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Alterations in extracellular matrix components in transplant glomerulopathy

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Abstract The distribution pattern of extracellular matrix (ECM) components in transplant glomerulopathy was studied in relation to light microscopic features, actin expression of mesangial cells, and intraglomerular inflammatory cells. Nine cases of mild (group I) and nine cases of severe (group II) transplant glomerulopathy were stained with antisera against fibronectin (FN), tenascin (TN), collagen types III and IV, smooth muscle actin, CD45RO, CD68, and Ki-67 antigen. The composition of ECM was similar in the two groups. The expanded mesangium was diffusely stained by type-IV collagen, FN and TN, and focally and weakly stained by type-III collagen and smooth muscle actin. Type-IV collagen was linearly stained along the capillary walls, imparting a double-contour feature, whereas FN and TN showed granular staining along the capillary walls. CD68 positive cells were increased in severe transplant glomerulopathy, but this increase was not related to ECM deposition. These findings suggest that increased glomerular deposition of normal and abnormal ECM components participate in the evolution of transplant glomerulopathy.

Key words Transplant glomerulopathy · Extracellular matrix · Actin · Inflammatory cells

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Introduction

Transplant glomerulopathy is characterized by mesangial widening and thickening of glomerular capillary walls in chronically rejecting renal allografts [10]. Extracellular matrix (ECM) may accumulate in the glomeruli of transplant glomerulopathy; however, little is known about the composition of ECM [6, 18]. Deposition of various ECM components has been reported in the glomeruli of pre-eclamptic nephropathy, showing similar light microscopic features to transplant glomerulopathy [4, 13]. In contrast to the resolution of increased matrix after delivery in pre-eclamptic nephropathy, the glomerular lesion in transplant glomerulopathy persists and may even progress to complete obsolescence of glomeruli. It is unclear whether this progress to glomerulosclerosis is related to the deposition of abnormal matrix or the persistence of injury. Fibronectin (FN) and tenascin (TN) are normal components of the glomerular mesangium, whereas the deposition of type-III collagen is considered to be abnormal [5, 17]. In addition to increased matrix, intraglomerular hypercellularity is occasionally found in transplant glomerulopathy. It may be related to a transition from transplant glomerulitis to transplant glomerulopathy or intraglomerular proliferative activity. It may also be related to the deposition of ECM by either a direct or indirect mechanism. We studied the glomerular staining pattern of type-III and -IV collagens, FN and TN in transplant glomerulopathy, and sought a possible link between light microscopic features and ECM deposition, actin expression of mesangial cells, and intraglomerular infiltrate.

Materials and methods

A total of 670 renal allograft biopsies were examined between 1 July 1992 and 31 December 1997 in the Department of Pathology, Yonsei University College of Medicine. Transplant glomerulopathy was diagnosed in 54 cases. Biopsies containing less than six nonsclerotic glomeruli per section, or cases with overlapping glomerulonephritis (four cases of IgA nephropathy, one case each of

Table 1 Demographic findings

	Group I	Group II
Number of cases	9	9
Age, gender ratio (male:female)	36.7±11.6, 8:1	41.3±11.0, 8:1
Proteinuria (g/24 h)	2.0±1.2*	7.8±6.3*
Serum creatinine at time of biopsy (mg/dl)	2.2±0.8	3.3±6.3
Time to biopsy (months)	67.3±37.9	82.5±71.8
HLA match		
A	1.1±0.3	1.3±0.5
B	0.2±0.3	0.6±0.5
DR	0.9±0.3	1.1±0.4

* $P<0.05$

membranous nephropathy and membranoproliferative glomerulonephritis) were excluded. Among the remaining cases, nine cases of mild (group I: less than 25% of nonsclerotic glomeruli involved by transplant glomerulopathy) and nine cases of severe (group II: more than 50% of nonsclerotic glomeruli involved) transplant glomerulopathy were randomly selected. There was no significant difference between the two groups with regard to the age and gender of donors and recipients, HLA match, and serum creatinine level. However, 24-h urinary protein excretion at the time of biopsy was significantly higher in group II (Table 1). Allograft biopsies were diagnosed according to the Banff schema [14]. The degree of tubular atrophy, interstitial fibrosis and interstitial inflammation were graded semi-quantitatively on a scale from 0 to 3 (0 negative; 1 <25% of cortex involved; 2 <50% cortex involved; 3 ≥50% of cortex involved). The number of glomeruli and the percentages of global and segmental sclerosis were recorded.

Stains for type-III and -IV collagens, FN, TN, and smooth muscle actin were performed on formalin-fixed, paraffin-embedded 4-μm-thick renal sections, using the LSAB kit (Dako, Glostrup, Denmark). The extent and intensity of staining were graded as follows: 0 negative; 1+ weakly positive; 2+ positive; and 3+ strongly positive. For the identification of intraglomerular inflammatory cells and proliferating cells, antibodies against CD45RO, CD68, and Ki-67 antigens were used. The number of positive cells were counted and expressed as mean±SD per nonsclerotic glomerulus. As controls, six cases of renal tissue unaffected by renal cell carcinoma and two cases of idiopathic hematuria were used. Comparison of data was performed using the Mann-Whitney test and Kruskal Wallis test, and P value <0.05 was considered significant.

Results

Light microscopy

The percentage of segmental sclerosis was significantly higher in group II than in group I ($P<0.01$). However, the degree of tubulointerstitial lesion was similar between the two groups (Table 2). Four cases showed increased inflammatory cells in a few glomeruli. Three cases contained mononuclear cell infiltration and the remaining case showed a mixed mononuclear and polymorphonuclear cell infiltrate, which was not associated with either acute tubulointerstitial rejection or glomerular endothelial swelling.

Immunohistochemistry

The glomeruli of control kidneys showed diffuse, linear staining of type-IV collagen along the glomerular basement membrane. It was also minimally present in the mesangium. Type-III collagen was negative in the glomeruli. FN was weakly stained along the glomerular capillary walls and in the mesangium, whereas TN was mostly negative or weakly positive near the vascular hilum. There were only a few CD45RO and CD68 positive cells in the glomeruli.

Table 2 Light microscopic findings

	Group I	Group II
Number of cases	9	9
Number of glomeruli	12.2±4.2	12.1±7.7
% Global sclerosis	21.2±21.4	17.6±16.3
% Segmental sclerosis	7.7±7.8*	25.7±17.6*
Injury score		
Interstitial inflammation	1.1±0.8	1.1±0.6
Tubular atrophy	1.1±0.6	1.4±1.2
Interstitial fibrosis	1.3±1.0	1.7±1.1

* $P<0.01$

In the glomeruli showing characteristic features of transplant glomerulopathy, type-IV collagen was positive in the mesangium and along both the original and newly-formed subendothelial basement membrane. Most intensive staining for type-IV collagen was present in areas of capillary collapse. Type-III collagen was weakly positive in the mesangium, but was strongly positive in the sclerotic glomeruli. FN was diffusely and strongly stained along the capillary walls and in a coarsely granular pattern in the mesangium. Globally sclerotic glomeruli were minimally stained for FN. Mesangial TN staining was roughly parallel to that of FN, but less in its intensity and extent. TN was strongly positive in the subendothelial area of thickened capillary walls in five cases in group I and in four cases in group II. There was no significant difference in the intensity between the two groups. The subendothelial hyalin and sclerotic area were not stained by TN. The glomerular mesangium was stained weakly with smooth muscle actin. A variable but increased deposition of ECM was also present in the apparently normal glomeruli in the sections of transplant glomerulopathy (Table 3). CD45RO and CD68 positive cells were occasionally present in the glomerular capillaries, whereas CD68 and Ki-67 positive cells were mainly noted in areas of parietal epithelial proliferation (Table 4).

In addition to glomerular staining, ECM deposition was present in other tissue components. Type-IV collagen was present along the tubular basement membrane and in

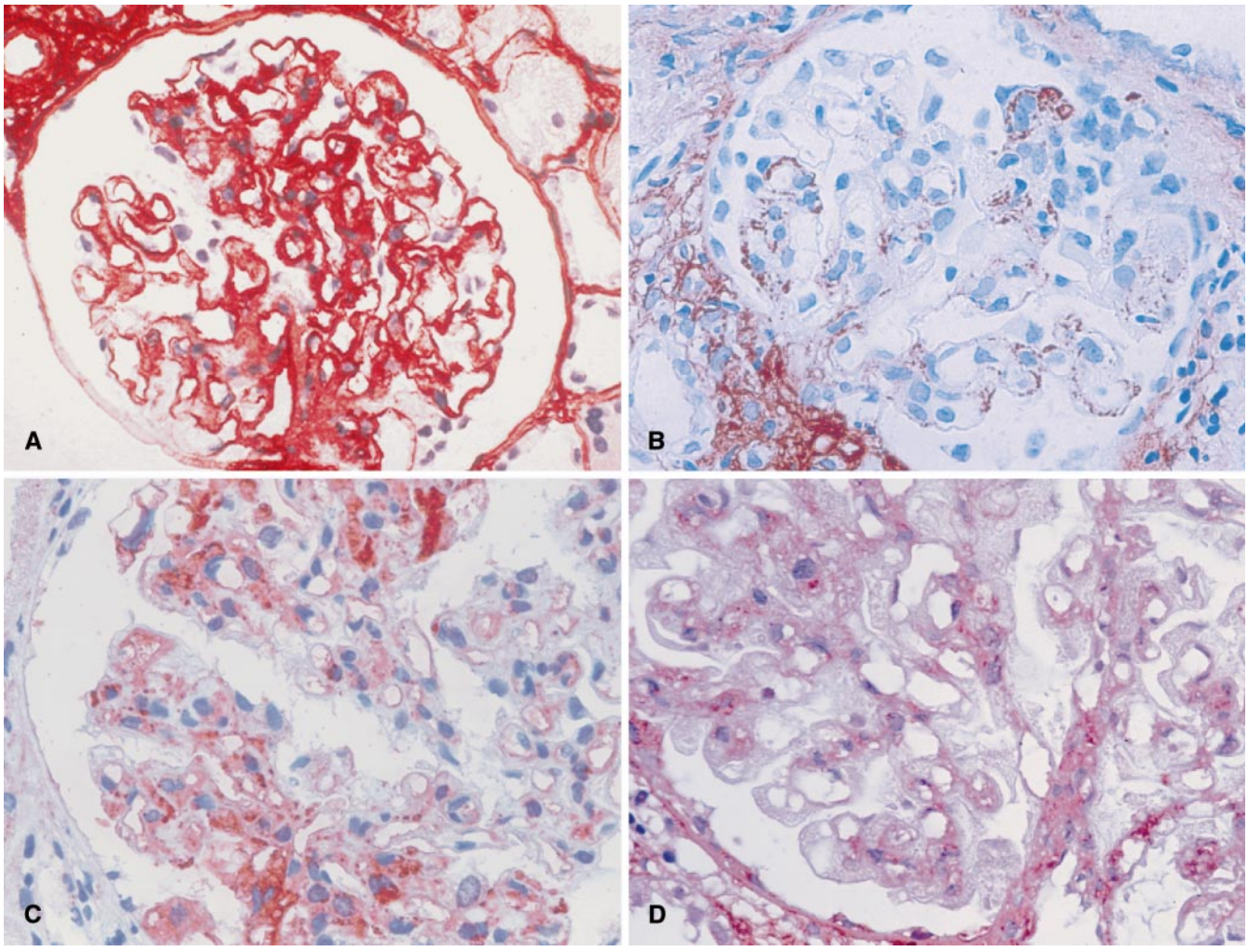

Fig. 1 Immunohistochemistry of transplant glomerulopathy (**A–D**, ×400). Note a double-contour feature of type-IV collagen (**A**) and tenascin staining (**B**) along the peripheral capillary wall. Strong staining of fibronectin (**C**) and weak staining of type-III collagen (**D**) are noted in the glomerular mesangium

the vessels of normal and chronically rejecting kidneys. Interstitium and blood vessels were also strongly positive for type-III collagen, FN and TN in cases with transplant glomerulopathy. The staining intensity of TN in the interstitium was not proportional to the severity of inflammatory infiltrate. Vascular TN reactivity was prominent mostly in the perivascular connective tissue and minimally in the mural stroma. Smooth muscle actin was positive not only in the vessels, but also in the fibrotic interstitium. The interstitial infiltrate was positive for CD45RO or CD68, as well as for Ki-67 antigen. Ki-67 positive cells were occasionally found in tubular epithelium.

Correlation between light microscopic and immunohistochemical findings

There was no significant relationship between the severity of glomerular lesion and ECM deposition. The number of CD68 positive cells showed statistical significance among controls, group I, and group II, being the highest in group II. There was a minimal increase of smooth muscle actin staining in the glomeruli of group II.

Discussion

The deposition of ECM has been reported in the context of chronic renal allograft rejection, mostly in the interstitium and large vessels [1, 12, 15]. Glomerular deposition of ECM largely accords with that of the interstitium. The glomeruli showing features of transplant glomerulopathy were stained intensely with type-IV collagen, FN and TN, both in the glomerular capillary walls and in the mesangium. A double-contour feature of glomerular capillary walls was evident with staining against type-IV collagen, suggesting a prolonged injury to endothelial cells enough to produce new basement membrane material. Glomerular mesangial and endothelial cells can synthesize several types of ECM, including collagens, laminin, and TN [3, 9]. Presence of type-III collagen in the glomeruli with intact Bowman's capsule supports the fact that type-III collagen is produced by the mesangial cells [16]. Moreover, trapping of plasma

Table 3 Extracellular matrix components. Sources and dilutions of antisera used: type-IV collagen (Dako, Glostrup, Denmark), dilution 1:50; type-III collagen (Southern Biotech Associates Inc, Birmingham, Ala.), dilution 1:20; fibronectin (Dako), dilution 1:200; tenascin (Dako), dilution 1:100. *GCW* glomerular capillary wall; *Mes* mesangium; *0* negative; *1+* weakly positive; *2+* positive; *3+* strongly positive

	Control		Group I		Group II	
	GCW	Mes	GCW	Mes	GCW	Mes
Type-IV collagen	1+	1+	2+~3+	1+	2+~3+	1+
Type-III collagen	0	0	0	0~1+	0	0~1+
Fibronectin	0~1+	1+	2+~3+	3+	2+~3+	3+
Tenascin	0~1+	1+	2+~3+	1+	2+~3+	1+

Table 4 Intraglomerular inflammatory cells, proliferating cells, and mesangial actin reactivity. Sources and dilutions of antisera used: CD45RO (Dako), dilution 1:75; CD68 (Dako), dilution 1:75; Ki-67 (Immunotech, Marseille, France), dilution 1:100; smooth muscle actin (Dako), dilution 1:50. *0* negative; *1+* weakly positive; *2+* positive

	Control	Group I	Group II
CD45RO	0.5±0.3*	0.4±0.8	1.0±1.6
CD68	0.3±0.3**	1.0±1.3**	1.9±1.4**
Ki-67	0.1±0.0	0.2±0.3	0.4±1.1
Smooth muscle actin	0~1+	1+	1+~2+

* Number of positive cells per nonsclerotic glomerulus
** $P<0.05$

protein may be involved in the glomerular lesion. IgM and C3 were deposited in the glomeruli in 55.6% and 22.2% of our cases, respectively. Taken together, increased glomerular deposition of normal and abnormal ECM components as well as plasma proteins participates in the evolution of transplant glomerulopathy and may lead to glomerulosclerosis. In addition to glomeruli showing features of transplant glomerulopathy, apparently normal glomeruli also showed increased deposition of ECM, which suggests a rather widespread glomerular injury in chronic rejection [8] and also a way to more serious glomerular pathology.

As a marker of mesangial alteration, smooth muscle actin staining was used. Actin expression has been reported in the mesangial cells in various conditions, either with mesangial proliferation or with increased matrix deposition [2, 7], as well as in chronic rejection [11]. Actin staining was localized in the glomerular axial area and its staining was slightly increased in group II. Although increased glomerular staining for smooth muscle actin correlated with poor graft function [8], shrinkage of mesangial cells and mesangial sclerosis in an advanced glomerular lesion may actually lessen the staining degree and its extent.

It is difficult to speculate on the role of inflammatory cells in ECM deposition in transplant glomerulopathy. Although CD68 positive cells tended to increase in severe glomerular lesions, they showed neither a specific pattern nor were they associated with an increased ECM deposition. Since Ki-67 positive cells were limited in areas of parietal epithelial proliferation, intraglomerular proliferative activity seemed to be minimal.

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