Distribution of fibronectin in normal and diseased human kidneys

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SUMMARY The distribution of fibronectin in 42 renal biopsies was investigated using an indirect immunoperoxidase technique on paraffin sections of formalin-fixed material. Biopsies were obtained from normal human kidneys and from patients with a variety of glomerular disorders. In normal glomeruli, fibronectin was present in Bowman's capsule, in the mesangium, and outlining peripheral capillary loops. A significant increase in fibronectin was observed in most types of glomerulopathy with a distribution closely related to the histopathological diagnosis. Fibronectin was diminished or absent in segmental scars, old diabetic nodules, and hyalinised glomeruli.

Fibronectin is a dimeric glycoprotein with a molecular weight of 450 000 daltons. Two forms have been characterised, a soluble form present in plasma and other extracellular body fluids, originally isolated by Morrison *et al.* in 1948¹ and called 'cold-insoluble globulin', and a form associated with the cell surface of a number of cultured cell lines, also known as 'large external transformation sensitive protein' (LETS).² *In vitro* studies suggest that fibronectin is important in adhesion of cell to cell and cell to substratum.³ It binds specifically to fibrinogen and fibrin,^{4,5} collagen,^{6,7} and heparin.⁸ *In vivo*, fibronectin and reticulin fibres co-distribute in most normal tissues.⁹ In models of experimental hepatic fibrosis produced by trauma or toxins, fibronectin and collagen are produced and deposited at the site of initial injury within hours, and both proteins are then incorporated into the resulting connective tissue septa (Burns *et al.*, unpublished observations).

Recent immunohistochemical studies on glomerular fibronectin have demonstrated that it is a component of the mesangium of normal human glomeruli and is present in increased amounts in the expanded mesangium of a variety of glomerular disorders.¹⁰⁻¹² While the observations of these authors for the most part concur, there are a few areas of disagreement. In normal human glomeruli, Pettersson and Colvin found fibronectin not only in the mesangium but also in capillary basement membranes,¹¹ whereas Weiss *et al.*¹² and Stenman and Vaheri⁹ found it in mesangium alone. In ad-

dition, the increased glomerular fibronectin in membranoproliferative glomerulonephritis noted by Pettersson and Colvin¹¹ and Weiss *et al.*¹² was contrary to the findings of Scheinman *et al.*¹⁰ The present study was undertaken in order to try to resolve some of these discrepancies.

Material and methods

Forty-two renal biopsies were included in the study. Of these, four biopsies were of normal renal tissue obtained from donor kidneys at the time of renal transplant. The remaining 38 were from patients with the following histopathological diagnoses: acute diffuse glomerulonephritis (3), minimal change (4), IgA nephropathy (3), focal segmental proliferative glomerulonephritis (6), diabetic nephropathy (5), mesangiocapillary (membranoproliferative) glomerulonephritis (4), membranous glomerulonephritis (4), malignant hypertension (3), amyloidosis (2), diffuse crescentic nephritis (3), and disseminated intravascular coagulation (1).

The isolation and characterisation of the fibronectin antigen and the rabbit anti-fibronectin IgG derived from it have been described elsewhere.^{13,14}

Immunohistochemical staining for fibronectin was conducted on sections of formalin-fixed, paraffin-embedded renal tissue by an indirect immunoperoxidase technique following pepsin digestion.¹⁴ Dewaxed, hydrated 3 μ m paraffin sections were incubated for 10 minutes at 37°C in distilled water, followed by a further 2 hours' incubation at the same temperature in a 0.4% solution of pepsin (Sigma Cat.

No. P-7012) in 0.01N HCl. After washing in running cold water for 10 minutes, the sections were rinsed in 0.01 M phosphate buffered saline, pH 7.2 (PBS), and treated with rabbit anti-fibronectin IgG (75 µg/ml) for 30 minutes at 22°C. They were then washed twice in PBS and treated with a 1:50 dilution of horseradish peroxidase labelled swine anti-rabbit IgG (Dakopatts, Mercia Brocades Ltd) for a further 30 minutes at 22°C. After further washings in PBS the horseradish peroxidase was developed with diaminobenzidine tetrahydrochloride and hydrogen peroxide in buffer.¹⁵ In some instances, staining for fibronectin was also performed by the indirect immunofluorescence technique on sections of snap-frozen renal tissue. These preparations were examined by epi-illumination using a Leitz Ortholux fluorescence microscope and blue light excitation.

Control sections in both procedures were incubated with either non-immune rabbit IgG or with fibronectin absorbed rabbit anti-fibronectin IgG. All sera, both immune and otherwise, were diluted with a 1.4% solution of ovalbumin in PBS.

Results

The results are summarised in the Table.

An identical staining pattern for fibronectin was observed in immunoperoxidase stained paraffin sections and corresponding immunofluorescent stained frozen sections. All controls were negative.

In normal kidney, fibronectin was present in mesangium and as a fine line around peripheral

capillary loops of glomeruli (Figs 1 and 2). There was positive staining of Bowman's capsule and of the interstitium with a peritubular distribution. Vessel walls were stained most conspicuously on their intimal aspect. In one of the 'normal' renal biopsies, subcapsular renal cortex contained two ischaemic glomeruli which showed shrinkage but not total hyalinisation of the tuft and intracapsular fibrosis. These had increased fibronectin both within the shrunken tuft and in the fibrosing capsule.

In all conditions associated with an expanded mesangium observable on routinely stained paraffin sections, there was a corresponding increase in mesangial fibronectin. This was most pronounced in acute diffuse glomerulonephritis (Figs 3 and 4), mesangiocapillary glomerulonephritis (Fig. 5), and diabetic glomerulopathy. In these three conditions there was also a considerable increase of fibronectin in peripheral capillary walls.

Only two of four cases of membranous glomerulonephritis showed increased fibronectin in thickened glomerular basement membranes (Fig. 6). In one of these cases it was unevenly distributed, being most marked on the endothelial aspect. The remaining two cases of membranous glomerulonephritis and all those with minimal change nephropathy showed no significant difference in fibronectin distribution from normal kidneys.

All cases of crescentic glomerulonephritis had conspicuous staining for fibronectin between proliferating crescentic cells (Fig. 7). Glomerular tuft staining was variable.

Results*

Diagnosis	No. of biopsies	Mesangium	Glomerular capillary wall	Vessels	Interstitium	Special features
Normal	4	+	+	+	+	1 case with two ischaemic glomeruli having +++ mesangial staining
Acute diffuse proliferative GN	3	+++	++	++(2) +(1)	++(1) +(2)	
Minimal change	4	+	+	+	+	
IgA disease	3	++	+	+	+	
Focal segmental proliferative GN	6	+ to +++	+	+	+(4) ++(2)	Increased staining in segmental lesions. One case with +++ in crescents
Diabetic	5	++ to +++	+ to +++	+	+ to ++	Early nodules +++ Older nodules + or - Hyalinised glomeruli -
Mesangiocapillary (membranoproliferative)	4	+++	+++	+	++	
Membranous	4	+	+(2) ++ (1) +++ (1)	+	+(3) ++ (1)	
Diffuse crescentic	3	+ to ++	+	+	++	+++ in crescents
Amyloid	2	- to ++	- to ++	+	+	Amyloid deposits negative ++ staining around them
Malignant hypertension	3	- to +++	+ to ++	+ to ++	++	Negative segmental scars and hyalinised glomeruli
Disseminated intravascular coagulation	1	+	++	+	+	+++ intracapillary thrombi

*Assessment of fibronectin staining was graded subjectively on a scale - to +++. Numbers in parentheses refer to the number of cases showing the change.

Fig. 1 *Normal glomerulus showing staining for fibronectin in mesangium and capillary loops. Rabbit anti-fibronectin IgG and horseradish peroxidase labelled swine anti-rabbit IgG. $\times 480$*

Fig. 2 *Normal glomerulus showing fibronectin in mesangium and capillary loops. Rabbit anti-fibronectin IgG and FITC-labelled swine anti-rabbit IgG. $\times 500$*

Fig. 3 Glomerulus in acute diffuse proliferative glomerulonephritis with increased staining for fibronectin in mesangium and capillary loops. Rabbit anti-fibronectin IgG and horseradish peroxidase labelled swine anti-rabbit IgG. $\times 480$

Fig. 4 Glomerulus in acute diffuse proliferative glomerulonephritis showing absence of staining with fibronectin-absorbed antiserum. Fibronectin-absorbed rabbit anti-fibronectin IgG and horseradish peroxidase labelled swine anti-rabbit IgG. $\times 480$

Fig. 5 Glomerulus in mesangiocapillary glomerulonephritis with increased mesangial and capillary wall staining for fibronectin. Rabbit anti-fibronectin IgG and horseradish peroxidase labelled swine anti-rabbit IgG. $\times 480$

Fig. 6 Glomerulus in membranous glomerulonephritis with increased staining for fibronectin in peripheral capillary walls. Rabbit anti-fibronectin IgG and horseradish peroxidase labelled swine anti-rabbit IgG. $\times 480$

Fig. 7 Glomerulus in crescentic glomerulonephritis with strongly positive staining for fibronectin between crescentic cells. Rabbit anti-fibronectin IgG and horseradish peroxidase labelled swine anti-rabbit IgG. $\times 480$

Fig. 8 Glomerulus in diabetic glomerulopathy showing a negatively stained large nodule and smaller nodules with varying degrees of positivity for fibronectin. Rabbit anti-fibronectin IgG and horseradish peroxidase labelled swine anti-rabbit IgG. $\times 480$

In renal amyloidosis, fibronectin surrounded the deposits of amyloid.

Hyalinised glomeruli, old segmental scars, and old diabetic nodules were all negative for fibronectin (Fig. 8). In contrast, glomeruli undergoing sclerosis, early segmental lesions, and some diabetic nodules (Fig. 8) were strongly positive for fibronectin.

Interstitial fibronectin was increased in severe glomerulonephritides and in end-stage renal disease. In vessels, fibronectin appeared to be concentrated in the intima. An increase was observed in intimal fibrosis and focally in small arteries and arterioles in malignant hypertension and acute diffuse glomerulonephritis.

Discussion

The distribution of fibronectin in normal and diseased human kidneys has previously been studied using immunological techniques applied to sections of frozen renal tissue. Formalin-fixed, paraffin-processed renal tissue does not react with antibody to fibronectin without prior enzymic reactivation of the antigen. In this study this was achieved by exposure to a 0.4% pepsin solution. Subsequent immunohistochemical staining gave results identical with those produced by immunofluorescent staining of frozen sections and enabled a more precise localisation of fibronectin within optimally preserved renal tissue. The method also had the advantage of rendering more material available for investigation.

This study has confirmed some previous observations regarding renal fibronectin distribution and has provided additional evidence for its presence in peripheral capillary walls of normal human glomeruli. This observation corresponds with immunohistochemical demonstration at the ultrastructural level of fibronectin along cell surfaces abutting glomerular capillary basement membranes.¹⁶ Additional evidence is also provided for increased glomerular fibronectin in mesangiocapillary glomerulonephritis, as in other types of glomerulopathy with an expanded mesangium. The reason for previous slightly differing results is not clear, though it may be due, in part, to differing methods of fixation.¹¹

The observation of negatively staining hyalinised glomeruli and old segmental scars¹⁰ has been confirmed, and, in addition, it was noted that ageing diabetic nodules lose their positivity for fibronectin. In all these situations the actively progressing lesion contains a considerable quantity of fibronectin, which gradually diminishes as scar tissue is laid down.

In the acute stages of damage, increased glomerular fibronectin may be present irrespective of the nature of the injurious process. Thus both ischaemi-

cally and immunologically damaged glomeruli contain increased quantities. The two exceptions are minimal change glomerulonephritis and some cases of membranous glomerulonephritis where staining for glomerular fibronectin does not differ significantly from that in normal kidneys. This indicates that accumulation of fibronectin does not directly relate to the occurrence of proteinuria. Whether the increased amount in diseased glomeruli is derived from plasma or by local production is uncertain. The considerable quantities present in conditions such as acute diffuse proliferative glomerulonephritis suggest that it may be an exudative phenomenon and plasma-derived. An alternative hypothesis is that glomerular cells produce increased fibronectin as a non-specific response to injury. In this context, it is of interest to note that production of fibronectin by glomerular cells in culture has been demonstrated.¹⁶ Whatever its source, it may well account for part of the mesangial expansion in glomerulonephritis.

Since fibronectin has an affinity for fibrin, its presence in sites of fibrin deposition such as glomerular crescents and intracapillary thrombi might be predicted. This has been discussed by Pettersson and Colvin, who originally observed the considerable quantity to be found between proliferating crescentic cells.¹¹

The role of fibronectin in relation to altered glomerular function in disease and in the reparative process requires further investigation. *In vitro* studies have suggested that it may be important in cell-collagen interaction and in collagen deposition.¹⁷ In the damaged glomerulus it might be involved in basement membrane regeneration or in the formation of scar tissue.

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