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Aging-Associated Changes in Renal Extracellular Matrix

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The composition of renal extracellular matrices was examined in 6-, 12-, 18-, and 24-month-old rats by immunofluorescence microscopy. No change in composition of tubular basement membrane was detected. Increased immunostaining for laminin chains B1 and s-laminin and thrombospondin characterized the thickened glomerular basement membrane. Interstitial collagens I and III were not detected in globally sclerotic glomeruli. The major change noted in the aged rat kidney at 24 months was generalized expansion of the interstitium by thrombospondin and fibronectin. In areas of tubular atrophy there was new expression of extra domain A (EDA)$^+$ fibronectin. Collagens I and III were detected focally in the interstitium adjacent to areas of tubular atrophy, but otherwise collagens I, III, and IV and laminin did not contribute to the interstitial fibrosis. Interstitial fibrosis was detectable at 18 months of age and preceded the development of sclerotic glomeruli, tubular atrophy, or accumulations of interstitial collagen. These changes in extracellular matrix composition distinguish the aging kidney from other sclerotic forms of renal disease. (Am J Pathol 1995, 146:742–752)

Glomerulosclerosis and tubulointerstitial fibrosis are hallmarks of progressive forms of renal diseases of diverse etiologies and are frequently present in kidneys from aged animals and man.[1–8] As seen by routine stains and light microscopy, the amorphous accumulation of extracellular matrix (ECM) material appears relatively uniform from one renal disease to the next. Thus, it has been assumed that scar formation is a final common pathway with end-stage lesions having similar composition. Recent studies of ECM have demonstrated that the actual composition of ECM is relatively unique to each tissue site and that some renal basement membranes contain matrix protein isoforms that have limited distribution in the body.[9–14] Furthermore, studies of specific diseases have shown that matrix expansion can result from excessive accumulation of constituents normally present or from the new expression of various matrix proteins in a particular tissue compartment.[6,7,15,16] Given the recent studies that demonstrate the profound effects of ECM on cell attachment, phenotype, differentiation, and secretory profile,[17–19] it is important to define the composition of ECM in normal and diseased states. As basic knowledge of growth factor and cytokine regulation of ECM synthesis grows, this will allow the development of strategies to alter or prevent the accumulation of abnormal matrix proteins with the hope of ultimately protecting renal function.

Recently, a large number of species- and chain-specific antibodies and cDNA probes to ECM proteins have become available. With the development of these tools the molecular heterogeneity and complexity of ECMs has become apparent.[9,10,20–22] Likewise, similar complexity exists within the kidney as well as within the glomerulus. In the present study, renal ECM composition was defined by the use of specific antibodies, and aging-associated changes were characterized. These studies demonstrate that, in addition to the previously described increase in glomerular basement membrane (GBM) thickness,[23] marked interstitial fibrosis develops, which precedes the development of focal glomerulosclerosis or tubular atrophy.

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Materials and Methods

Reagents and Antibodies

The following antibodies were used for indirect immunofluorescence microscopy: rabbit anti-rat tail collagen I (AB755), rabbit anti-rat collagen III (AB757, Chemicon, Temecula, CA), monoclonal murine anti-bovine type IV collagen M2 (specific for human COL4A3, SciMedx, Denville, NJ), rabbit anti-mouse collagen IV (T40261, Biodesign, Kennebunkport, ME), monoclonal murine anti-human laminin (LM) A (A006, Telios Pharmaceutical, San Diego, CA), monoclonal murine anti-human laminin B2 (A005, Telios Pharmaceutical), rabbit anti-mouse laminin B1B2 (UBI 06–164, Upstate Biotechnology, Lake Placid, NY), monoclonal murine anti-s-laminin, also designated laminin B3 or B1s[18] (C1, C4, D5, kindly provided by Dr. Joshua Sanes,[24]) murine monoclonal antibodies to fibronectin (FN) isoforms ED-A (IST-9) and ED-B (BC-1) (kindly provided by Dr. Luciano Zardi,[25]) fluorescein-conjugated rabbit anti-rat immunoglobulin (Ig)G, rabbit anti-mouse IgG, and goat anti-rabbit IgG (ICN Immunobiologicals, Lisle, IL). Antibodies produced in our laboratory included rabbit anti-rat albumin, rabbit anti-EHS laminin (Collaborative Research, Bedford, MA), rabbit anti-rat thrombospondin (TSP),[26] rabbit anti-rat fibronectin,[16] rabbit anti-mouse laminin A peptide (FDAYDTSDKETKQGETC, amino acids (aa) 2412 to 2427), rabbit anti-mouse laminin B1 peptide (VIGPYRETVDSVEKKVNEIC, aa 1211 to 1230), and rabbit anti-mouse laminin B2 peptides (DDMVTDQAFEDRUKEAEREVC, aa 1061 to 1080 and EAENELKRKQDDADQDMC, aa 1468 to 1484), and rabbit anti-rat s-laminin (B1s) (SAQSRAREAEKQLREQVC, aa 1693 to 1709). These antibodies were tested for antigen specificity by Western blot and enzyme-linked immunosorbent assay.

Experimental Design

Fisher 344 rats were obtained from the National Institute on Aging (Bethesda, MD) at 6, 12, 18, and 24 months of age. These animals are barrier based and the most widely used rodent model of aging. One week after arrival in our laboratory, three animals at each age were weighed and bled, and a 24-hour urine specimen was collected. Animals were housed in metabolic cages with water and food provided *ad libitum*. Urine volumes were measured so that 24-hour urinary protein excretions rates could be calculated. Animals were killed, and kidneys were removed and processed for light, electron, and immunofluorescence microscopy as described below.

Light Microscopy and Electron Microscopy

Routine histological analysis was performed to confirm the aging-related GBM thickening and glomerular and interstitial fibrosis that has been described previously[1–3] in other rat strains. For light microscopy, renal tissue was fixed in alcoholic Bouins solution, embedded in paraffin, sectioned at 4 μ, and stained with periodic acid Schiff by routine methods. Glomerular diameter was estimated with an eyepiece micrometer. Fifteen glomeruli were sized per animal. For electron microscopy, tissue was fixed in 2.5% glutaraldehyde, washed in cacodylate buffer, embedded in Epon, post-fixed with osmium tetroxide, stained with uranyl acetate, and examined with a JEM, Jeol-100S electron microscope.

Immunofluorescence Microscopy

Renal tissue was snap frozen in precooled isopentane with liquid nitrogen, embedded in OCT compound (TissueTek), and sectioned at 4 μ. Tissue was fixed with −20 C acetone and methanol and stained with the designated antibody, followed by the appropriate fluorescein isothiocyanate-conjugated second antibody. Staining was for 20 minutes at room temperature followed by three 3-minute washes with phosphate-buffered saline. Sections were coverslipped with phosphate-buffered saline-glycerol and examined with a Leitz epi-fluorescence microscope equipped with a 100-W Osram mercury lamp. Controls included preimmune serum from each species represented by the primary antibodies. Slides were randomized, coded, and scored (0 to 4+ intensity, normal or expanded area) by two independent observers who had no knowledge of sample identity. Scores for each observer were averaged and the mean score for each group was determined. (These means, rounded to whole integers, are shown in Tables 2 and 3.)

Blood and Urine Studies

Serum was assayed for urea nitrogen and creatinine with a Beckman autoanalyzer. Urine protein excretion was measured by sulfosalicylic acid precipitation as previously described.[27] The concentration of protein

(mg/ml) is multiplied by the volume of urine per day to calculate the urinary protein excretion (mg/day).

Statistical Analysis

For quantitative data, group means were compared by one-way analysis of variance with subgroup testing by contrasts. Results are expressed as the group mean ± 1 SD. For immunofluorescence data, statistical comparisons between rats of different age groups were performed by the Mann-Whitney test. Comparisons in which no statistical differences were noted in fluorescence intensity are designated in the Table as NC (no change). Mean intensity scores are given in Table 3 in those cases for which a significant change ($P < 0.05$) was identified. No quantitative method was devised to note a change in the area or pattern of staining. Examples of these changes are described in the text and examples are provided in the micrographs.

Results

The results of serum creatinine and urea nitrogen, urinary protein excretion, and glomerular diameters are summarized in Table 1. Aged animals (24 months) developed increases in urinary protein excretion without an increase in serum creatinine or urea nitrogen. A significant increase in glomerular diameter ($P < 0.01$, analysis of variance) was noted between 6 and 12 months of age while animals were still growing; however, no additional increase was present thereafter. At 24 months of age the mean glomerular diameter was not significantly different from 18-month-old rats; however, the SD for 24-month-old rats was high. Glomerular diameters varied from 112 to 250 μ. Thus, it appeared that sclerosis of some glomeruli was accompanied by the development of compensatory hypertrophy in other glomeruli. There was no evidence that generalized hypertrophy preceded the development of sclerosis once animals had stopped growing.

Before 24 months of age no significant changes in glomerular structure were demonstrated by light microscopy. At 24 months of age some small and globally sclerotic glomeruli were observed. Generalized changes in mesangial cellularity or glomerular ECM accumulation were not observed. Electron microscopy demonstrated a three- to four-fold increase in GBM thickness between 6 and 24 months of age as described previously.[23] Similarly, a generalized increase in the interstitial accumulation of ECM developed by 24 months of age. In addition, focal areas of tubular atrophy and tubular dilatation were apparent. In these areas significant ECM accumulation within adjacent interstitium occurred (Figure 1). Thus, the animals in this study that were examined for ECM composition demonstrated the changes characteristic of the aging kidney.

The pattern of immunostaining of various renal ECM compartments in young rats (6 months of age) is shown in Table 2. No changes in intensity or pattern of staining were discerned at 12 months of age. Although each of the ECM compartments contains collagen and a variety of associated glycoproteins, there are some notable differences. GBM is rich in type IV collagen and sLM with significantly lesser amounts of other LM forms or FN. TSP is not routinely detected in normal GBM. In contrast to GBM, mesangial matrix (MM) stains predominantly with antibodies to FN and one of the monoclonal antibodies to sLM (C4). TSP and LM are also easily demonstrated. Faint staining of the MM for the ED-A isoform of FN is detectable. Tubular basement membrane (TBM) is rich in type IV collagen, LM, and FN. The proximal tubular TBM is notably different in its intensity of staining with polyclonal anti-LM and in staining for the A chain of LM as compared with the distal tubule. FN isoforms ED-A and ED-B are not normally expressed in TBM or the

Table 1. *Animal Characteristics*

Group	Age (months)	Weight (g)*	Serum creatine (mg/dl)†	Serum urea nitrogen (mg/dl)†	Urinary protein excretion (mg/day)‡	Glomerular diameter (range; μ)§
1	6	346 ± 16	0.67 ± 0.06	15 ± 3	1.7 ± 1.6	123 ± 5 (*100–150*)
2	12	430 ± 41	0.70 ± 0.10	14 ± 1	7.6 ± 6.5	153 ± 5 (*137–175*)
3	18	435 ± 49	0.73 ± 0.06	15 ± 3	5.7 ± 2.3	143 ± 5 (*137–158*)
4	24	444 ± 16	0.85 ± 0.11	16 ± 3	65.6 ± 74	168 ± 25 (*112–250*)

Results are expressed as group mean ± 1 SD; $n = 3$ per group.
*$P < 0.05$ for group 1 < 2, 3, and 4.
†No significant difference between groups.
‡$P < 0.05$ for group 4 > 1, 2, and 3.
§$P < 0.01$ for group 1 < 2, 3, and 4.

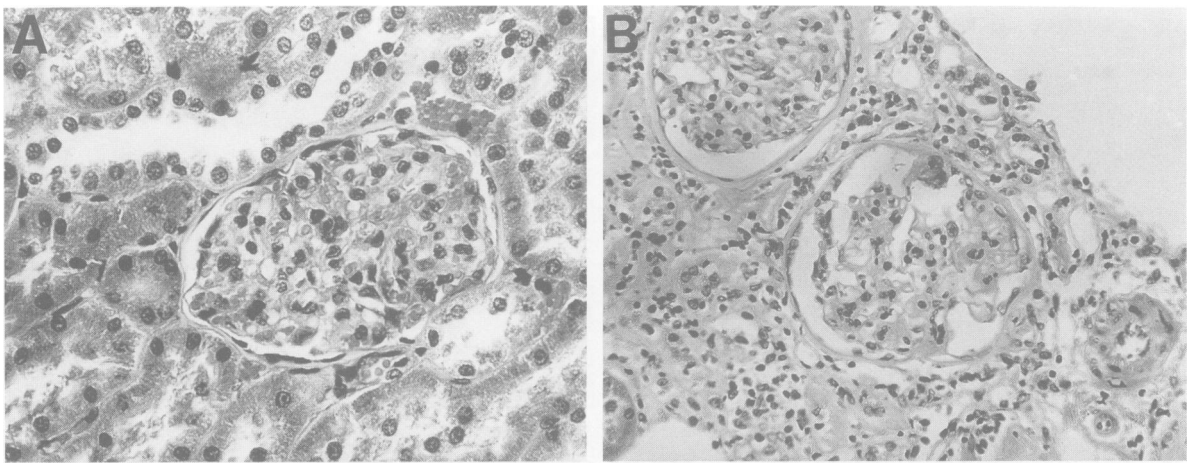

Figure 1. *Light micrographs showing significant focal interstitial fibrosis and thickening of Bowman's capsule in a 24-month-old rat* (**B**) *as compared with a 12-month-old rat* (**A**). *Stained with periodic acid Schiff; original magnification,* ×*250.*

Table 2. *Structure-Specific ECM Composition (Young Rat)*

	INT	TBM-PT	TBM-DT	BC	GBM	MM
Col I	Trace	–	–	–	–	–
Col III	+	+	–	–	–	–
Col IV α1	–	+++	+++	+++	+++	+
Col IV M2	–	++	++	++	+++	+
LM	–	+++	++	+	+	++
LM A	–	+	–	–	Trace	–
LM B1	–	–	–	–	++	+
LM B2	–	++	++	+	+	++
LM B1B2	–	++	++	–	++	+
sLM (C1)	–	–	–	–	+	++
sLM (C4)	–	–	–	–	+	+++
sLM (D5)	–	–	–	–	++++	Trace
FN	++	++	++	++	++	++++
FN ED-A	–	–	–	–	–	++
FN ED-B	–	–	–	–	–	–
TSP	++	+	+	+++	Trace	+++

INT, interstitium; TBM-PT, TBM of proximal tubule; TBM-DT, TBM of distal tubule; BC, Bowman's capsule; Col, collagen. Results represent the group mean (n = 3) after averaging the scores assigned by the two independent observers.

renal interstitium. It is also of note that the LM B1 peptide recognized by the polyclonal antibody used in this study is detected in glomerular LM, but it is not demonstrated in TBM.

Bowman's capsule stains more prominently with antibodies to FN and TSP than to collagen IV and LM. In the normal kidney the interstitial space between adjacent tubules is small in amount. It contains scanty amounts of collagens I and III. The predominant immunoreactive proteins are FN and TSP, which extend around peritubular capillaries. This gives a chicken-wire appearance to the staining pattern (Figure 2) as compared with collagen IV and LM (Figure 3), which show circular staining of TBM without apparent extension into the interstitial space.

The directional changes in intensity of staining for ECM proteins with age are summarized in Table 3. The major change in the glomerulus is an increase in the thickness of the GBM. The intensity of staining for FN and collagen IV are slightly increased in association with GBM thickening. The major change in GBM composition is a significant increase in staining for LM. GBM normally stains with antibodies to LMB2 and sLM. Thickened GBM in 24-month-old rats showed increases in staining for LMB1, LMB2, and sLM. The changes in staining for the LMB chains were more evident than the changes in intensity of LMA. In addition to these changes TSP was easily detected in the aged GBM. The changes in intensity of staining of GBM for sLM and LMB1 are shown in Figure 4. These data show accumulation primarily of LM and TSP in the aged, thickened GBM. In contrast to significant changes in the GBM, no notable changes in the amount or composition of MM were observed. The intensity of staining for FN, TSP, and LM was diminished slightly at 24 months of age. There was no change in the intensity of staining for collagen IV. No significant staining for collagens I and III within MM

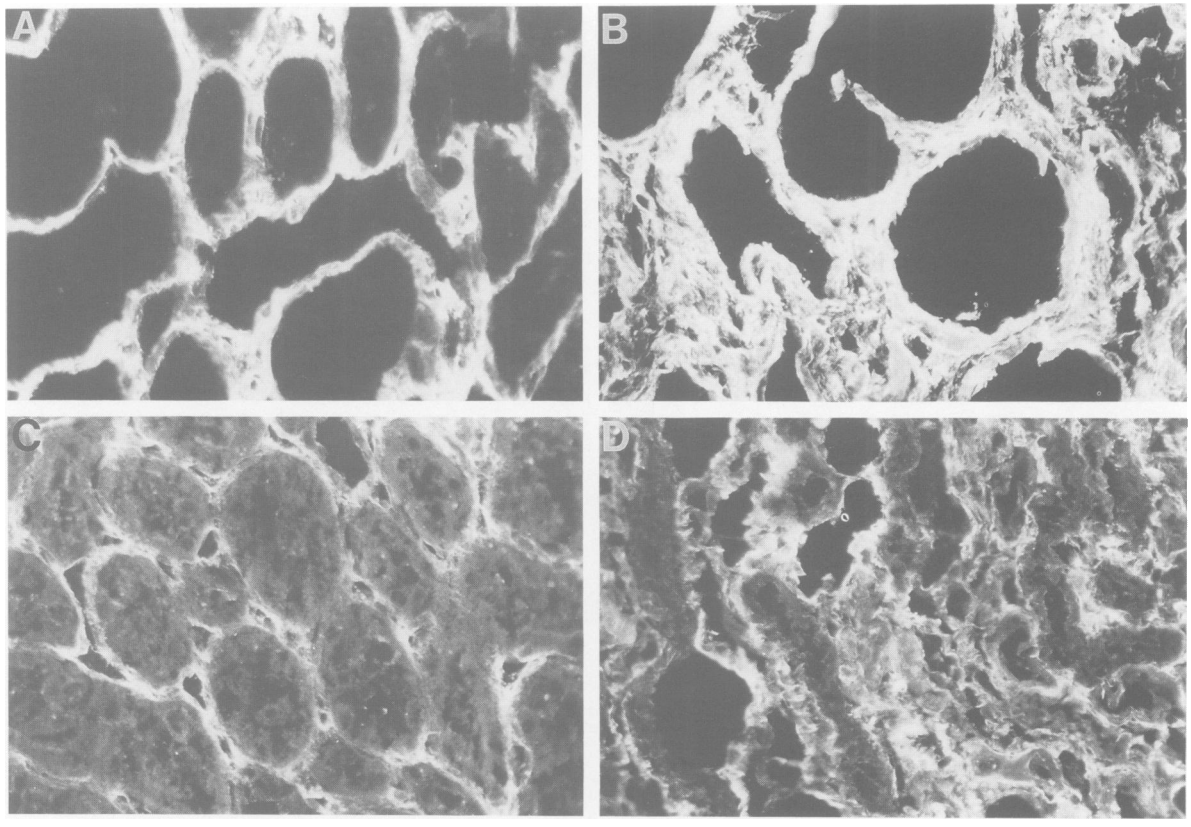

Figure 2. *FN and TSP staining of TBM and the interstitial space adjacent to tubules is shown for young (A and C, 6 months) and old (B and D, 24 months) rats. Note the prominent staining for FN (A and B) and TSP (C and D) in TBM and interstitium and around peritubular capillaries. Original magnification, ×250.*

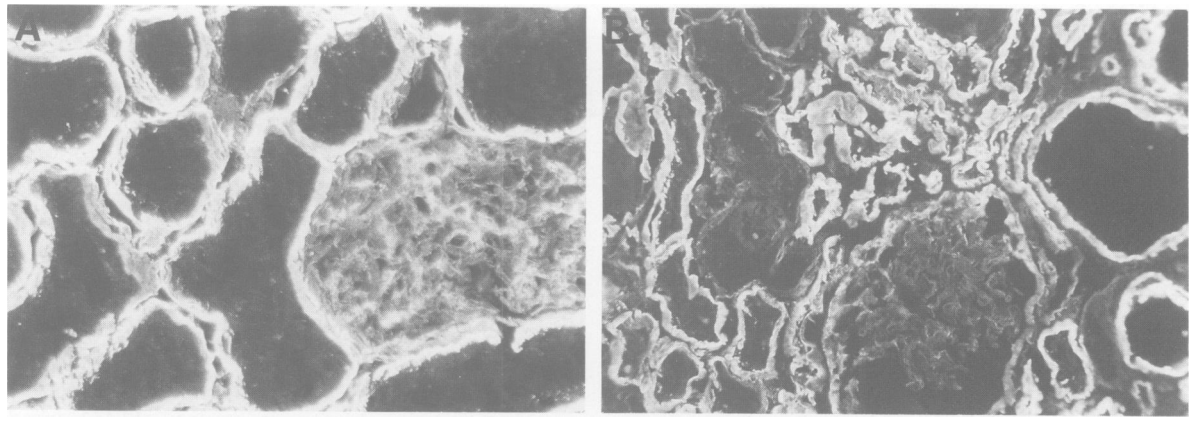

Figure 3. *Micrographs of TBM from young (A, 6 months) and old (B, 24 months) rats stained with rabbit anti-EHS LM show comparable thickness and intensity of staining for LM. Note the continuous staining of TBM without apparent extension of LM into adjacent interstitial space or around peritubular capillaries. Slightly diminished intensity of LM staining is also apparent in glomeruli from old (B) as compared with younger rats (A). A generalized increase in the thickness of TBM is not apparent in old rats (see TBM at left of photo). Focal areas of tubular atrophy in old rats (lower right hand corner of B) were associated with corrugation and thickening of TBM, which stained brightly for LM. Immunoreactivity for LM was not present in expanded, fibrotic areas adjacent to atrophic tubules. Original magnification, ×250.*

were observed. There was no evidence for changes in isoform expression of FN or LM.

Significant increases in the thickness of Bowman's capsule were noted with aging. The increase was a generalized change that was not limited to either small sclerotic glomeruli or large glomeruli. There was no significant change in the intensity of staining for LM or collagen IV within Bowman's capsule. The major immunoreactive proteins detected in Bowman's capsule are TSP and FN. The predominant change in the thickened Bowman's capsule seen in 24-month-old rats was a marked increase in intensity of staining for

Table 3. *Aging-Related Changes in ECM Composition*

	INT	TBM	BC	GBM	MM
Col I	Trace/1+†	NC	NC	NC	–/tr
Col III	+/+++†	NC	NC	NC	NC
Col IV α1	NC	NC*	NC	+++/+++	NC
Col IV M2	NC	NC*	NC	NC	NC
LM	NC	NC*	NC	+/++	++/+
LM A	NC	NC	NC	Trace/+	NC
LM B1	NC	NC	NC	++/+++	+/++
LM B2	NC	NC*	NC	+/+++	++/+
LM B1B2	NC	NC*	NC	++/+++	NC
sLM (C1)	NC	NC	NC	+/++	++/+
sLM (C4)	NC	NC	NC	+/+++	+++/++
sLM (D5)	NC	NC	NC	NC	NC
FN	++/++++	NC	NC	++/+++	++++/+++
FN ED-A	–/+†	–/+++†	NC	NC	NC
FN ED-B	NC	NC	NC	NC	NC
TSP	++/++++	NC	+++/++++	–/++	+++/+

INT, interstitium; BC, Bowman's capsule; Col, collagen; NC, no change. Intensity scores are shown for 6-month-old/24-month-old rats.
*Changes in staining intensity and thickness only in focal areas of interstitial fibrosis; no generalized increase.
†In focal areas of interstitial fibrosis only.

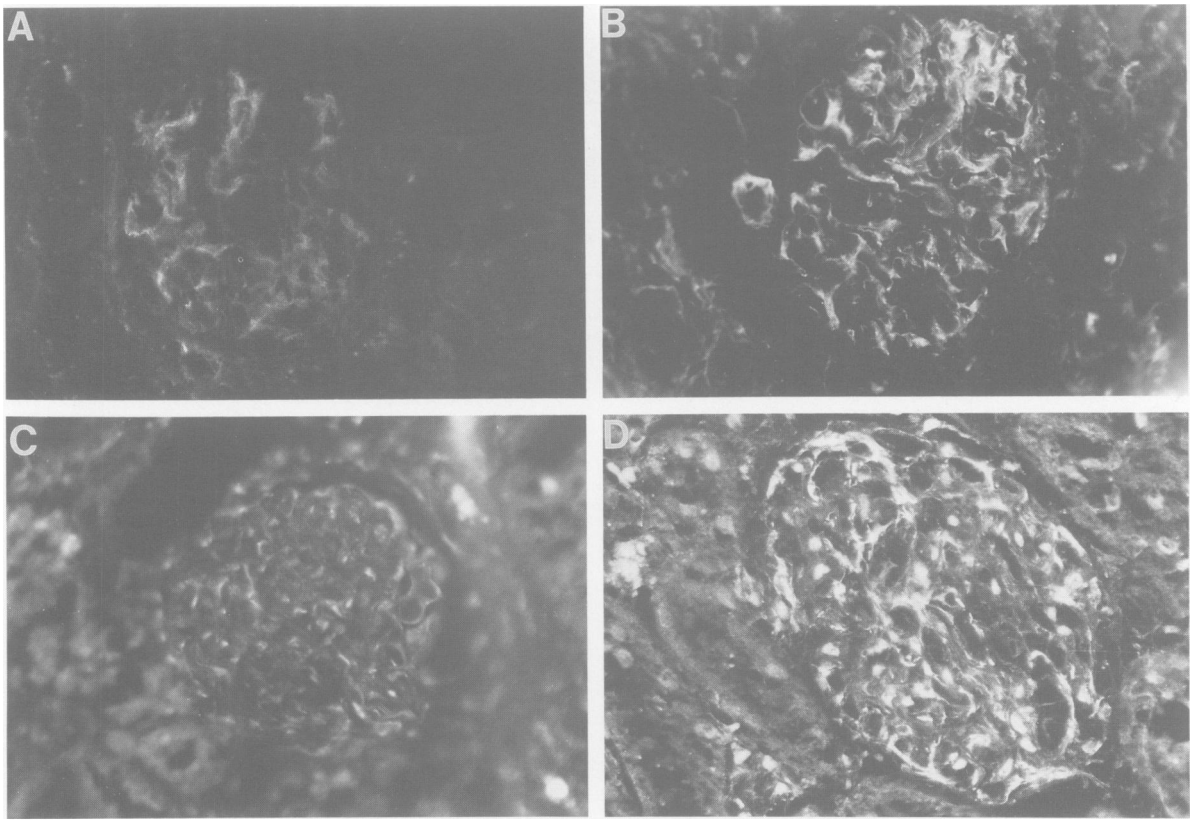

Figure 4. *Immunofluorescence micrographs showing GBM thickening in aged rats with increase in the intensity of staining for sLM (C4) in 12-month-old* (**A**) *and 24-month-old* (**B**) *and LM B1 in 6-month-old* (**C**) *and 24-month-old* (**D**) *rats. Original magnification, ×400.*

TSP. Although a generalized increase in intensity of staining for FN was not observed, some glomeruli in 24-month-old rats had bright staining for the ED-A isoform of FN in Bowman's capsule. These changes are shown in Figures 5 and 6.

The modest, generalized thickening of TBM that was noted by electron microscopy was not discernible by immunofluorescence microscopy, and no generalized changes in TBM composition were noted. In small focal areas of tubular atrophy, TBM appears very thickened as it becomes corrugated, presumably from tubular collapse. Although these changes are easily detected by immunostaining for LM and collagen IV, there was no evidence for a change in the isoform of LM or collagen IV in TBM of atrophic tubules. However, TBM of some atrophic tubules did

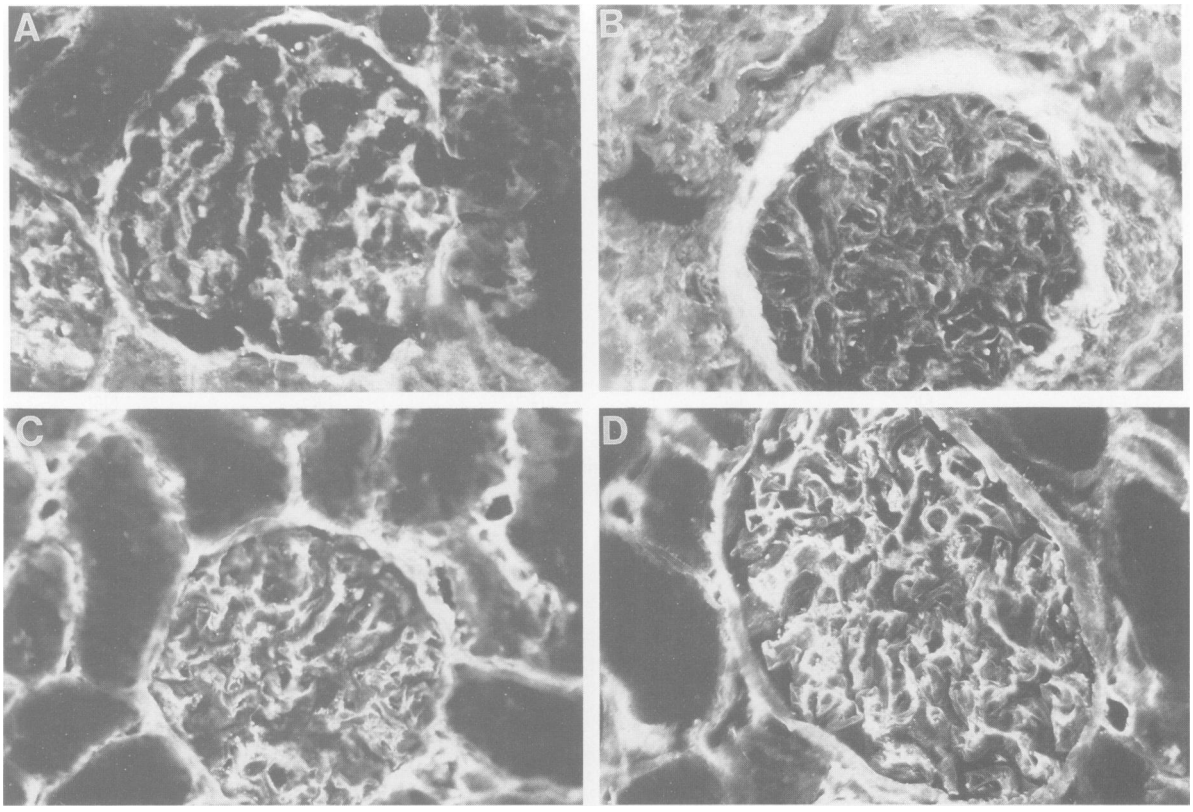

Figure 5. *Immunofluorescence micrographs showing thickening of Bowman's capsule with age. Significant staining for TSP* (**A**, *6 months;* **B**, *24 months) is apparent in Bowman's capsule of the aged rat. Also note in* **A** *the bright staining for TSP in MM with scant GBM staining. In contrast, the aged rat* (**B**) *shows more distinct GBM staining for TSP and less prominent staining in MM. The staining pattern for FN is shown in* **C** *and* **D**. *Significant thickening of Bowman's capsule is also shown in* **D** (*24 months*) *as compared with* **C** (*12 months*), *but without an apparent change in the intensity of staining for FN. There is comparable intensity of staining for FN in MM, but increased staining of GBM at 24 months* (**D**) *as compared with 12 months* (**C**). *Original magnification,* ×*400.*

stain brightly with antibodies to the ED-A isoform of FN (Figure 6).

The most striking change in all kidneys from 18- and 24-month-old rats was a generalized increase in the interstitium (Figures 2 and 3). The interstitial space is small in young rats and stains for both FN and TSP. LM and collagen IV appear to be confined to the TBM. Similarly, the expanded interstitium of 18- and 24-month-old rats contained only FN and TSP. Serum did not appear to be the source of these proteins as the absence of interstitial albumin or IgG exclude generalized accumulation of serum proteins in the interstitial space. ED-A FN was not generally detected throughout the interstitium; however, new expression of ED-A FN was demonstrated at 18 and 24 months of age (Figure 6). Areas of marked accumulation of ECM within the interstitium occurred adjacent to areas of tubular atrophy. In these areas the composition of ECM differed from that observed in the generally expanded interstitium of aged rats. Focal staining for collagen III was sometimes evident in these areas and, rarely, focal collections of collagen I. Focal collections of ED-A FN were seen in these areas when adjacent atrophic tubules were also expressing this FN isoform.

Discussion

The findings in this study demonstrate heterogeneity and complexity of ECM compartments within rat kidney. Some of these findings have been described previously.[21–23,28–31] In particular, novel isoforms of collagen IV and LM are differentially expressed in various renal ECM compartments and are altered with disease. Collagen IV is a family of heterotrimeric proteins with various isoforms determined by selective use of collagen IV chains $\alpha 1$ to $\alpha 5$. Most basement membranes contain $\alpha 1(IV)_2\ \alpha 2(IV)_1$. In humans GBM contains significant amounts of other isoforms that include $\alpha 3$ to $\alpha 5$ chains.[13] These novel isoforms are not normally detected in MM, TBM, or other compartments within the human kidney. In contrast to humans, the monoclonal antibody M2, which specifically rec-

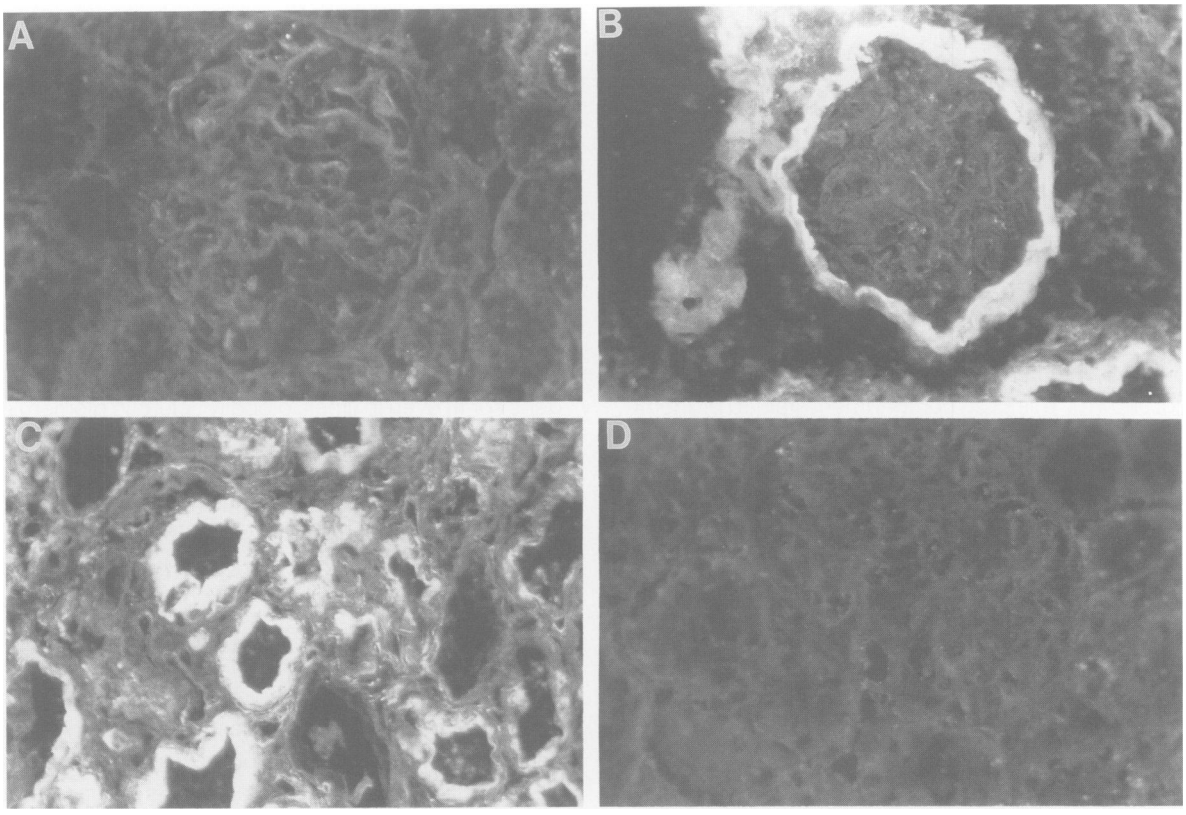

Figure 6. *The pattern of staining for FN isoforms are shown. Modest intensity of staining of MM with antibodies to FN ED-A (IST-9) is shown for old* (**A**, *24 months*) *rats. Note the generalized absence of immunoreactivity for this FN isoform in the interstitium and Bowman's capsule. Similar patterns of staining were observed in all age groups. Some glomeruli from aged rats* (**B**, *24 months*) *demonstrated intense staining for FN ED-A in Bowman's capsule. Also, significant immunoreactivity for FN ED-A was seen in TBM* (**C**, *24 months*) *in areas of significant tubular atrophy. Absence of staining for FN ED-B (BC-1) is shown in* **D** (*18-month-old rat*). *Original magnification, ×250.*

ognizes human COL4A3, stains all basement membranes of rat kidney. Rat homologues of the novel collagen IV chains have not yet been sequenced; thus, the significance of the staining pattern in rats is not completely understood. Yet, at the present, using available antibodies to collagen IV, we have no evidence that alterations in collagen IV isoform expression occurs in aged rat kidneys.

The LM family of proteins is similarly complex. Classical laminin isolated from the EHS sarcoma is a heterotrimer of AB1B2. A growing number of A chain alternatives have been described (reviewed in reference 18) and, with the exception of merosin,[29,32] sequence information is not yet available for all of these proteins. Similarly, several B chain homologues, including sLM, have been identified.[12,24,29] Thus, the final composition of the LM molecule is highly variable, tissue specific, and regulated during differentiation and in disease states.[22,29,33] The present study demonstrates that comparable degrees of complexity of the LM molecule exist within the renal ECM compartments. Positive staining of glomerular LM with antibodies to sLM (B3) and a B1 peptide, and absence of these epitopes in tubular LM, is evidence of unique LM isoform expression within the kidney. A number of unique functions have been attributed to these novel LM chains.[18] Understanding their specific distribution in tissues and with disease is the next step in attempts to understand the relationship of these proteins to function in health and disease.

In regards to the composition of renal ECM of aged rats, there appear to be three major changes that occur. The first is a generalized thickening of GBM and TBM, with the degree of thickening of GBM being somewhat greater.[23] As GBM thickens, the intensity of staining for LM increases out of proportion to the intensity of staining for collagen IV or FN. A significant increase in the intensity of staining for B1 peptide and the epitope of sLM recognized by monoclonal antibody C4 was seen. Comparable changes in the intensity of staining for B2 and A were not noted, suggesting that the thickened GBM of aging may occur as a result of accumulation of a unique LM isoform or disordered LM chain synthesis and assembly of the heterotrimer. Confirmation of this possibility must await additional information and reagents to further define this complex molecule. In addition to the in-

crease in immunostaining for LM in the aged GBM, TSP was also detected. No changes were observed in the composition of TBM with age.

The second major change that was noted was a significant increase in the amount of ECM within the renal interstitium. This was independent of the modest increase in thickness of the TBM. It was detected at 18 months of age before the presence of significant glomerulosclerosis or tubular atrophy. Significant inflammatory cellular infiltrate within the interstitium was not observed by light microscopy, nor were macrophages detected by immunostaining for ED-1 (data not shown). This ECM compartment stained predominantly for FN and TSP. TBM components LM and collagen IV were not detected in the interstitial space. Interstitial collagens I and III were notably absent from the interstitium with the exception of a few focal areas (discussed below). Electron microscopy showed a loose network appearance to the interstitium without evidence of interstitial collagen fibrils. The absence of staining for albumin and IgG within the interstitium argues against simple trapping of serum proteins as the cause of FN and TSP accumulation. Although FN and TSP have many known functions, a significant amount of data indicate their association with blood vessels, and in concert they appear to regulate angiogenesis.[34,35] Given the abundant blood vessels and peritubular capillaries supported within the interstitium and careful studies from aged humans showing corkscrew and other small vessel changes within the renal interstitium,[36] additional studies are needed to determine the potential relationship between interstitial fibrosis and angiogenesis. Takazakura et al[36] have suggested that glomerulosclerosis results after arteriolar occlusion or afferent to efferent arteriolar shunting with subsequent interruption of glomerular perfusion. Thus it is possible that angiodysplasia is a primary event that affects glomerular perfusion.

The third change observed within the aging rat kidney was focal areas of tubular atrophy and interstitial scar. Separate from the generalized changes described above, small infrequent areas of marked structural change were noted. In association with tubular atrophy, TBM appeared significantly thickened as they became corrugated from collapse. The only change in their composition was the appearance of bright staining for the ED-A isoform of FN. In other studies expression of this isoform has been associated with tumor invasiveness,[37] development,[38] and cellular phenotype.[34] Significant expansion of the interstitium was noted adjacent to areas of tubular atrophy. In addition to intense staining for FN and TSP in these areas, there was focal accumulation of ED-A FN and collagens I and III. Occasional ED-1-positive macrophages were also detected in these areas and interstitial collagen fibrils were seen by electron microscopy.

Aside from the changes described above, it is important to note the absence of other changes. There was no evidence of generalized nodular accumulation of MM. The intensity of staining for individual ECM proteins within the MM was unchanged or minimally diminished and there was no evidence of significant accumulation of collagen IV or LM as noted in the GBM. Furthermore, there was no evidence of collagens I or III even in small, globally sclerotic glomeruli. This distinguishes the aging kidney from other forms of glomerulosclerosis in which significant accumulations of collagens occur.[16] Similarly, the absence of generalized accumulation of collagens I and III within the interstitium is in contrast to cyclosporine toxicity[39] and other forms of interstitial renal disease. For these reasons it appears that the changes in ECM that characterize the aging kidney are relatively unique and will require additional study to elucidate the mechanisms responsible for these changes. Changes in ECM composition in these rats occurred in association with GBM thickening and interstitial fibrosis as has been well described in other rat strains and man; thus, we suspect these changes to be aging related. Extension of these observations to other rat strains and man should exclude the possibility of a disease process unique to Fisher 344 rats.

In summary, previous studies have shown increased glomerular diameter and GBM and TBM thickening with age in rats and man.[23] Similar changes were seen here. It has been suggested that generalized hypertrophy initiates sclerosis which then proceeds to compensatory hypertrophy in nonsclerotic glomeruli thereby leading to progressive glomerulosclerosis. Alternatively, Takazakura et al[36] have suggested that vascular changes are responsible for the glomerulosclerosis. Although the analysis presented here was not designed to address this question, generalized hypertrophy paralleled body growth and then appeared stable. With the exception of changes in GBM thickness and composition that developed by 18 months, significant MM expansion and nodular glomerulosclerosis was not evident at 18 months of age. Small sclerotic glomeruli, as well as very large glomeruli, were relatively infrequent at 24 months of age. Most glomeruli had a normal appearance. Unlike other forms of renal disease[16] for which glomerular accumulation of collagen occurs with a light microscopic appearance of sclerosis, generalized collagen accumulation was not observed and there was a paucity of interstitial collagen in any lo-

cation within the kidney. The most striking change in the ECM noted in this study was generalized expansion of the interstitium. This was the result of noncollagenous ECM proteins. This study provides new information characterizing the unique composition of various renal ECM compartments and characterizes the changes in glomerular and tubulointerstitial ECM with aging. These findings demonstrate that the pattern and composition of ECM accumulation within the aging kidney differs from other forms of renal scarring. Demonstration of generalized accumulation of FN and TSP suggests that an undefined interstitial process is a primary and important event in the development of progressive renal disease in aging rats. Additional studies are needed to better understand the renal interstitium and the relevance of these findings to aging man.

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References

1. Couser WG, Stilmant MM: Mesangial lesions and focal glomerular sclerosis in the aging rat. Lab Invest 1975, 33:491–501
2. Yumura W, Sugino N, Nagasawa R, Kubo S, Hirokawa K, Maruyama N: Age-associated changes in renal glomeruli of mice. Exp Gerontol 1989, 24:237–249
3. Brandis A, Bianchi G, Reale E, Helmchen U, Kuhn K, Claus H, Duzzi L: Age-dependent glomerulosclerosis and proteinuria occurring in rats of the Milan normotensive strain and not in rats of the Milan hypertensive strain. Lab Invest 1986, 55:234–243
4. Bruneval P, Foidart JM, Nochy D, Camilleri JP, Bariety J: Glomerular matrix proteins in nodular glomerulosclerosis in association with light chain deposition disease and diabetes mellitus. Hum Pathol 1985, 16:477–484
5. Ainsworth SK, Hirsch HZ, Brackett NC, Jr, Brissie RM, Williams AV, Jr, Hennigar GR: Diabetic glomerulonephropathy: histopathologic, immunofluorescent, and ultrastructural studies of 16 cases. Hum Pathol 1982, 13:470–478
6. Striker LM, Killen PD, Chi E, Striker GE. The composition of glomerulosclerosis. I. Studies in focal sclerosis, crescentic glomerulonephritis, and membranoproliferative glomerulonephritis. Lab Invest 1984, 51:181–192
7. Yoshioka K, Takemura T, Tohda M, Akano N, Miyamoto H, Ooshima A, Maki S: Glomerular localization of type III collagen in human kidney disease. Kidney Int 1989, 35:1203–1211
8. Nakamura T, Ebihara I, Tomino Y, Koide H, Kikuchi K, Koiso K: Gene expression of growth-related proteins and ECM constituents in response to unilateral nephrectomy. Am J Physiol 1992, 363:389–396
9. Laurie GW, Leblond CP, Inoue S, Martin GR, Chung A: Fine structure of the glomerular basement membrane and immunolocalization of five basement membrane components to the lamina densa (basal lamina) and its extensions in both glomeruli and tubules of the rat kidney. Am J Anat 1984, 169:463–481
10. Martinez-Hernandez A, Chung AE: The ultrastructural localization of two basement membrane components: entactin and laminin in rat tissues. J Histochem Cytochem 1984, 32:289–298
11. Laurie GW, Horikoshi S, Killen PD, Segui-Real B, Yamada Y: *In situ* hybridization reveals temporal and spatial changes in cellular expression of mRNA for a laminin receptor, laminin, and basement membrane (type IV) collagen in the developing kidney. J Cell Biol 1989, 109:1351–1362
12. Ekblom M, Klein G, Mugrauer G, Fecker L, Deutzmann R, Timpl R, Ekblom P: Transient and locally restricted expression of laminin A chain mRNA by developing epithelial cells during kidney organogenesis. Cell 1990, 60:337–346
13. Saus J, Wieslander J, Langeveld JP, Quinones S, Hudson BG: Identification of the Goodpasture antigen as the α3(IV) chain of collagen IV. J Biol Chem 1988, 263:13374–13380
14. Butkowski RJ, Wieslander J, Kleppel M, Michael AF, Fish AJ: Basement membrane collagen in the kidney: regional localization of novel chains related to collagen IV. Kidney Int 1989, 35:1195–1202
15. Goyal M, Wiggins R: Fibronectin mRNA and protein accumulation, distribution, and breakdown in rabbit anti-glomerular basement membrane disease. J Am Soc Nephrol 1991, 1:1334–1342
16. Abrass CK, Peterson CV, Raugi GJ: Phenotypic expression of collagen types in mesangial matrix of diabetic and nondiabetic rats. Diabetes 1988, 37:1695–1702
17. Furcht LT: Critical factors controlling angiogenesis: cell products, cell matrix, and growth factors. Lab Invest 1986, 55:505–509
18. Kleinman HK, Weeks BS, Schnaper HW, Kibbey MC, Yamamura K, Grant DS: The laminins: a family of basement membrane glycoproteins important in cell differentiation and tumor metastases. Vitamins Horm 1993, 47:161–186
19. Juliano RL, Haskill S: Signal transduction from the extracellular matrix. J Cell Biol 1993, 120:577–584
20. Madri J, Roll F, Furthmayr H, Foidart J: Ultrastructural localization of fibronectin and laminin in the basement membranes of the murine kidney. J Cell Biol 1980, 86: 682–687
21. Desjardins M, Bendayan M: Heterogeneous distribution of type IV collagen, entactin, heparan sulfate proteoglycan, and laminin among renal basement

membranes as demonstrated by quantitative immunocytochemistry. J Histochem Cytochem 1989, 37:885–897
22. Sanes JR, Engvall E, Butkowski R, Hunter DD: Molecular heterogeneity of basal laminae: isoforms of laminin and collagen IV at the neuromuscular junction and elsewhere. J Cell Biol 1990, 111:1685–1699
23. Goldstein RS, Tarloff JB, Hook JB: Age-related nephropathy in laboratory rats. FASEB J 1988, 2:2241–2251
24. Sanes JR, Hunter DD, Green TL, Merlie JP: S-Laminin. Cold Spring Harbor Symp Quant Biol 1990, LV:419–430
25. Carnemolla B, Balza E, Siri A, Zardi L, Nicotra MR, Bigotti A, Natali PG: A tumor-associated fibronectin isoform generated by alternative splicing of messenger RNA precursors. J Cell Biol 1989, 108:1139–1148
26. Raugi G, Olerud JE, Gown AM: Thrombospondin in early human wound tissue. J Invest Dermatol 1987, 89:551–554
27. Kingsbury FB, Clark CP: The rapid determination of albumin in urine. J Lab Clin Med 1926, 11:981–989
28. Stout RW: The role of insulin in atherosclerosis in diabetics and nondiabetics. Diabetes 1981, 30:S54–S57
29. Engvall E, Earwicker D, Haaparanta T, Ruoslahti E, Sanes JR: Distribution and isolation of four laminin variants: tissue restricted distribution of heterotrimers assembled from five different subunits. Cell Regul 1990, 1:731–740
30. Abrahamson DR, Irwin MH, St John PL, Perry EW, Accaritti MA, Heck LW, Couchman JR: Selective immunoreactivities of kidney basement membranes to monoclonal antibodies against laminin: localization of the end of the long arm and the short arms to discrete microdomains. J Cell Biol 1989, 109:3477–3491
31. Courtoy PJ, Timpl R, Farquhar MG: Comparative distribution of laminin, type IV collagen and fibronectin in the rat glomerulus. J Histochem Cytochem 1982, 30: 874–886
32. Paulsson M, Saladin K: Mouse heart laminin. J Biol Chem 1989, 264:18726–18732
33. Wewer UM, Engvall E, Paulsson M, Yamada Y, Albrechtsen R: Laminin A, B1, B2, S and M subunits in the postnatal rat liver development and after partial hepatectomy. Lab Invest 1992, 66:378–389
34. Glukhova MA, Frid MG, Shekhonin BV, Vasilevskaya TD, Grunwald J, Saginati M, Koteliansky VE: Expression of extra domain A fibronectin sequence in vascular smooth muscle cells is phenotype dependent. J Cell Biol 1989, 109:357–366
35. Tolsma SS, Volpert OV, Good DJ, Frazier WA, Polverini PJ, Bouck N: Peptides derived from two separate domains of the matrix protein thrombospondin-1 have anti-angiogenic activity. J Cell Biol 1993, 122:497–511
36. Takazakura E, Sawabu N, Handa A, Takada A, Shinoda A, Takeuchi J: Intrarenal vascular changes with age and disease. Kidney Int 1972, 2:224–230
37. Oyama F, Herohashi S, Shimosato Y, Titani K, Sekiguchi K: Deregulation of alternative splicing of fibronectin pre-mRNA in malignant human liver tumors. J Biol Chem 1989, 264:10331–10334
38. DeSimone DW, Norton PA, Hynes RO: Identification and characterization of alternatively spliced fibronectin mRNAs expressed in early xenopus embryos. Dev Biol 1992, 149:357–369
39. Nast CC, Adler SG, Artishevsky A, Kressler CT, Ahmed K, Anderson PS: Cyclosporine induces elevated procollagen α1 (I) mRNA levels in the rat renal cortex. Kidney Int 1991, 39:631–638