

## The glomerular distribution of laminin and fibronectin in glomerulonephritis

L. Nakapoulou, K. Stefanaki, P.M. Zeis, J. Papadakis, J. Boletis, G. Vosnidis and P. Davaris

Department of Pathology, Medical School, University of Athens, Athens, Greece

**Summary.** Laminin (LAM) and fibronectin (FI) are regarded as major components of the glomerular extracellular matrix

The aim of this study was to define the distribution of LAM and FI in primary glomerulonephritis (GN) and GN of systemic lupus erythematosus (SLE) and to correlate the type of glomerular disorders with possible changes in the expression of these components. Normal portions of kidney tissue from 10 patients with renal tumors and sixty-six renal biopsies obtained from patients with GN were studied by the immunoperoxidase-antiperoxidase (PAP) method for the detection of LAM and FI. Twelve patients had membranous GN (MGN), 8 mesangiocapillary GN (MCGN), 21 mesangioproliferative GN (MPGN), including 11 cases of IgA nephropathy, 11 focal segmental glomerulosclerosis (FSGS) and 14 had SLE. In MGN, LAM was detected more intensely than FI along the glomerular basement membranes (GBM), in subepithelial GBM protrusions and in the newly-formed GBM. On the contrary, FI was intensely expressed in the mesangium. LAM and FI expression was pronounced in stages II and III of MGN. In MCGN, LAM and FI were diffusely expressed along the GBM and in the mesangium. The distribution of the two antigens in MPGN and FSGS was similar to that seen in normal glomeruli. However, the FI staining reaction was more intense in severe mesangioproliferative lesions, mainly observed in the cases of IgA-nephropathy. There were no differences in the distribution of LAM and FI between primary and SLE GN. The antigen staining pattern was pronounced in the membranous and mesangiocapillary lesions of SLE GN. The crescents observed in 7 cases contained increased amounts of both LAM and FI, while the adhesions with Bowman's capsule seen in 9 cases demonstrated increased amounts of LAM. In contrast, the large adhesions observed in 2 cases and the sclerotic lesions in 4 cases contained only small amounts of LAM.

In conclusion, the increased LAM and FI glomerular expression mainly in MGN and MCGN, and the FI overexpression in severe mesangioproliferative lesions of IgA nephropathy suggest that the disturbance of extracellular glomerular matrix is probably due to the damage of glomerular cells or the involvement of the above components in immune-complex formation.

**Key words:** Fibronectin, Laminin, Glomerulonephritis, Immunoperoxidase method

### Introduction

Laminin (LAM) and fibronectin (FI) are non collagenous glycoproteins and are regarded as major components of extracellular matrix (Stenham and Vaheri, 1978; Ekblom et al., 1982).

The normal glomerular basement membrane (GBM) is a specialized extracellular matrix, composed of type IV collagen, LAM, heparan sulphate proteoglycan and small amounts of entactin, nidogen and chondroitin sulphate proteoglycans (Martínez-Hernández and Amenta, 1983; Timpl et al., 1983a; Martínez-Hernández and Chung, 1984; Oikawa et al., 1989; Van den Heuvel et al., 1989). LAM the predominant glycoprotein in the GBM binds to type IV collagen and has cell-adhesive properties (Foidart et al., 1980; Timpl et al., 1983b; Laurie et al., 1984; Mounier et al., 1986). According to previous data, LAM is preferentially localized in the lamina rara (Foidart et al., 1980) and the subendothelial part of GBM. Nevertheless, recent immunohistochemical studies indicate that LAM is distributed throughout the GBM (Laurie et al., 1984; Abrahamson, 1986) and in the mesangium (Scheinman et al., 1980; Courtoy et al., 1982; Shinkai et al., 1990; Weeks et al., 1991).

FI is mainly observed in renal interstitium, vessel walls, and glomerular mesangium (Dixon et al., 1980; Linder et al., 1980; Courtoy et al., 1982). Several authors have reported FI detection along the subendothelial part of GBM (Pettersson and Colvin, 1978; Courtoy et al., 1982; Fouser and Michael, 1987) but it has not been

fully established whether this glycoprotein is an intrinsic component of mature GBM or an exogenous serum-derived element (Martínez-Hernández and Amenta, 1983).

Several investigators have reported changes in the distribution or quantity of extracellular components in glomerular lesions of patients with renal disease (Schiffer et al., 1981; Fukatsu et al., 1988; Funabiki et al., 1990) and experimental models (Matsuo et al., 1986). However, there is some controversy about the alteration of the extracellular matrix in glomerulonephritis.

Therefore, the aim of this study was to investigate the nature of the glomerular extracellular matrix in primary glomerulonephritis (GN) and GN of systemic lupus erythematosus (SLE) by assessment of LAM and FI immunohistochemical distribution.

#### Materials and methods

Percutaneous needle renal biopsies were obtained from 66 patients with various types of GN. Fifty-two patients suffered from primary forms of the disease and 14 from GN of SLE (Table 1). Histologically normal kidney tissue was obtained from 10 patients with renal tumor. The specimens were fixed in buffered formalin and embedded in paraffin.

Serial sections were cut at 4  $\mu$ m, and stained with haematoxylin eosin, PAS, Silver-Methenamine and Trichrome Masson. In all cases diagnosis of the type of glomerulonephritis was based on the characteristic findings by light and immunofluorescence microscopy.

The immunoperoxidase-antiperoxidase (PAP) method (Sternberg, 1979) was used on paraffin sections for the detection of LAM and FI. Deparaffinized and rehydrated paraffin sections were treated with methanol containing 0.3%  $H_2O_2$  in order to block the endogenous peroxidase. After incubation with 0.01% pronase for 60 min, in order to unmask the antigenic determinants, sections were treated with the following antibodies:

- a) normal swine serum (1:20, Dakopatts, Denmark)
- b) primary antibodies: polyclonal rabbit anti-LAM antibody (1:60, Eurodiagnostics); and polyclonal rabbit anti-FI antibody (1:50, Dakopatts, Denmark).
- c) Swine anti-rabbit IgG (1:40, Dakopatts, Denmark).
- d) Peroxidase anti-peroxidase complex (PAP) (1:60, Dakopatts, Denmark).

Sections were thoroughly rinsed with Tris Buffer solution (pH 7.6) between reaction steps. Finally, slides were immersed in Tris buffer solution (pH 7.6) containing 0.6 mg/ml Diaminobenzidine and 0.03%  $H_2O_2$  to visualize immunoreactivity. Sections incubated with normal rabbit serum in place of the primary antibody were used as negative controls.

The extraglomerular vessels and the mesangium in every case were used as positive controls for LAM and FI respectively. The positive staining for both antigens was classified as faint (+), moderate (++) and intense (+++).

#### Results

In the ten cases of normal kidney tissue, LAM was detected along the glomerular and tubular basement membranes, in the mesangium and extraglomerular arteriolar walls. On the other hand, FI was expressed mainly in the mesangium and less frequently in the extraglomerular arteriolar walls.

In all cases of membranous glomerulonephritis (MGN) LAM and FI were detected along the GBM, in subepithelial basement-membrane protrusions (spikes) in newly-formed basement membrane material above the subepithelial deposits and less intensely in the mesangium (Fig. 1).

The staining intensity of LAM was more pronounced in stages II and III of MGN (Table 1). Although FI was also expressed along the GBM, the staining appeared less conspicuous than for LAM. FI was expressed with increased staining intensity in the mesangium (Table 2). In mesangiocapillary glomerulonephritis (MCGN) both LAM and FI were expressed along the basement

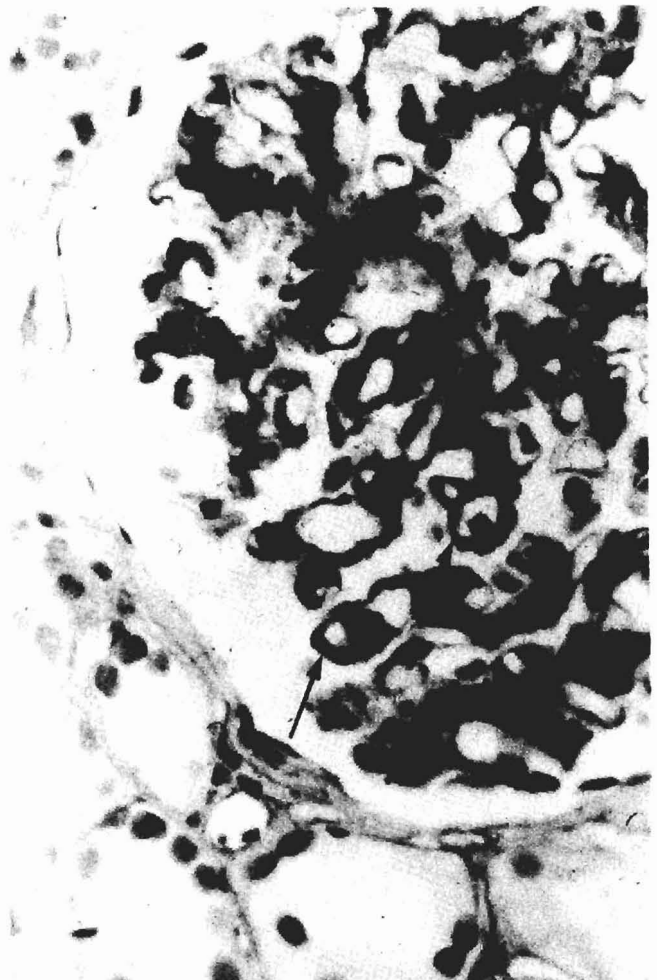


Fig. 1. LAM expression in MGN along some basement membranes (arrows) and in the mesangium. PAP, x 500

# Laminin and fibronectin in glomerulonephritis

membranes and in the mesangium (Fig. 2).

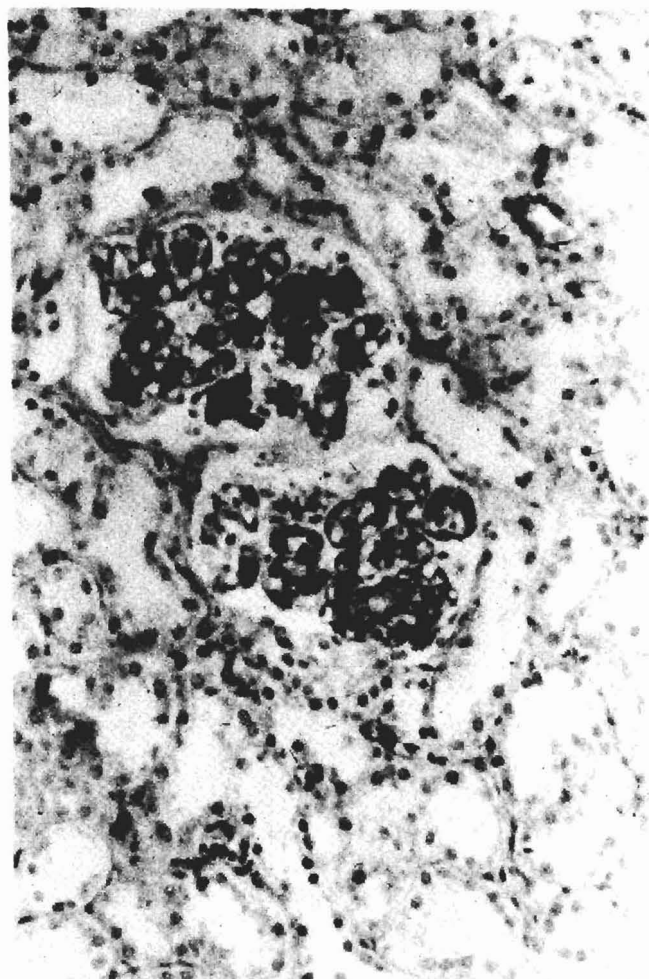
The distribution of the two antigens in mesangio-proliferative glomerulonephritis (MPGN) and focal

segmental glomerulosclerosis (FSGS) was similar to that seen in normal glomeruli. However, in the severe mesangioproliferative lesions mainly observed in IgA

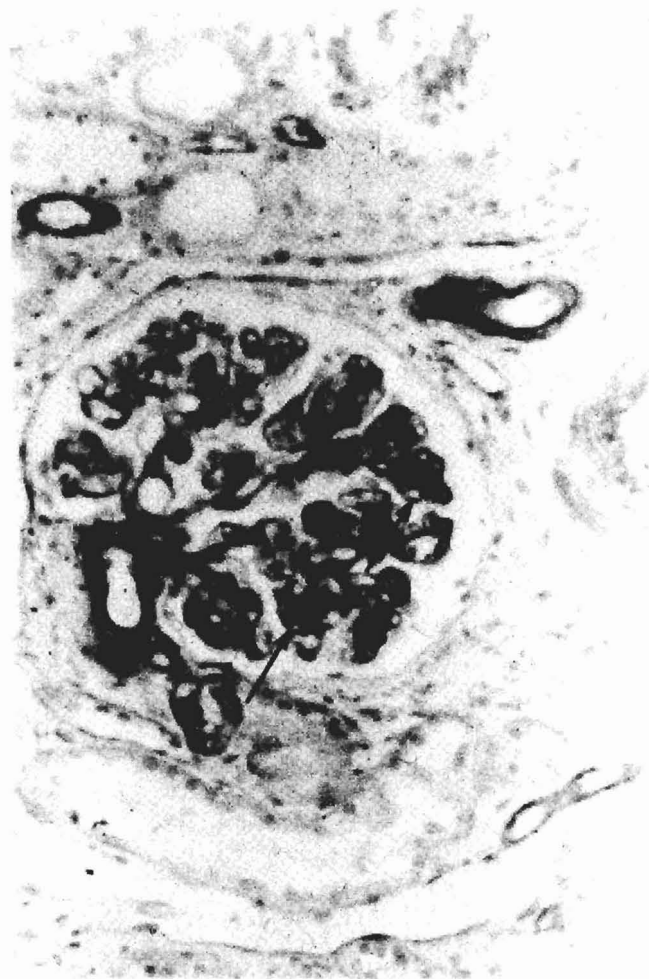
**Table 1.** Distribution of laminin in various types of glomerulonephritis (GN).

TYPE OF GN	NUMBER OF CASES	GBM*			MESANGIUM			CR*			SYN*			SCL*		
		+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++
Membranous GN (MGN)	12															
Stage I	2	1	1	2												
Stage II	6	2	2	2							1	1				
Stage III	4		2	2	2	2										
Mesangiocapillary GN (MCGN)	8	2	5	1	2	3	3									
Mesangioproliferative GN (MPGN)	10	6	4		6	2						1				
IgA-nephropathy	11	10	1		5	2	1	1			2	1		1	1	
Focal segmental glomerulosclerosis (FSG)	11	8	3		3	1	1				2				1	
Systemic lupus erythematosus (SLE)	14							3	3						1	
Stage III	3	2	1		2											
Stage IV	6	1	3	2	1	4	1					1				
Stage V	5	1	2	2	2	1	2									

\*: GBM, glomerular basement membranes; CR, crescents; SYN, synechiae with Bowman's capsule; SCL, sclerotic lesions.



**Fig. 2.** LAM expression in MCGN along the basement membranes and in the mesangium. PAP. x 250



**Fig. 3.** FI expression in MPGN, mainly in the severe mesangio-proliferative lesions (arrows) and in the vessel walls. PAP. x 300

### Laminin and fibronectin in glomerulonephritis

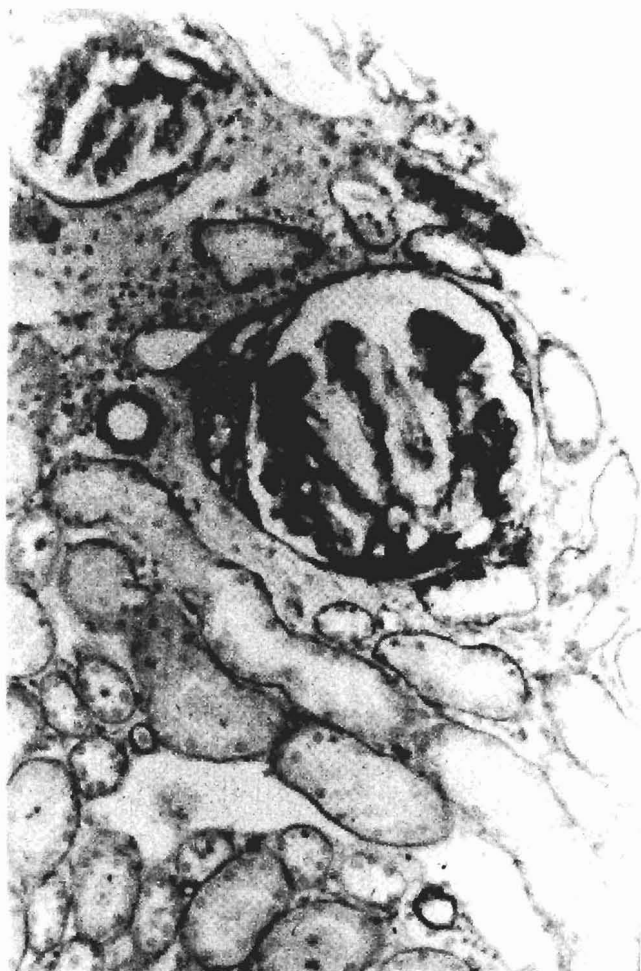
**Table 2.** Distribution of fibronectin in various types of glomerulonephritis (GN).

TYPE OF GN	NUMBER OF CASES	GBM*			MESANGIUM			CR*			SYN*			SCL*		
		+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++
Membranous GN (MGN)	12															
Stage I	2	1	1		1		1									
Stage II	6	2	4		2	2	1				1					
Stage III	4	3	1		4											
Mesangiocapillary GN (MCGN)	8	2	4	2	4	3	1									
Mesangioproliferative GN (MPGN)	10	10			3	6					1					
IgA-nephropathy	11	10	1		4	7	1	1	1						1	
Focal segmental glomerulosclerosis (FSG)	11	11			8	3									1	
Systemic lupus erythematosus (SLE)	14							1	3	2						
Stage III	3	3			1	2										
Stage IV	6	4	2		1	4	1									
Stage V	5	3	2													

\*: GBM, glomerular basement membrane; CR, crescents; SYN, synechiae with Bowman's capsule; SCL, sclerotic lesions.

nephropathy the FI expression was more pronounced (Fig. 3).

There was no difference in the distribution of LAM



**Fig. 4.** FI expression in MPGN, mainly in the severe mesangio-proliferative lesions and in a small crescent (star). PAP. x 300

and FI between GN of SLE and primary glomerulonephritis.

The staining pattern of the two antigens was more intense in the membranous and mesangiocapillary lesions of SLE glomerulonephritis.

Notably, all cellular and fibrocellular crescents observed in 7 cases, contained increased amounts of both LAM and FI. The staining for FI was particularly intense. In contrast, the adhesions with Bowman's capsule seen in 9 cases, demonstrated increased amounts of LAM and in only 2 cases they faintly expressed small amounts of FI.

Moreover, the large adhesions observed in 2 cases and the sclerotic lesions in 4 cases contained small amounts of LAM and stained faintly with FI (Tables 1, 2).

### Discussion

The GBM and the mesangial matrix represent the key structural elements of the renal glomerulus. The GBM is formed by endothelial and epithelial cells, which contribute to the orderly maintenance of the mature GBM through biosynthesis and degradation of specific components (Sariola et al., 1984). Recently, several lines of evidence indicate that glomerular cells display surface receptors that bind basement membrane components like LAM, FI and type IV collagen (Abrahamson et al., 1988). These receptors are extremely important in the development and maintenance of normal glomerular architecture, as they act cooperatively to cement endothelial and epithelial cells to GBM. The distribution of the extracellular structural components is altered in some experimental models of GN (Matsuo et al., 1986; Okuda et al., 1992).

Although several immunohistochemical studies on extracellular matrix components have been conducted, the distribution of these components in various types of GN has not been fully determined (Schiffer et al., 1981; Martínez-Hernández and Amenta, 1983; Yoshioka et al., 1989).



In the present study, all the cases of MGN expressed LAM more intensely than FI along the GBM, in subepithelial basement membranes protrusions (spikes) and in newly formed material above the subepithelial deposits. However, FI expression was more pronounced in the mesangial areas. The staining intensity for both antigens was more pronounced in stages II and III of MGN. Our findings referring to the distribution and expression of LAM are compatible with those observed in MGN by Fukatsu et al. (1988). In the literature, FI expression in MGN is a matter of controversy. Linder et al. (1980), reported decreased expression of the antigen in the mesangium of the 4 cases of MGN studied, while Petterson and Colvin (1978) did not observe any reduction in mesangial FI of their MGN cases. The discrepancy of FI expression in MGN between our results and the above data, may be due to the greater sensitivity of the immunoperoxidase method used in our study than that of the immunofluorescence performed by the other authors in addition to the few cases included in their series. Moreover, another possible explanation could be that renal biopsies with MGN might have been obtained from patients during different stages of the disease process. Recent experimental studies concerning the distribution of type IV collagen, LAM and FI in passive Heymann nephritis suggest that the increased width of the GBM is not accompanied by an increase in the amount of structural GBM components or their synthetic rates (Fogel et al., 1991), although the above authors cannot exclude the possibility that increased matrix synthesis may occur as a late phenomenon, which supports our findings in advanced stages of MGN. In MCGN LAM and FI were detected along the GBM and in the mesangium. Striker et al. (1984) and Funabiki et al. (1990) have examined 4 and 15 cases of MCGN respectively and reported an increase in the components of glomerular extracellular matrix, in which LAM and FI were included. The distribution of LAM and FI in the above studies is comparable to ours.

The increased amounts of LAM and FI in MGN and MCGN may be due to the disturbance of the coordinate synthesis of GBM components by epithelial and mesangial cells, because of the immunological injury caused from the deposition or the *in situ* formation of immune complexes. Lake et al. (1985) and Cosio et al. (1990) have suggested that FI plays a key role in the initiation of the *in situ* formation of immune complexes and the progression of glomerular injury, which supports our findings.

The distribution of LAM and FI in MPGN and FSG was similar to that seen in normal glomeruli, as has been reported by other authors (Striker et al., 1985). However, FI expression was more pronounced in the severe mesangioproliferative lesions, mainly observed in the cases of IgA nephropathy. Several serological studies have been conducted referring to the circulating antibodies in IgA-nephropathy (Shinkai et al., 1990). Moreover, Jenette et al. (1991) suggest that the presence of FI in the circulating IgA FI aggregates may play an

important role in the preferential mesangial deposition of the immune complexes, since the above aggregates have the same unusual predominance of  $\lambda$  to  $\kappa$  chains as that observed in the glomerular deposits of IgA-nephropathy.

The increased expression of FI in the severe mesangioproliferative lesions of our IgA nephropathy cases is supported by recent experimental observations suggesting that immune complexes containing antigens that bind to FI localize in the mesangium and cause GN (Cosio et al., 1990).

There were no differences in the distribution of LAM and FI between our cases of SLE GN with membranous type lesions and primary MGN, a finding also observed by Fukatsu et al. (1988). The staining pattern of the two antigens was more intense in the membranous and mesangiocapillary lesions of SLE GN.

Notably, all cellular and fibrocellular crescents in our cases contained increased amounts of both LAM and FI. The staining for FI was particularly intense. The pronounced expression of the above antigens in the crescents observed in our cases and the detection of collagen type IV in them, as reported by other authors (Foidart et al., 1980; Striker et al., 1984; Funabiki et al., 1990), indicate an involvement of epithelial cells in the development of crescents.

In contrast, the crescents studied in cases with vasculitis were mainly composed of extraglomerular matrix components like collagen type III (Striker et al., 1984; Yoshioka et al., 1989) which supports the proposed theory, that fibroblasts migrate into the glomeruli from the interstitium when Bowman's capsule is disrupted. The large adhesions with Bowman's capsule and the sclerotic lesions observed in our cases contained small amounts of LAM. Our observation, as well as the detection of collagen type III reported by other authors (Striker et al., 1984; Yoshioka et al., 1989; Funabiki et al., 1990) in combination with recent experimental findings (Okuda et al., 1992), suggest that extraglomerular matrix components are closely linked to the progression of glomerulosclerosis. The immunohistochemical results of our study, indicate that the increased amounts of LAM and FI, mainly in membranous and MCGN, as well as in MPGN may be due to their overproduction caused by damage in the glomerular cells or their participation in the formation of immune complexes. Further investigation is necessary to define if the possible disturbance of LAM and FI surface receptors is involved in the pathogenesis of these types of GN.

## References

- Abrahamson D.R. (1986). Recent studies on the structure and pathology of basement membranes. *J. Pathol.* 149, 257-278.
- Abrahamson D.R., Woods A. and Couchman J.R. (1988). Cell surface receptors for kidney basement membranes. *Am. J. Kidney Dis.* XII, 78-83.
- Cosio F.G., Mahan J.D. and Sedmak D.D. (1990). Experimental glomerulonephritis induced by antigen that binds to glomerular

# Laminin and fibronectin in glomerulonephritis

- fibronectin. *Am. J. Kidney Dis.* 15, 160-168.
- Courtney P.J., Timpl R. and Farquhar M.G. (1982). Comparative distribution of laminin, type IV collagen and fibronectin in the rat glomerulus. *J. Histochem. Cytochem.* 30, 874-876.
- Dixon A.J., Burns J., Dunnhill M.S. and McGee J. (1980). Distribution of fibronectin in normal and diseased human kidneys. *J. Clin. Pathol.* 33, 1021-1028.
- Eklom P., Miettinen M., Rapola J. and Foidart J.M.I. (1982). Demonstration of laminin basement membrane glycoprotein in routinely processed formalin-fixed human tissues. *Histochemistry* 75, 301-307.
- Fogel M., Boyd C., Leardkamolkarn V., Abrahamson D., Minto A. and Salant D. (1991). Glomerular basement membrane expansion in passive Heymann nephritis. Absence of increased synthesis of type IV collagen, laminin and fibronectin. *Am. J. Pathol.* 138, 465-475.
- Foidart J.M., Bere E.W. Jr., Yaar M., Rennard S.J., Gullino M., Martin G.R. and Katz S.I. (1980). Distribution and immunoelectron microscopic localization of laminin, a non-collagenous basement membrane glycoprotein. *Lab. Invest.* 42, 336-342.
- Fouser L.S. and Michael A.F. (1987). Antigens of the human glomerular basement membrane. *Springer Seminar Immunopathol.* 9, 317-339.
- Fukatsu A., Matsuo S., Killen P.D., Martin G.R., Andres G.A. and Brentjens J.R. (1988). The glomerular distribution of type IV collagen and laminin in human membranous glomerulonephritis. *Hum. Pathol.* 19, 64-68.
- Funabiki K., Horikoshi S., Tomino Y., Nagai Y. and Koide H. (1990). Immunohistochemical analysis of extracellular components in the glomerular sclerosis of patients with glomerulonephritis. *Clin. Nephrol.* 34, 239-246.
- Jenette J.C., Wieslander J., Tuttle R., Falk R.J. and the Glomerular Disease Collaborative Network (1991). Serum IgA-fibronectin aggregates in patients with IgA-nephropathy and Henoch-Schönlein purpura: diagnostic value and pathogenic implications. *Am. J. Kidney Dis.* XVIII, 466-471.
- Lake R.A., Morgan A., Henderson B. and Staines N.A. (1985). A key role for fibronectin in the sequential binding of native d.s. DNA and monoclonal anti-DNA antibodies to components of the extracellular matrix. Its possible significance in glomerulonephritis. *Immunology* 54, 389-395.
- Laurie G.W., Leblond C.P. and Inoue S. (1984). Fine structure of the glomerular basement membrane and immunolocalization of fine basement membrane components to the lamina densa (basal lamina) and its extensions in both glomeruli and tubules of the rat kidney. *Am. J. Anat.* 169, 453-481.
- Linder E., Miettinen A. and Törnroth T. (1980). Fibronectin as a marker for the glomerular mesangium in immunohistology of kidney biopsies. *Lab. Invest.* 42, 70-75.
- Martínez-Hernández A. and Amenta P.S. (1983). The basement membrane in pathology. *Lab. Invest.* 48, 656-677.
- Martínez-Hernández A. and Chung A.E. (1984). The ultrastructure localization of two major basement membrane components: entactin and laminin in rat tissues. *J. Histochem. Cytochem.* 32, 289-298.
- Matsuo S., Brentjens J.R., Andres G.A., Foidart J.M., Martin G.R. and Martínez-Hernández A. (1986). Distribution of basement membrane antigens in glomeruli of mice with autoimmune glomerulonephritis. *Am. J. Pathol.* 122, 36-49.
- Mounier F., Foidart J.M. and Gubler M.C. (1986). Distribution of extracellular matrix glycoproteins during normal development of human kidney. An immunohistochemical study. *Lab. Invest.* 54, 394-401.
- Oikawa T., Sayama K., Matsuda Y., Fukimoto Y., Iwaguchi T. and Matsuzawa A. (1989). Characterization of two possible forms of type IV collagen from human kidney cortex. *Biochem. Int.* 19, 615-624.
- Okuda S., Kanai H., Tamaki K., Onoyama F. and Fujishima M. (1992). Synthesis of fibronectin by isolated glomeruli from nephrectomized hypertensive rats. *Nephron* 61, 456-462.
- Petterson E.E. and Colvin R.B. (1978). Cold insoluble globulin (fibronectin. LETS protein) in normal and diseased human glomeruli papain sensitive attachment to normal glomeruli and deposition in crescents. *Clin. Immunol. Immunopathol.* 11, 425-436.
- Sariola H., Timpl R. and Von der Mark K. (1984). Dual origin of glomerular basement membrane. *Dev. Biol.* 101, 86-96.
- Scheinman J.I., Foidart J.M., Gehron-Robey P., Fish A.J. and Michael A.F. (1980). The immunohistology of glomerular antigens. IV. Laminin, a defined non-collagen basement membrane glycoprotein. *Clin. Immunol. Immunopathol.* 15, 175-189.
- Schiffer M.S., Michael A.F., Kim Y. and Fish A.J. (1981). Distribution of glomerular basement membrane antigens in diseased human kidneys. *Lab. Invest.* 44, 234-240.
- Shinkai Y., Karai M., Osawa G., Sato M. and Koshikawa S. (1990). Antimouse laminin antibodies in IgA nephropathy and various glomerular diseases. *Nephron* 56, 285-296.
- Stenham S. and Vaheiri A. (1978). Distribution of a major connective tissue protein, fibronectin in normal human tissues. *J. Exp. Med.* 147, 1054-1064.
- Sternberg L.A. (1979). The unlabelled antibody peroxidase-antiperoxidase (PAP) method. In: *Immunohistochemistry*. Sternberg L.A. (ed). John Wiley and Sons, New York, pp 297-321.
- Striker L., Killen P., Chi E. and Striker G. (1984). The composition of glomerulosclerosis. I. Studies in focal sclerosis crescentic glomerulonephritis and membranoproliferative glomerulonephritis. *Lab. Invest.* 51, 181-192.
- Timpl R., Driadek M., Fujiwara S., Nowack H. and Wick G. (1983a). Nidogen: a new self-aggregating basement membrane protein. *Eur. J. Biochem.* 137, 455-465.
- Timpl R., Engle J. and Matin J. (1983b). Laminin-a multi-functional protein of basement membrane. *Trends Biochem. Sci.* 8, 207-209.
- Van den Heuvel L., Van den Born J., Van de Velden J., Veerkam J.H., Monnens L., Schroder C.H. and Berden J.H.M. (1989). Isolation and partial characterization of heparan sulphate proteoglycan from the human glomerular membrane. *Biochem. J.* 264, 457-465.
- Weeks B.S., Kopp J.B., Horikoshi S., Cannon F.B., Carrett M., Kleinman H.K. and Klotman P.E. (1991). Adult and fetal human mesangial cells interact with specific laminin domains. *Am. J. Physiol.* 261, F688-F695.
- Yoshioka K., Takemura T., Tohda M., Akano N., Miyamoto H., Ooshima A. and Maki S. (1989). Glomerular localization of type III collagen in human kidney disease. *Kidney Int.* 35, 1203-1211.