

# Differential expression of laminin isoforms in diabetic nephropathy and other renal diseases

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**Laminin a non-collagenous glycoprotein is a major component of the renal glomerular basement membrane and mesangium. Thus far eleven distinct chains have been described, permutations of which make up 15 laminin isoforms. Laminin molecules interact with cells and other matrix molecules during organ development and differentiation. We studied the distribution of laminin isoforms in patients with type 1 diabetic nephropathy, membranous nephropathy, membranoproliferative glomerulonephritis and IgA nephropathy/ Henoch–Schönlein purpura. Immunofluorescence microscopic studies with laminin-chain-specific antibodies to the  $\alpha$ 1,  $\alpha$ 2,  $\alpha$ 5,  $\beta$ 1,  $\beta$ 2 and  $\gamma$ 1 chains detected  $\alpha$ 2,  $\beta$ 1 and  $\gamma$ 1 chain expression in the normal mesangium and  $\alpha$ 5,  $\beta$ 2 and  $\gamma$ 1 in normal glomerular basement membrane. Significantly, constituents of the glomerular basement membrane,  $\alpha$ 5,  $\beta$ 2 and  $\gamma$ 1 chains were overexpressed in kidneys with diabetic nephropathy. Initially the constituents of the mesangium increased commensurate with the degree of mesangial expansion and degree of diabetic nephropathy. Reduction in  $\alpha$ 2 chain intensity was observed with severe mesangial expansion and in the areas of nodular glomerulosclerosis. In addition, with late disease aberrant expression of  $\alpha$ 2 and  $\beta$ 2 chains was observed in the mesangium. Glomerular basement membrane in renal disease overexpressed molecules normally present in that location. In summary, the alterations in basement membrane composition in various renal diseases seem to not only reflect the balance between synthesis and degradation of normal basement membrane constituents, but also their aberrant expression.**

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Laminin is a major non-collagenous glycoprotein component of glomerular basement membrane and mesangium and consists of three different polypeptide chains designated  $\alpha$ ,  $\beta$  and  $\gamma$ . Laminin was first isolated from EHS mouse tumor and subsequently found to be a cross-shaped disulfide-bonded heterotrimer composed of a 400-kDa  $\alpha$ 1 chain along with  $\beta$ 1 and  $\gamma$ 1 chains of approximately 200 kDa. Eleven distinct laminin chains have been described to date (five  $\alpha$ , three  $\beta$  and three  $\gamma$  chains). These chains can combine into at least 15 different laminin isoforms, Lm-111 to Lm-523.<sup>1–3</sup>

Laminins are involved in multiple interactions with other basement membrane components and cells and play important roles in the supramolecular assembly of extracellular matrix and the stability of basement membrane.<sup>3–5</sup> Laminins also help mediate various cellular functions, including growth, differentiation and attachment. The distribution of laminin chains in the glomerulus is site-specific and associated with the presence of different isoforms in the glomerular basement membrane and the mesangium. Therefore the  $\alpha$ 5,  $\beta$ 2 and  $\gamma$ 1 chains are present in normal adult human glomerular basement membrane, whereas  $\alpha$ 1,  $\alpha$ 2 and  $\beta$ 1 chains are not.<sup>2,6–8</sup> Only  $\alpha$ 2,  $\alpha$ 4,  $\beta$ 1 and  $\gamma$ 1 chains are present in the normal adult mesangium.<sup>2,9</sup> Therefore, glomerular basement membrane and mesangium in normal adult human kidneys expresses different isoforms of laminin: Lm-521 ( $\alpha$ 5,  $\beta$ 2 and  $\gamma$ 1) in glomerular basement membrane and Lm-211/411 ( $\alpha$ 2/ $\alpha$ 4,  $\beta$ 1 and  $\gamma$ 1) in the mesangium.

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In addition, there is considerable variation in the distribution of laminin isoforms in the developing kidney. Nephrogenesis is a complex process during which the unique elements of the glomerular basement membrane, mesangial matrix, tubular basement membrane and blood vessels come together. In developing rodent glomeruli, the transition of type IV collagen isoforms is accompanied by the transition of laminin isoforms.<sup>2,10</sup> Although collagen  $\alpha 1(IV)$  and  $\alpha 2(IV)$  are found in all developing

**Table 1** Specificity and source of antibodies

Antibodies	Specificity	Source	References
161EB7	Laminin $\alpha 1$	I Virtanen	8
2G9	Laminin $\alpha 2$	E Engvall/Chemicon	30
5H2/H7	Laminin $\alpha 2$	E Engvall	30
4C7	Laminin $\alpha 5$	E Engvall/Chemicon	31
3E5	Laminin $\beta 1$	E Engvall/Chemicon	31
C4	Laminin $\beta 2$	JR Sanes/Hybridoma Bank	32
D5	Laminin $\beta 2$	JR Sanes/Hybridoma Bank	32
2E8	Laminin $\gamma 1$	Hybridoma Bank	
102	$\alpha 1(IV)NC$	University of MN laboratory	33

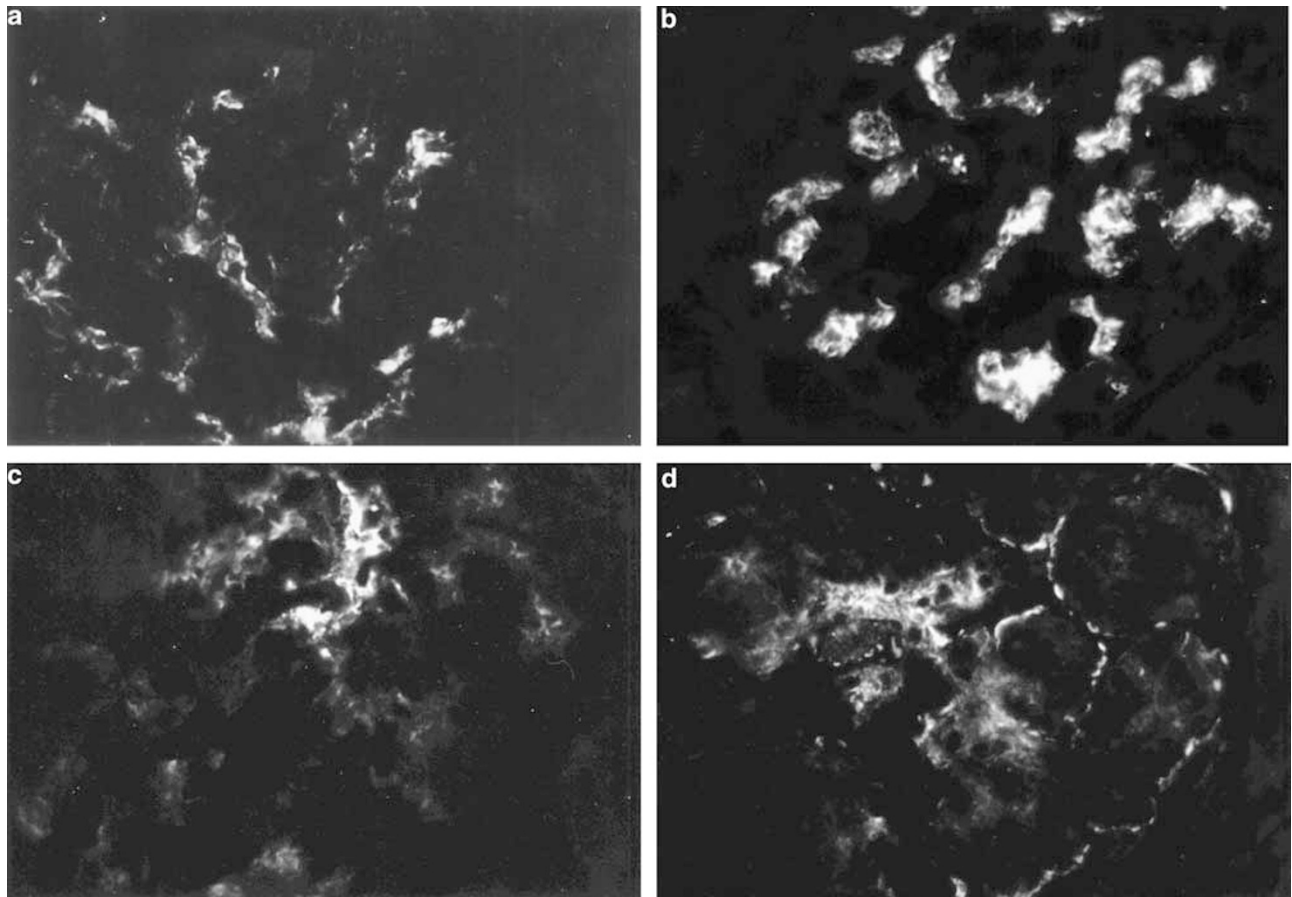
Abbreviation:  $\alpha 1(IV)NC$  = non-collagenous domain of  $\alpha 1$  chain of type IV collagen.

glomerular basement membrane together with laminin  $\beta 1$  chain, the expression of laminin  $\beta 2$  chain is associated with collagen  $\alpha 3-5(IV)$  in the glomerular

**Table 2** Laminin chain distribution in human kidneys

Antibody to	$\alpha 1$	$\alpha 2$	$\alpha 5$	$\beta 1$	$\beta 2$	$\gamma 1$
<i>GBM</i>						
Normal	-	-	+	-	+	+
Diabetic nephropathy	-	-	↑	-	↑	↑
Membranous nephropathy	-	-	↑	-	↑	↑
Membranoproliferative glomerulonephritis	-	-	→	-	→	→
Henoch-Schönlein purpura/IgA nephropathy	-	-	→	-	→	→
<i>Mesangium</i>						
Normal	-	+	-	+	-	+
Mild diabetic nephropathy	-	↑↑	↑	↑↑	↑	↑↑
Severe diabetic nephropathy	-	↓/-	↑	↑↑	↑	↑↑
Membranoproliferative glomerulonephritis	-	↓/-	↑	↑↑	↑	↑↑
Henoch-Schönlein purpura/IgA nephropathy	-	↑↑	↑	↑↑	↑	↑↑
Membranous nephropathy	-	→/↑	→/↑	→/↑	→/↑	→/↑

- absent; + present; ↑ increase; ↓ decrease; → no change.



**Figure 1** Immunofluorescence microscopy of glomeruli immunoreacted for Lm  $\alpha 2$  chain ( $\times 550$ ). (a) Normal kidney; (b) mild diabetic nephropathy; (c) severe diabetic nephropathy; (d) membranoproliferative glomerulonephritis. In the normal glomerulus, there is a variable immunoreaction in the mesangium. In mild diabetic nephropathy, there is a diffuse increase in mesangial staining. In contrast, note the decrease to disappearance of mesangial staining in severe diabetic nephropathy and membranoproliferative glomerulonephritis.

basement membrane of more mature glomeruli. In other words, transition of collagen  $\alpha 1-2(\text{IV})$  to  $\alpha 3-5(\text{IV})$  during the glomerular basement membrane development is associated with the transition of laminin chains from  $\beta 1$  to  $\beta 2$ . Mutant mice lacking the COL4A3 gene for  $\alpha 3(\text{IV})$  also lack  $\alpha 3-5(\text{IV})$  chains in the glomerular basement membrane with persistence of  $\alpha 1-2(\text{IV})$ .<sup>11</sup> Nonetheless, the laminin  $\beta 1$  to  $\beta 2$  switch occurs in rodents<sup>11</sup> and in man.<sup>6</sup> This suggests that collagen IV and laminin networks in glomerular basement membrane are regulated independently. Mutant mice lacking laminin  $\beta 2$  chain show structurally intact glomerular basement membrane M with normal developmental transition of type IV collagen isoforms.<sup>12</sup> The severe proteinuria in these mice suggests that the laminin  $\beta 2$  chain has an important role in glomerular permselectivity through the maintenance of normal glomerular basement membrane structure or normal podocyte maturation. Recent studies show that glomerular basement membrane is a real barrier for albumin as proteinuria precedes podocyte abnormality in *Lam $\beta 2$ <sup>-1/-1</sup>* mice.<sup>13</sup> Taken together, the transitions of laminin and type IV collagen isoforms, which

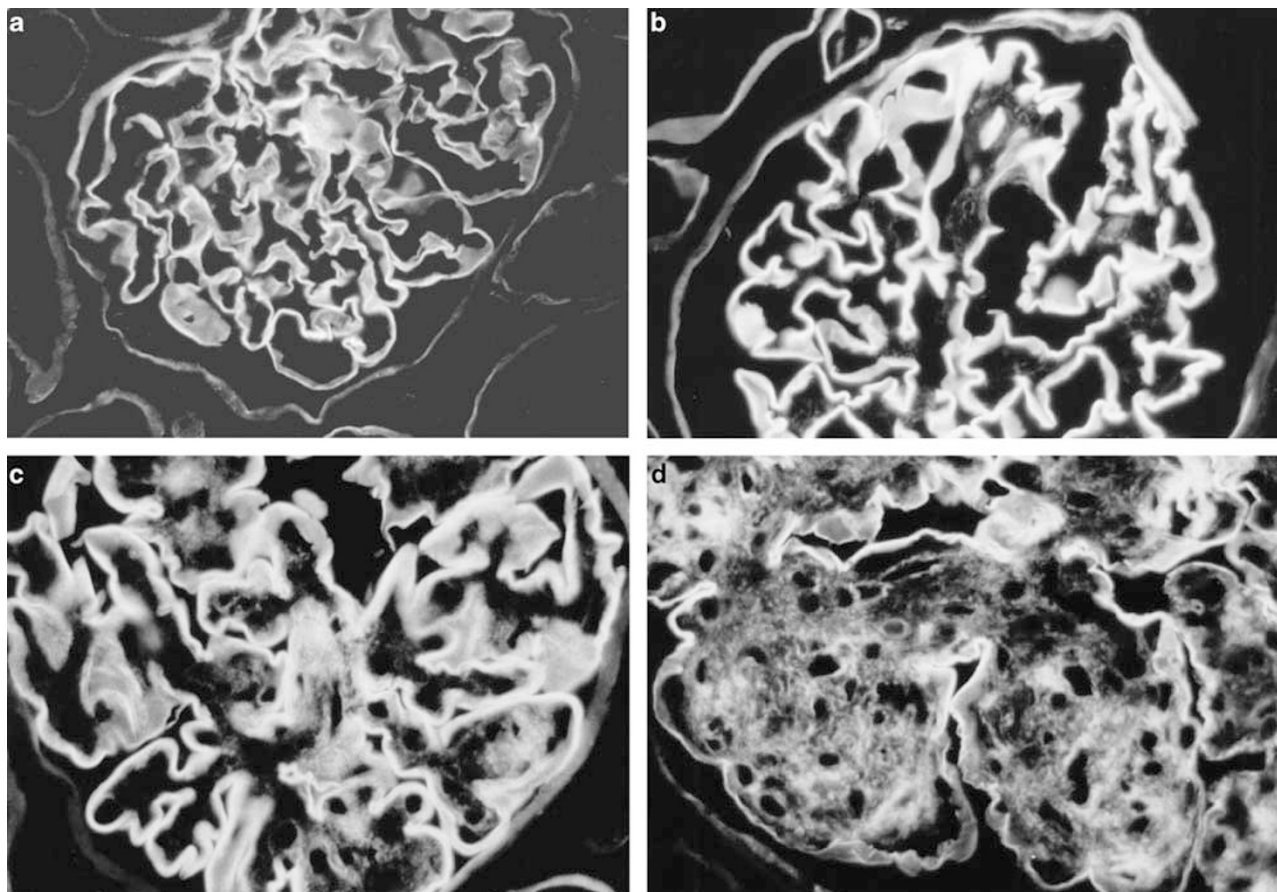
normally coincide, appear to have specific roles in various stages of renal development. In this report, we examined the distribution of laminin isoforms in normal human kidneys and in various disease states.

## Materials and methods

Kidney tissues were obtained from 12 patients with overt type 1 diabetic nephropathy, 5 patients with type I idiopathic membranoproliferative glomerulonephritis, 12 patients with Henoch-Schönlein purpura/IgA nephropathy, 5 patients with idiopathic membranous nephropathy and 14 normal controls including three adult kidney donors (age range, 3 to 59 years). The frozen material used in this study was residue of biopsies obtained for diagnostic purposes. This study was performed with the permission of the Institutional Review Board of the University of Minnesota.

## Antibodies

The monoclonal antibodies used in this study are listed in Table 1. FITC-conjugated and



**Figure 2** Immunofluorescence microscopy of glomeruli immunoreacted for Lm  $\alpha 5$  chain ( $\times 550$ ). (a) Normal kidney; (b) mild diabetic nephropathy; (c) severe diabetic nephropathy; (d) membranoproliferative glomerulonephritis. In the normal glomerulus the Lm  $\alpha 5$  chain overlies the glomerular basement membrane but is not detected in the mesangium. In diabetic nephropathy the Lm  $\alpha 5$  chain overlies the thickened glomerular basement membrane. There is only a segmental reaction in the mesangium in mild diabetic nephropathy, which progresses to diffuse staining as the mesangium expands further in severe diabetic nephropathy. In membranoproliferative glomerulonephritis, the Lm  $\alpha 5$  chain is detected in the expanded mesangium and glomerular basement membrane.

tetramethylrhodamine isothiocyanate (RITC)-conjugated goat anti-mouse IgG were obtained from Caltag (South San Francisco, CA, USA) and ICN-Cappel (Irvine, CA, USA); FITC-conjugated goat anti-rat IgG from Pel Freeze (Rogers, AR, USA) and Jackson Laboratories (West Grove, PA, USA); FITC-conjugated goat anti-human IgG from Kallestedt (Austin, TX, USA) and ICN-Cappel. All secondary antibodies were absorbed with normal human serum.

### Immunohistological Methods

Immunofluorescence studies were carried out as previously described.<sup>14</sup> Kidney tissues were snap-frozen in isopentane precooled in liquid nitrogen, sectioned at 4  $\mu\text{m}$  in a cryostat at constant room temperature (25°C) and humidity (30%), and fixed with acetone for 10 min. The slides were exposed to primary antibodies followed by FITC-conjugated secondary antibodies. Dual fluorochrome labeling was performed using mouse monoclonal anti-laminin  $\alpha 2$  chain detected with rhodamine-conjugated goat anti-mouse IgG, followed by rat monoclonal anti-type IV collagen ( $\alpha 1(\text{IV})\text{NC}$ , where NC denotes non-collagenous) detected with FITC-conjugated

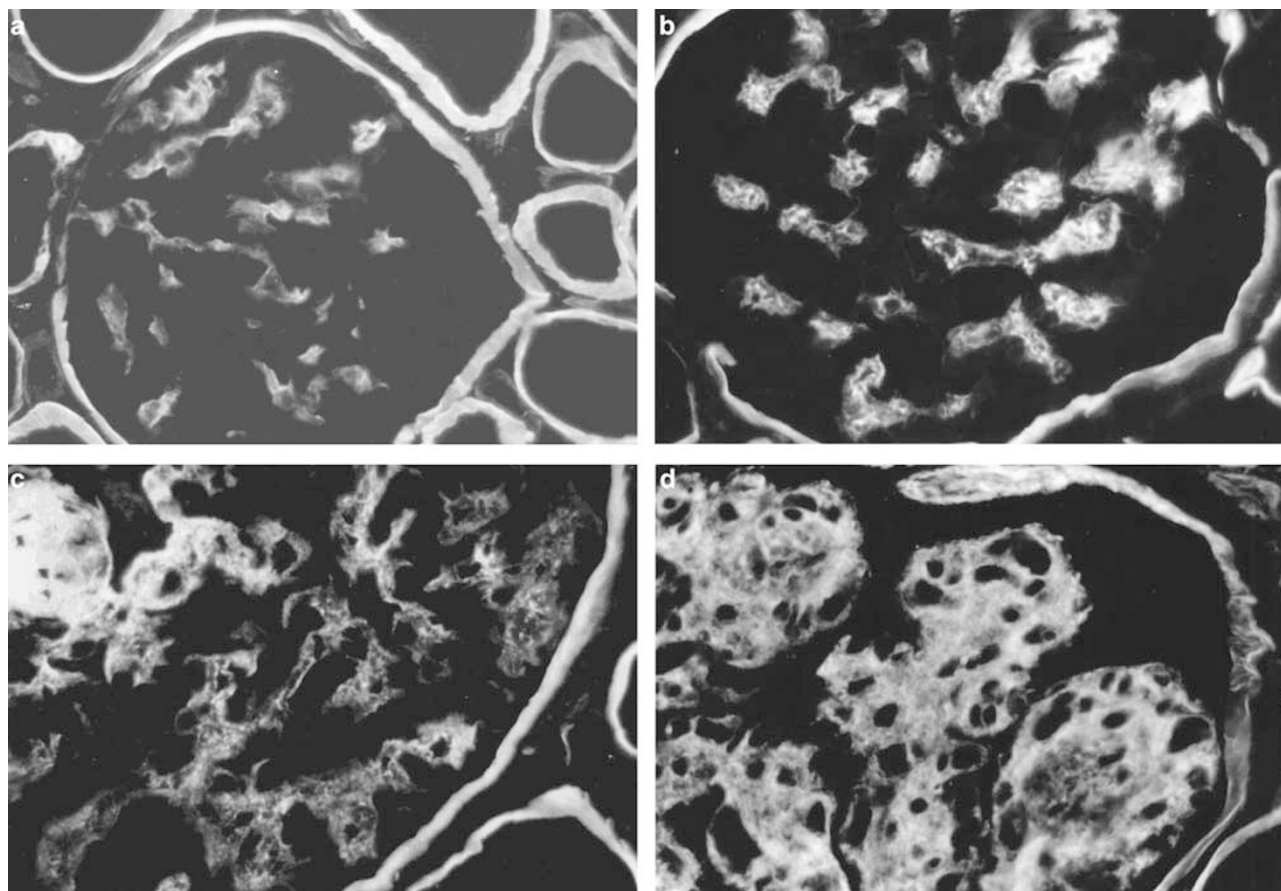
goat anti-rat IgG (absorbed with human and mouse sera). Appropriate controls were as previously described.<sup>14</sup> P-phenylenediamine in PBS glycerol was applied to fluorochrome-stained tissue sections to retard fluorescence quenching and sections were examined using epifluorescence microscope with appropriate filters (Carl Zeiss, Oberkochen, Germany).

### Morphometry

Routine stereological techniques, previously described in detail,<sup>15</sup> were used to measure mesangial fraction of glomerular volume in 12 patients with diabetic nephropathy. Mesangial fraction of glomerular volume in patients with diabetic nephropathy ranged from 0.22 to 0.55 with normal being <0.2.

### Results

Immunoreactivity for Lm  $\alpha 5$ ,  $\beta 2$  and  $\gamma 1$  chains was found in the glomerular basement membrane in normal adult human kidneys, whereas Lm  $\alpha 1$ ,  $\alpha 2$



**Figure 3** Immunofluorescence microscopy of glomeruli immunoreacted for Lm  $\beta 1$  chain ( $\times 550$ ). (a) Normal kidney; (b) mild diabetic nephropathy; (c) severe diabetic nephropathy; (d) membranoproliferative glomerulonephritis. Lm  $\beta 1$  chain is detected in both the normal and expanded mesangium.

and  $\beta 1$  chains were absent (Table 2, Figures 1a–5a, Lm  $\alpha 1$  not shown). Only  $\alpha 2$ ,  $\beta 1$  and  $\gamma 1$  chains were present in the mesangium. The intensity and extent of mesangial immunoreactivity for laminin chains, in contrast to type IV collagen, were quite variable in different normal kidneys ranging from trace focal to intense diffuse labeling.

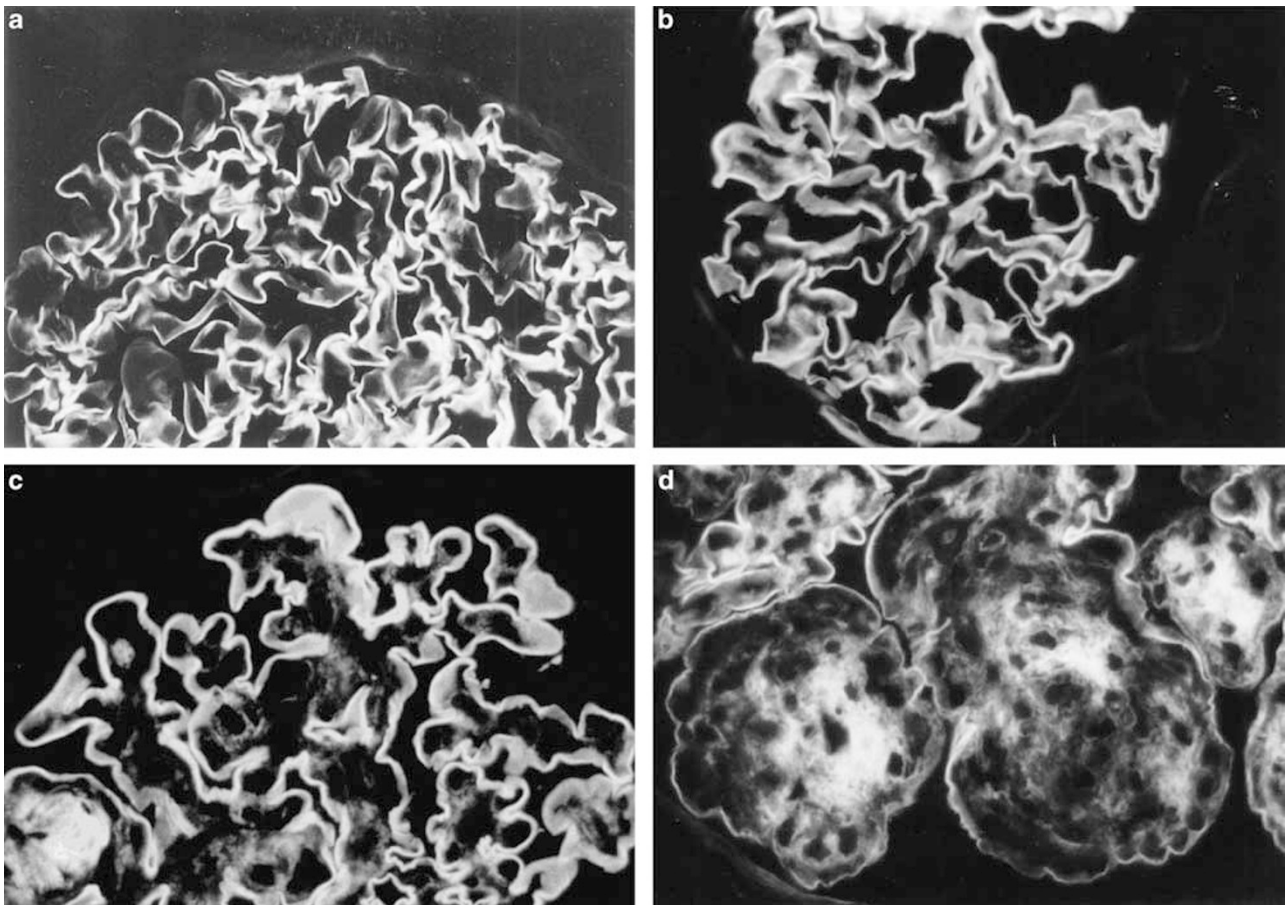
The thickened glomerular basement membrane in mild diabetic nephropathy showed increased reactivity (both the area and the intensity of the staining) only with antibodies to the normal laminin constituents of glomerular basement membrane, namely Lm  $\alpha 5$ ,  $\beta 2$  and  $\gamma 1$  chains (Table 2, Figures 1b–5b). Mild to moderate mesangial expansion in diabetic nephropathy, in contrast, was associated not only with the increased reactivity with antibodies to the normal laminin constituents of mesangial matrix, but also with the aberrant expression of normal glomerular basement membrane components, Lm  $\alpha 5$  and  $\beta 2$  chains (Table 2, Figures 1b–5b). As the mesangial expansion progressed further in diabetic nephropathy, there was a striking decrease or disappearance of Lm  $\alpha 2$  chain, whereas other laminin chains and type IV collagen [ $\alpha 1(\text{IV})\text{NC}$ ] continued to

increase (Figures 1c–5c and 6–8). These findings are summarized in Table 2. In the 12 diabetic nephropathy cases where mesangial fraction of glomerular volume was determined, the reduction in  $\alpha 2$  chain intensity was only noted when mesangial fraction of glomerular volume exceeded 0.45 (4 cases) and in the area of nodular glomerulosclerosis.

In membranous nephropathy, like in mild diabetic nephropathy, there was thickening of the glomerular basement membrane with increased reactivity of antibodies to the normal glomerular basement membrane constituents Lm  $\alpha 5$ ,  $\beta 2$  and  $\gamma 1$  chains (Table 2, figures not shown).

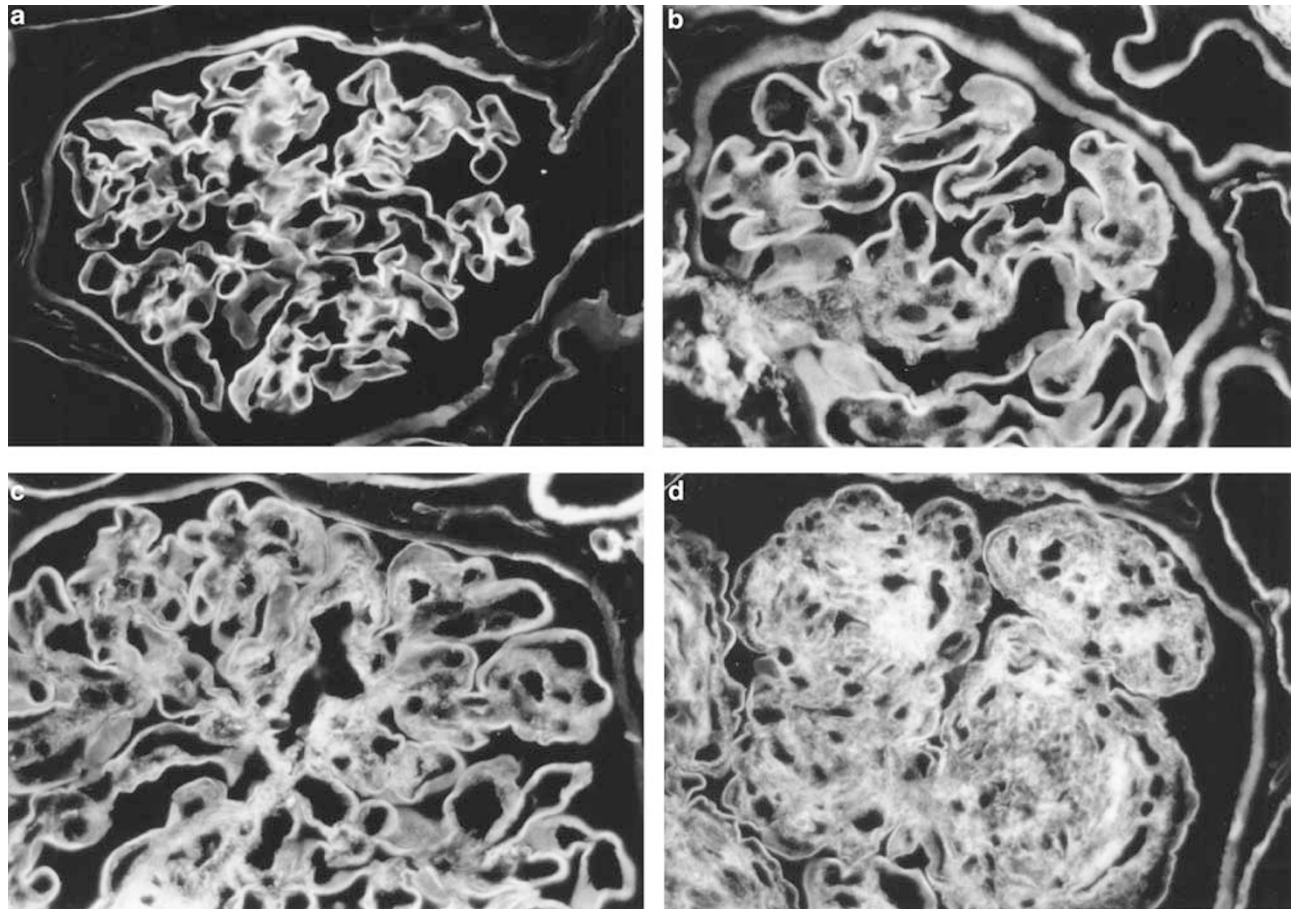
Cases of Henoch–Schönlein purpura/IgA nephropathy expressed increased normal mesangial constituents  $\alpha 2$ ,  $\beta 1$  and  $\gamma 1$  chains and aberrantly expressed Lm  $\alpha 5$  and  $\beta 2$  chains (Table 2, figures not shown).

Cases of membranoproliferative glomerulonephritis behaved similar to severe diabetic nephropathy, with a decrease or disappearance of Lm  $\alpha 2$  chain while overexpressing normal mesangial constituents  $\beta 1$  and  $\gamma 1$  and type IV collagen [ $\alpha 1(\text{IV})\text{NC}$ ] and aberrantly expressing  $\alpha 5$  and  $\beta 2$  chains (Table 2, Figures 1d–5d and 9).



**Figure 4** Immunofluorescence microscopy of glomeruli reacted for Lm  $\beta 2$  chain ( $\times 550$ ). (a) Normal kidney; (b) mild diabetic nephropathy; (c) severe diabetic nephropathy; (d) membranoproliferative glomerulonephritis. The distribution and pattern of glomerular immunoreactivity are similar to those of Lm  $\alpha 5$  chain.





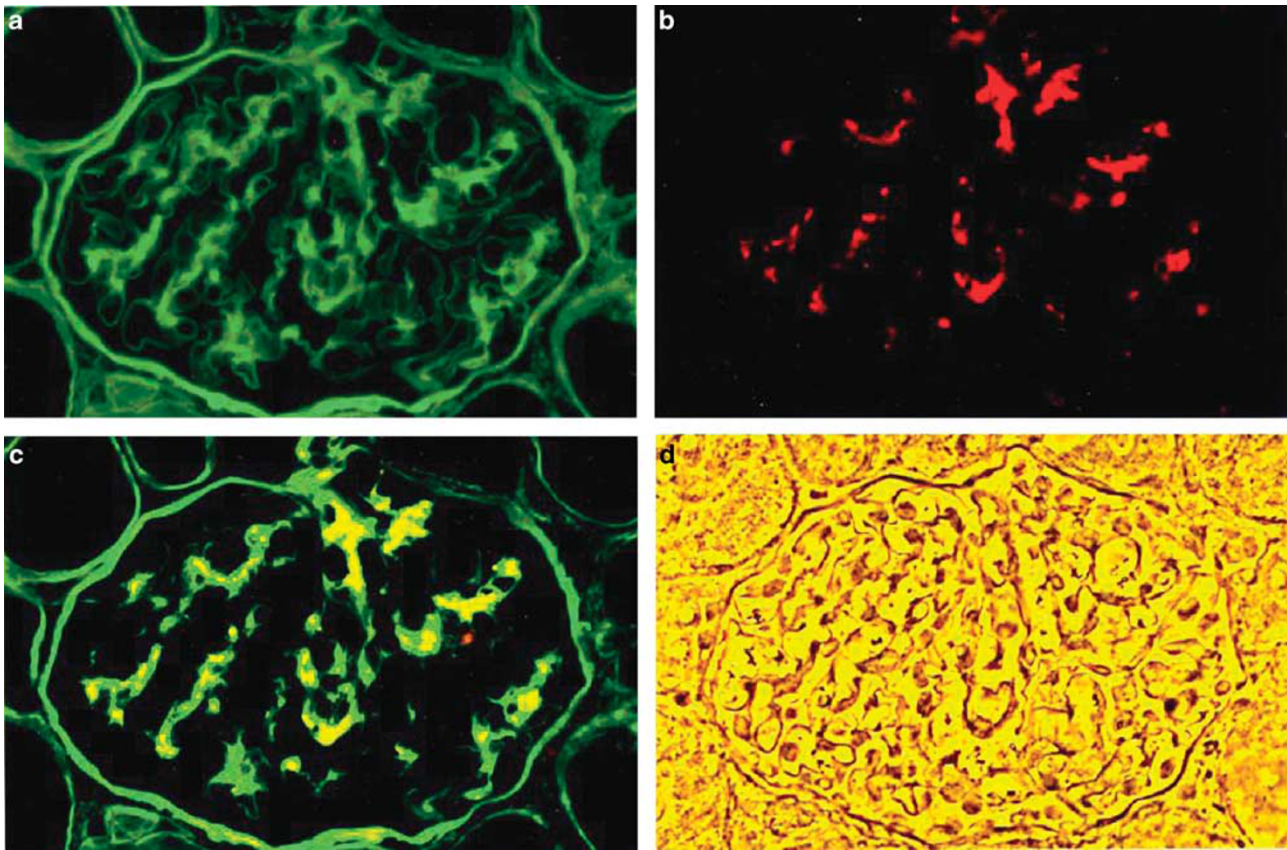
**Figure 5** Immunofluorescence microscopy of glomeruli immunoreacted for Lm  $\gamma$ 1 chain ( $\times 550$ ). (a) Normal kidney; (b) mild diabetic nephropathy; (c) severe diabetic nephropathy; (d) membranoproliferative glomerulonephritis. In normal glomeruli, the Lm  $\gamma$ 1 chain overlies both the glomerular basement membrane and the mesangium. In diabetic nephropathy, there is intense immunoreactivity in both the thickened glomerular basement membrane and the expanded mesangium. In membranoproliferative glomerulonephritis, there is intense reactivity in the expanded mesangium with positive staining reaction in glomerular basement membrane.

## Discussion

Glomerular structural–functional relationships have been demonstrated in a variety of kidney diseases.<sup>15–18</sup> There is a close correlation between mesangial expansion and renal function in diabetic nephropathy and membranoproliferative glomerulonephritis. These structural changes are due, at least in part, to alterations in the composition of extracellular matrix and basement membrane constituents, which reflect disturbances in the balance between extracellular matrix and basement membrane synthesis and degradation. Although it is generally believed that these alterations involve only the normal extracellular matrix and basement membrane constituents, more recent studies have shown aberrant expression of basement membrane proteins in various renal diseases. In human Alport syndrome and in the murine collagen COL4A3 knockout model, type IV collagen  $\alpha$ 1 and  $\alpha$ 2 chains, type V collagen, type VI collagen and laminin  $\alpha$ 2 chains are abnormally expressed in the glomerular basement membrane in association with the absence of the  $\alpha$ 3,  $\alpha$ 4 and  $\alpha$ 5 chains of type IV

collagen.<sup>8,9,19–22</sup> Lm-111 ( $\alpha$ 1,  $\beta$ 1 and  $\gamma$ 1), normally present only in the mesangium was detected in the glomerular basement membrane of sclerotic glomeruli in murine lupus nephritis.<sup>23</sup> Studies of membranous nephropathy have demonstrated increased expression in the glomerular basement membrane of normal constituents Lm  $\beta$ 2 and  $\alpha$ 1,  $\alpha$ 3 and  $\alpha$ 5 chains of type IV collagen and variable aberrant expression of Lm  $\beta$ 1.<sup>24</sup>

In our study, progressive mesangial expansion in diabetic nephropathy, membranoproliferative glomerulonephritis and Henoch–Schönlein purpura/IgA nephropathy was associated with aberrant mesangial expression of Lm  $\alpha$ 5 and  $\beta$ 2 chains normally expressed only in adult glomerular basement membrane and with the decrease and the disappearance of mesangium-specific laminin chains,  $\alpha$ 2 in marked mesangial expansion. This loss of normally expressed and aberrant expression of Lm isoforms may be deleterious for renal function. Earlier studies by Kikkawa *et al*<sup>25</sup> have demonstrated that mesangial cells organize the glomerular capillaries by adhering to a specific



**Figure 6** Dual fluorochrome staining of a normal glomerulus for (a) type IV collagen (FITC) and (b) Lm  $\alpha 2$  chain (RITC) ( $\times 550$ ). (a) Both the glomerular basement membrane and the mesangium react for type IV collagen; (b) diffuse staining of the mesangium for Lm  $\alpha 2$  chain; (c) when viewed under a filter system that permits visualization of both fluorochromes, there is an overlap (green and red to yellow/orange) in the mesangial localization of type IV collagen and Lm  $\alpha 2$  chain. (d) The same glomerulus is visualized by phase-contrast microscopy.

domain of Lm  $\alpha 5$  chain in the glomerular basement membrane. Thus, a disorganized laminin 521 with abnormal expression of glomerular basement membrane can likely lead to disorganization of glomerular capillaries and therefore to aberrant glomerular function.

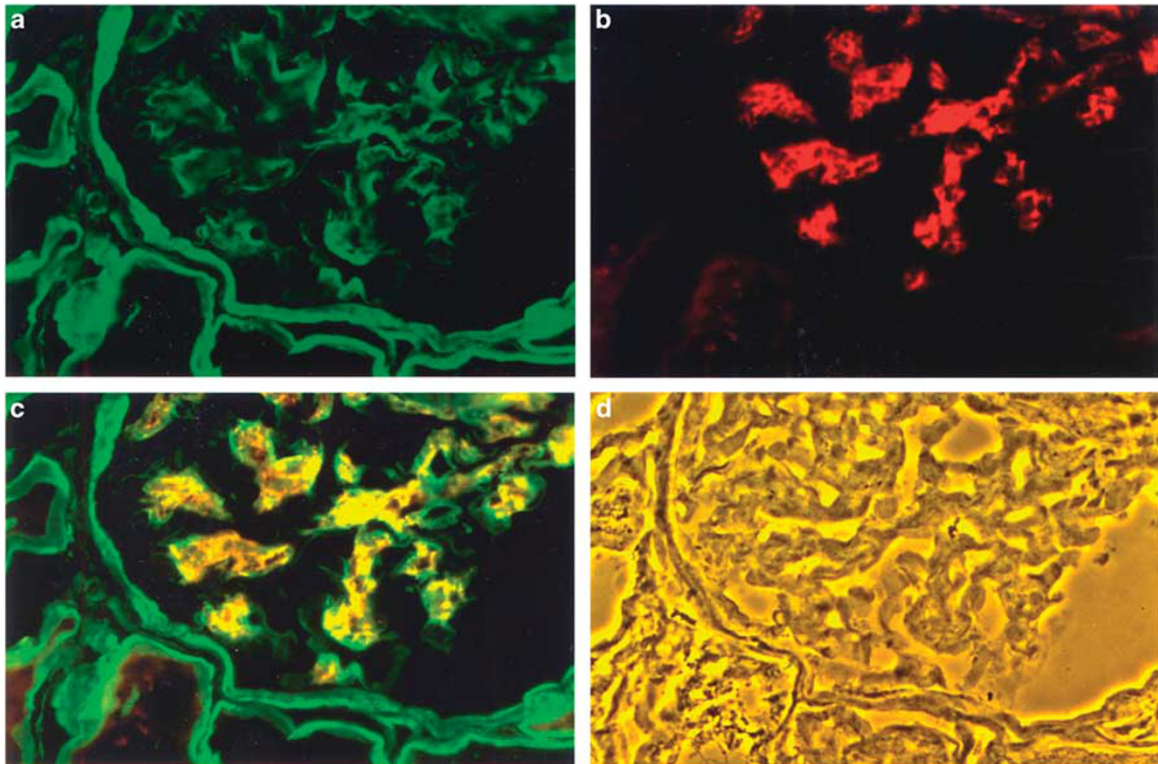
Phenotypic alterations of extracellular matrix synthetic profiles of glomerular cells have been observed during nephrogenesis and *in vitro* studies. Both laminin  $\alpha 5$  and  $\beta 2$  chains are expressed in the mesangium of early and late capillary stages of fetal glomeruli with the disappearance in mesangium of normal adult kidneys (personal observation). Mesangial cells *in vitro* synthesize significant amount of interstitial collagens, which are not present in normal mesangium, most likely reflecting dedifferentiation.<sup>26</sup> One of the explanations for the disappearance of laminin  $\beta 2$  in the markedly expanded mesangium could be due to the masking of an epitope occurring due to the progression of mesangial expansion. However, since the two monoclonal antibodies we used were against different epitopes of laminin  $\beta 2$  and both gave identical staining characteristics; the possibility of a 'hidden epitope' is negated. In addition, persistent expression of other laminin chains and type IV collagen in

markedly expanded mesangium makes the hidden epitope explanation unlikely. It has been suggested that the mesangium in disease states may be populated by cells originating from extraglomerular sites such as the bone marrow or the juxtaglomerular apparatus.<sup>27–29</sup> These translocated cells could express different matrix synthetic profiles, although it is equally plausible that resident mesangial cells undergo dedifferentiation due to disease related alterations in cytokines/growth factors or local architectural changes representing advancing disease processes.

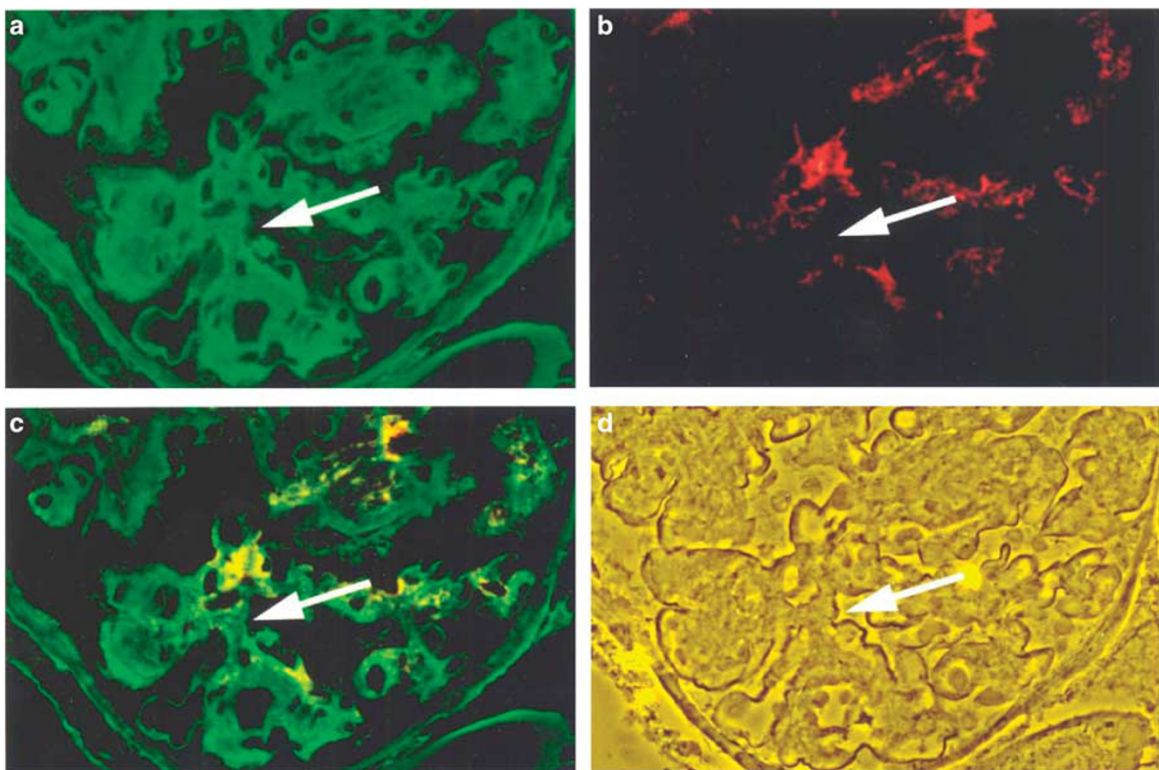
We believe that changes in the A2 isoform of laminin reflect the degree of mesangial matrix expansion. In other words, in early/mild mesangial expansion as occurs in mild diabetic nephropathy or Henoch–Schönlein purpura/IgA nephropathy, there is an increase in expression of the A2 isoform, whereas in severe diabetic nephropathy or membranoproliferative glomerulonephritis, there is a decrease. Although it is unclear as to why this happens, we speculate that this may be due to masking of an epitope for the A2 isoform of laminin or dedifferentiation of mesangial cells, which occurs as mesangial expansion progresses.

In summary, the alterations in basement membrane composition in various renal diseases seem to



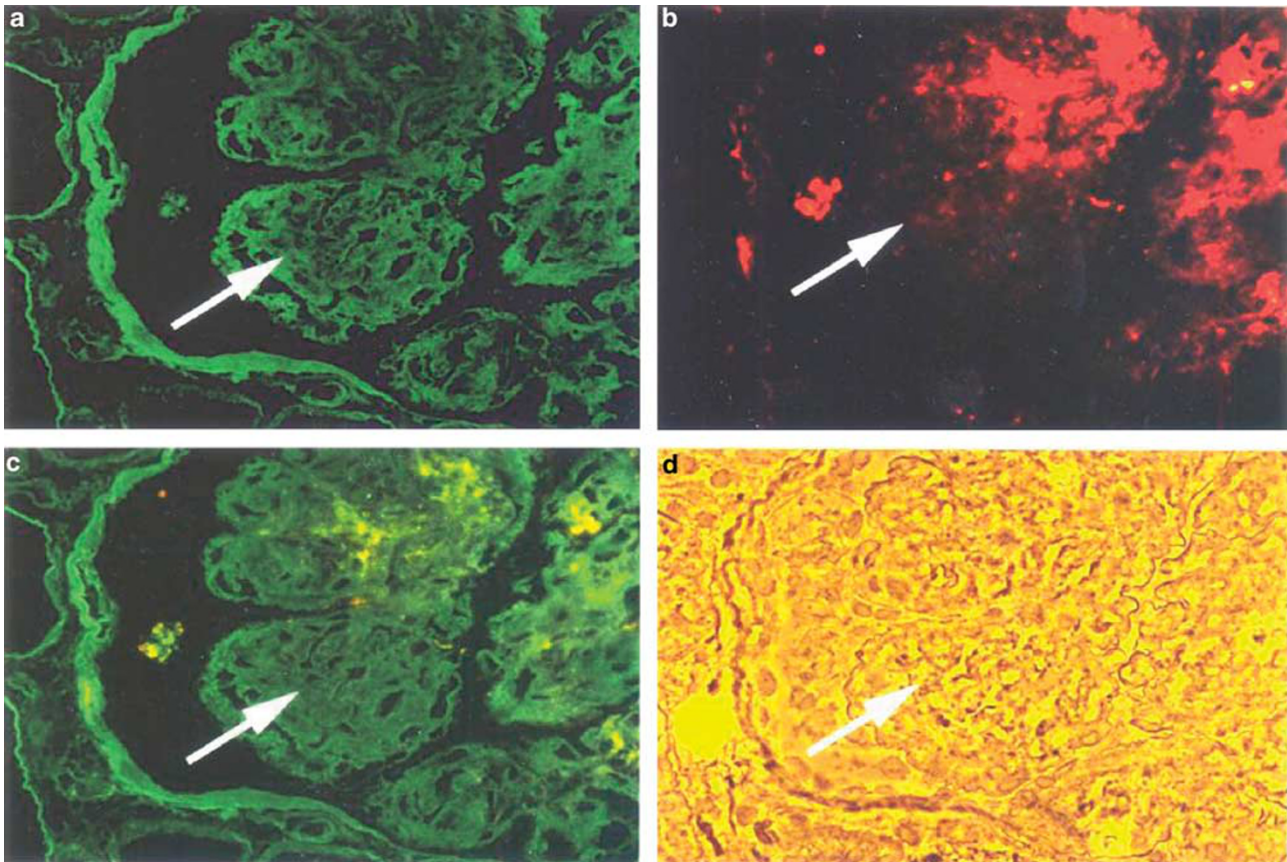


**Figure 7** Dual fluorochrome studies of a glomerulus from mild diabetic nephropathy stained for (a) type IV collagen (FITC) and (b) Lm  $\alpha 2$  chain (RITC) ( $\times 550$ ). (c) Note the increased and complete overlap of mesangial immunoreactivity for type IV collagen and Lm  $\alpha 2$  chain. (d) The same glomerulus is visualized by phase-contrast microscopy.



**Figure 8** Dual fluorochrome studies of a glomerulus from severe diabetic nephropathy stained for (a) type IV collagen (FITC) and (b) Lm  $\alpha 2$  chain (RITC) ( $\times 550$ ). While there is a diffuse increased mesangial staining for type IV collagen with glomerular basement membrane thickening, note the striking decrease to disappearance (arrow) of the Lm  $\alpha 2$  chain in the mesangium. (c) When both fluorochromes are visualized together, the area of loss is seen in green. (d) The same glomerulus is visualized by phase-contrast microscopy.





**Figure 9** Dual fluorochrome studies of a glomerulus from membranoproliferative glomerulonephritis stained for (a) type IV collagen (FITC) and (b) Lm  $\alpha 2$  chain (RITC) ( $\times 550$ ). (c) As in severe diabetic nephropathy, there is a decrease to disappearance (arrow) of the Lm  $\alpha 2$  chain in the mesangium, while diffuse increased mesangial immunoreactivity for type IV collagen persists. (d) The same glomerulus is visualized by phase-contrast microscopy.

not only reflect the balance between synthesis and degradation of normal basement membrane constituents, but also the aberrant new expression of basement membrane molecules. Thus, tissue remodeling may incorporate both an increase of normal constituents and emergence of abnormal constituents during disease exemplified by diabetes.

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## Disclosures/conflict of interest

The authors declare no conflict of interest.

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