reacted positively with an antibody against α1 (IV) (Adachi et al.

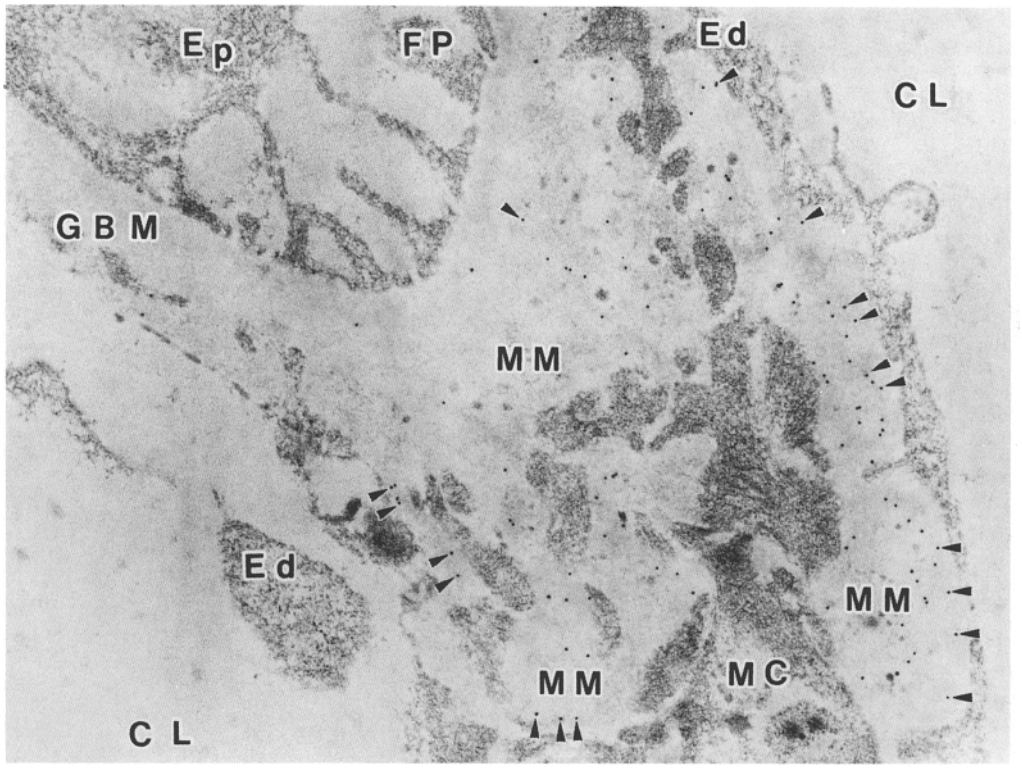

Fig. 2 Immunoelectron micrograph of glomerulus from control kidney showing the distribution of basement membrane-associated collagen. Note many gold particles restricted to the mesangial matrix (MM). Gold particles located in the periphery of the mesangial matrix are indicated by arrowheads. Ep, epithelial cell; FP, foot process; GBM, glomerular basement membrane; MC, mesangial cell; Ed, endothelial cell; CL, capillary lumen. (× 18,000)

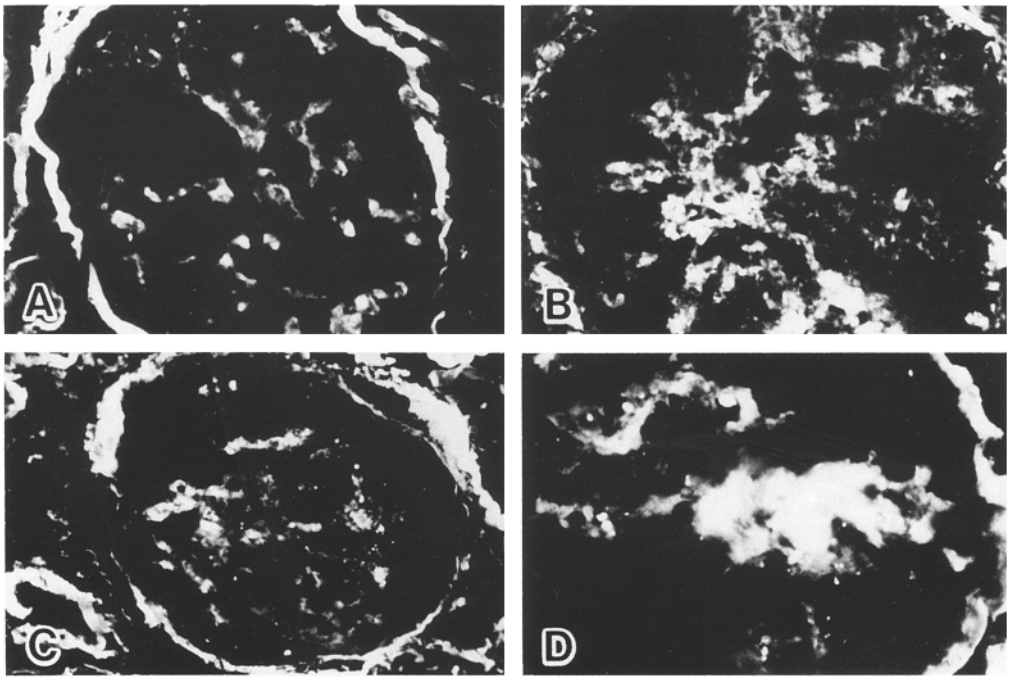

Fig. 3 Indirect immunofluorescent micrograph of kidney from patients with diabetic nephropathy. The distribution of basement membrane-associated collagen (BAC) is observed. Distribution of BAC in the mildly sclerosed glomerulus (**A**), moderately sclerosed glomerulus (**B**) and almost completely sclerosed and hyalinized glomerulus (**C**) and in the nodular lesion (**D**). (**A**, **B** × 400, **C** × 300, **D** × 1,000)

1989). In experimental liver cirrhosis in the snow monkey, there is prominent staining of sinusoids in contrast to the negative staining of these structures under normal conditions (unpublished observations).

In the present study, JK-132 showed a staining distinct from the localization of known chains of type IV collagen, reacting almost exclusively with the mesangial matrix. Regional localization of type IV collagen chains have been reported in the glomeruli (Butokowski et al. 1989), who observed mesangial and subendothelial localization of α1 and α2 chains of type IV collagen, in contrast to the dense staining of the glomerular capillary walls by α3 and α4 (IV). Using the same chain-specific antibodies to type IV collagen, we obtained similar results in the human kidney.

The similar distribution of collagen chains α1 and α2

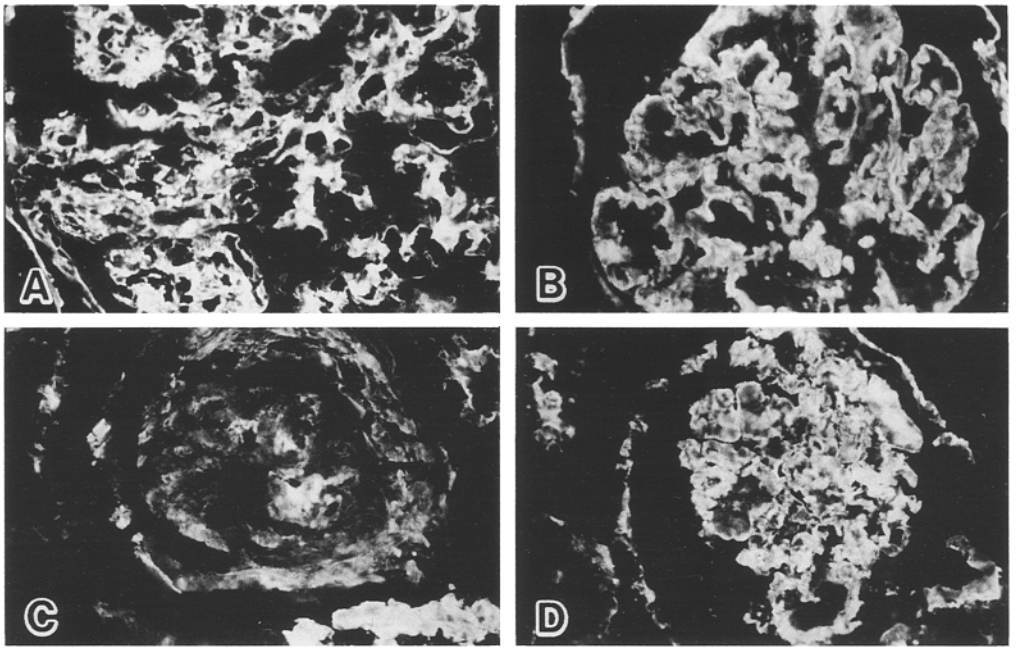

Fig. 4 Indirect immunofluorescent micrographs of kidneys from patients with diabetic nephropathy. The specimens were stained with anti-α2 (IV) antibody (**A**, **C**), anti-α3 (IV) antibody (**B**) and anti-α4 (IV) antibody (**D**). Increased mesangial staining for α2 (IV) is observed in a moderately sclerosed glomerulus (**A**). The intensity of staining is decreased in the hyalinized glomerulus (**C**). Lobular staining of α3 is observed in a moderately sclerosed glomerulus (**B**) staining for α4 is preserved in a hyalinized glomerulus (**D**). (**A**, **B** ×400, **C**, **D** ×300)

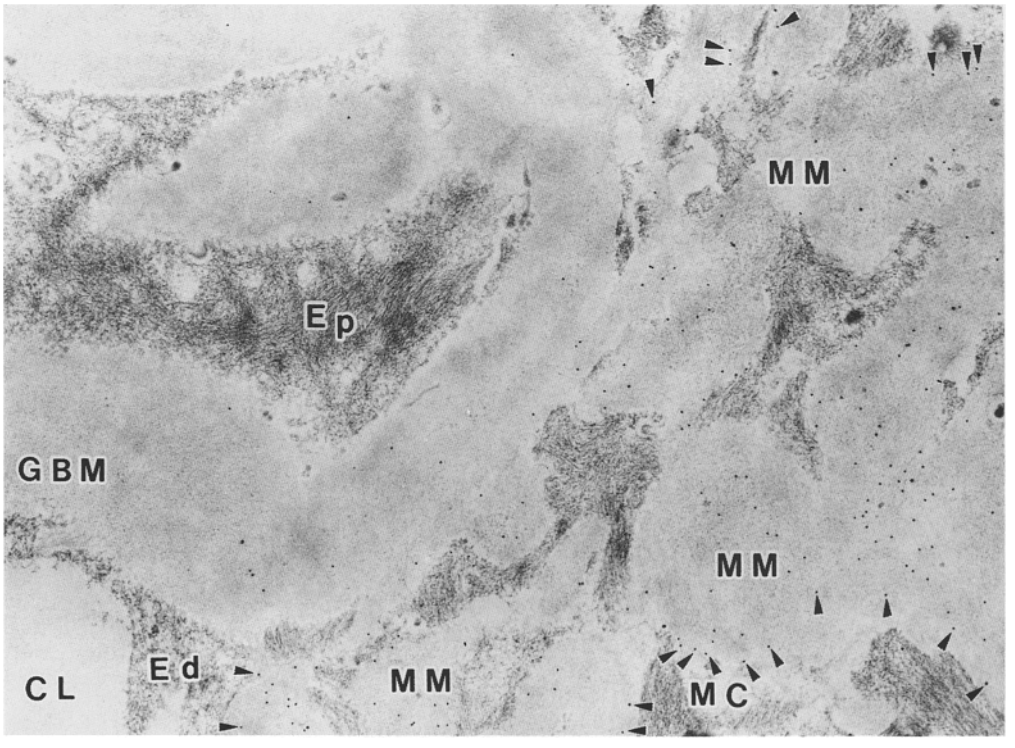

Fig. 5 Immunoelectron micrograph of the glomerulus from a patient with diabetic nephropathy. The ultrastructural distribution of basement membrane-associated collagen is seen. Note gold particles confined to the mesangial matrix (MM). Gold particles located in the periphery of the figure is indicated by arrowheads. Ep, epithelial cell; MC, mesangial cell, Ed, endothelial cell, GBM, glomerular basement membrane; CL, capillary lumen. (×15,000)

Table 3 Distribution of basement membrane-associated collagen and various chains of type IV collagen in normal control and in patients with diabetic nephropathy

	Capillary			Mesangium		
	Normal	Diabetes		Normal	Diabetes	
		Moderate	Advanced		Moderate	Advanced
BAC	(−)	(−)	(−)	(++)	(+++)	(++)
α1, α2 (IV)	(+)	(+)	(−)	(++)	(+++)	(+)
α3, α4 (IV)	(++)	(+++)	(++)	(−)	(−)	(−)

BAC, basement membrane-associated collagen

and of α3 and α4 may be attributed to the similarity of their respective genes. The α1 and α2 (IV) genes have been found together on chromosome 13 at the segment q34 (Griffin et al. 1987). These two genes were found to be arranged in opposite directions, head-to-head, separated only by a short region of 127 bp (Poschel et al. 1988). The α3 and α4 (IV) genes were also found together on chromosome 2 at q35–37 (Mariyama et al. 1992). The molecular structure of BAC and the isolation of BAC cDNA cloning are presently being studied.

Unique differences in the distribution of type IV collagen chains have been reported. Kleppel et al. (1989) and Kashtan et al. (1986) described the absence of novel chains of type IV collagen in the GBM of male patient with Alport's syndrome, whereas the traditional chains of type IV collagens are present. In membranous nephropathy the subepithelial spikes, and the thickened GBM in stage II and early stage III, consisted predominantly of novel type IV collagen. In late Stage III, an increase in the traditional type IV collagen molecules was observed in the subendothelial region (Kim et al. 1991a).

The difference in the localization of various collagen chains both in the normal kidney and in these various diseases may be explained by differential sites of synthesis of these chains. BAC may be synthesized by mesangial cells, α1 and α2 (IV) chains by endothelial/mesangial cells, and α3 and α 4 (IV) chains by visceral epithelial cells.

Although the pathogenesis of diabetic nephropathy is not completely understood, disturbed regulation of the synthesis or metabolism of ECM components has been reported. As diabetic glomerulosclerosis progresses, there is an increase in the glomerular ECM and a decline in renal function. In diabetic nephropathy, biochemical (Beisswenger and Spiro 1973; Kartunen et al. 1986), immunohistochemical (Falk et al. 1983; Nerlich and Schleichert 1991) and molecular biological studies (Ihm et al. 1992; Ledbetter et al. 1990) have indicated an increase in type IV collagen.

Immunohistochemically Falk et al. (1983) and Nerlich and Schleicher (1991) found an increase in type IV collagen in early and moderate diabetic nephropathy and a decrease in hyalinized glomeruli. BAC showed a very similar staining intensity to that of type IV collagen, the intensity in creasing as the glomerulosclerosis advanced but decreasing in advanced hyalinized glomeruli. However, BAC was stained in nodular lesions in contrast to the absence of staining with type IV collagen.

Recently, Kim et al. (1991b) demonstrated that antibodies to α3 and α4 (IV) reacted intensely with the thickened GBM but not with the mesangium in patients with insulin-dependent diabetes mellitus. In contrast, reactivity of the antibodies to α1 and α2 was prominent within the expanded mesangial matrix, although staining was significantly decreased in the peripheral capillary walls. We obtained similar results in our study of patients with NIDDM.

Our study further supports the view that expansion of the mesangial matrix and thickening of the GBM involve separate and distinct collagen components (Kim et al. 1991b) and that BAC is a good marker of mesangial matrix expansion in diabetic nephropathy.

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