

Glomerulosclerosis in Mice Transgenic for Growth Hormone. Increased Mesangial Extracellular Matrix Is Correlated with Kidney mRNA Levels

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Summary

Mice transgenic for growth hormone (GH) develop progressive glomerulosclerosis. The compositions of kidney extracellular matrix (ECM) and ECM mRNA were examined. The glomerulosclerotic areas in GH mice contained types I and IV collagen, laminin, and basement membrane heparan sulfate proteoglycan (HSPG), which increased with age. The type IV collagen, laminin B2, and HSPG mRNA levels in GH mice, measured by a solution hybridization RNase protection assay, were increased over normal littermates. These findings suggest that the accumulation of ECM components in the glomeruli of GH mice is regulated at the transcriptional level and that glomerulosclerosis is, in part, due to the excess production of ECM rather than simply a reduction in its turnover. The glomerular lesions in GH mice resemble diabetic nephropathy and may allow further dissection of the molecular basis of certain forms of glomerulosclerosis.

The accumulation of extracellular matrix in mesangial areas, glomerulosclerosis, is the hallmark of glomerular diseases, such as diabetes mellitus. It has not previously been possible to determine whether glomerulosclerosis is due to excess synthesis of extracellular matrix (ECM) components, a decrease in their turnover, or both. We developed a non-inflammatory model of progressive glomerulosclerosis in mice transgenic for bovine growth hormone (GH) (1, 2). The sclerotic glomeruli were disproportionately increased in size with respect to either total kidney or body weight (2).

We used immunofluorescence microscopy to determine the composition of the ECM, and measured kidney mRNA levels to determine the role of ECM synthesis in the development of the sclerotic lesions.

Materials and Methods

Animals. GH mice (Tg [Mt-1, GH] Bri 27) or human IGF-I (Tg [Mt-1, IGF-I] Bri 45) and littermate controls were previously described (1, 2). Immunohistology was performed at 7 and 19 wk, and whole kidney RNA was extracted from control and GH mice at 28 wk.

Antibodies. Guinea pig anti-mouse type I collagen, rabbit anti-mouse type IV collagen, and anti-mouse laminin (3) were provided by Dr. C. Little (University of Virginia). Rabbit anti-mouse type I collagen (NCI-79-43-IM) and rabbit anti-mouse type III collagen (NCI-79-61-IIIM) were obtained from Biomeasure

Bogden Laboratories, Hopkinton, MA. Rabbit anti-human type IV collagen and rat monoclonal anti-mouse heparan sulfate proteoglycan (HSPG) (HK-102) were described previously (4, 5).

Immunofluorescence Microscopy. Cryostat sections of kidneys were stained as described previously (2).

RNA Isolation and Northern Analysis. Total RNA was isolated from kidneys according to the method of Chomczynski and Sacchi (6). Equal amounts of RNA were subjected to electrophoresis under denaturing conditions through a 1% agarose/formamide gel, and transferred to Nytran (Schleicher & Schuell, Inc., Keene, NH). The filters were baked, prehybridized, hybridized to a ³²P-cDNA probe for rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (1.3 kbp) (7), washed, and exposed overnight to x-ray film.

Synthesis of RNA Probes. The cDNA subclone (p1234) of PstI-AvaI site from pFAC in the plasmid vector pGEM2 (Promega Corp., Madison, WI) codes for a portion of the major triple helical and the globular domain (NC1) of the mouse α 1 (IV) chain (8). A 1.7-kbp EcoRI-XbaI cDNA fragment of P1298 (coding for Laminin B2) was subcloned into Bluescript KS (9). A 2.0-kbp EcoRI cDNA fragment of mouse HSPG clone 5 (p1301) (10) was subcloned into the EcoRI site of pGEM-7Zf (+) (Promega Corp.) (pGMMPG1). An 850-bp XhoI genomic DNA fragment from pAZ1002 of the mouse α 2(I) procollagen gene (11) was subcloned into the XhoI site of pGEM-7Zf (+) (pGM101). The orientation of the plasmid inserts was analyzed by determination of the size of digestible fragments with restriction enzymes (data not shown).

Radiolabeled hybridizing probes were prepared by linearizing the constructs with ApaI (pGM101), PVUII (p1234), HindIII

(p1298), and XhoI (pGMPG1). The transcription reaction was performed according to published methods (12, 13). The transcript size was checked by electrophoresis of heat-denatured probes on a polyacrylamide/urea gel, followed by autoradiography. The 1-kb ladder, labeled by the replacement synthesis method using T4DNA polymerase (all restriction enzymes were purchased from Bethesda Research Laboratories, Gaithersburg, MD), was used as a control size marker.

Solution Hybridization Assays. Total RNA was hybridized with a ³²P-RNA probe by incubating for 10 min at 85°C, followed by 16 h at 45°C. The samples were digested with 40 μg/ml RNase A (Sigma Chemical Co., St. Louis, MO) and 2 μg/ml RNase T₁ (Pharmacia Fine Chemicals, Piscataway, NJ) for 1 h at 30°C, followed by incubation with SDS (0.56%) and proteinase K (280 μg/ml) for 30 min at 37°C. RNA was purified and the protected fragments were heat denatured and analyzed by electrophoresis on a polyacrylamide/urea gel (12). Autoradiograms were analyzed by densitometry.

The protected bands were of identical size in GH and control mice. The size of the RNA probe for α1(IV) was 350 nt and contained a 330-nt sequence encoding the globular (NC1) domain. The major protected band with this probe had a size of 330 nt. The RNA probe for the laminin B2 chain contained a coding sequence of 250 nt. The protected band had a size of 250 nt. The RNA probe for HSPG contained a coding sequence of 240 nt. The total size of the α2(I) RNA probe was 940 nt. It included the promoter, the first exon (210 nt), and the first intron. Using this α2(I) probe, the solution hybridization assay revealed a protected band of 210 nt. When mRNA was omitted during the hybridization procedure, no protected bands were detected. Additional bands were not found.

Statistical Analysis. Data were analyzed with an unpaired *t* test and expressed as the mean ± SE.

Results

Immunohistology. Type IV collagen, laminin, and HSPG antigens markedly and progressively increased in the mesangial areas of GH mice between 7 and 19 wk (Table 1 and Fig. 1). The peripheral glomerular basement membrane ECM components were not different in any mice.

Table 1. Composition of Extracellular Matrix

Mice		Col I [†] Mes	Col IV [§] Mes	Laminin Mes	HSPG Mes
GH	7W	+	+++	+++	+++
GH	19W	+++	++++	++++	++++
IGF-I	7W	-	tr	+	+
IGF-I	19W	-	tr	+	+
WT	7W	-	tr	+	+
WT	19W	-	tr	+	+

Col, collagen; Mes, mesangium.

-, tr, +, ++, +++: amount of staining.

* Guinea pig anti-mouse type I collagen.

† Rabbit anti-mouse type I collagen.

§ Rabbit anti-mouse type IV collagen.

|| Rabbit anti-human type IV collagen.

There was a significant accumulation of type I collagen in the sclerotic mesangium of GH mice. This was more pronounced at 19 wk than at 7 wk. Type I collagen was not present in the glomeruli of IGF-I or control mice. It was present at comparable levels in the interstitium of IGF-1 and control mice but was more prominent in the perivascular regions of the GH mice. Small amounts of type III collagen were found in the interstitial areas of all mice, but not in the glomeruli.

ECM mRNA Levels. The level of type IV collagen mRNA in GH mice was 3.83-fold higher than in control mice (Figs. 2 and 3). The level of laminin B2 chain mRNA in GH mice was approximately three times greater than that in control mice. The mRNA level of HSPG was also significantly increased in GH mice. A marked increase in the mRNA levels of α2(I) was detected in one of two GH mice tested, and it was high normal in the other. The mRNA level for GAPDH was identical in control and GH mice.

Discussion

This study provides the first evidence, at both the product and mRNA levels, that the development of glomerulosclerosis is associated with an increase in the net synthesis of ECM. There was also a change in the phenotype of the glomerular ECM.

The ECM composition has been examined in several sclerosing glomerular diseases in humans. The glomerulosclerotic areas were found to contain type IV collagen and laminin (4). Nonobese diabetic mice develop both type I diabetes mellitus and glomerulosclerosis. The glomerular lesions contain only normal GBM components (14). In the present study, the sclerotic zones in the glomeruli of GH mice were largely composed of basement membrane components. The enlarged mesangial regions also contained type I collagen, which increased in parallel with the glomerulosclerotic process. The stimuli for this phenotypic change are unknown, but the only human disease with a comparable finding is diabetes mellitus (15). Type III collagen was not found in the glomeruli of GH or control mice but was identified in the interstitium, a finding in agreement with our previous observations (4).

The increase in the levels of mRNA coding for ECM components in GH mice, but not for a house-keeping gene (GAPDH), supports the postulate that increased synthesis accompanies the accumulation of matrix and that it is specific for matrix. That type I collagen mRNA levels were not uniformly elevated may reflect the fact that we assayed total kidney RNA. Clarification of this point will require studies of mRNA from isolated glomeruli. Glomeruli are difficult to isolate from mice in sufficient number and purity by the usual techniques to obtain sufficient RNA for analysis (16). Therefore, we have begun to microdissect glomeruli and to use more sensitive techniques, such as the PCR.

Data that support the hypothesis that one of the mechanisms for the development of glomerulosclerosis may be an alteration in the regulation of ECM synthesis at the transcriptional level were obtained in a rabbit model of anti-GBM

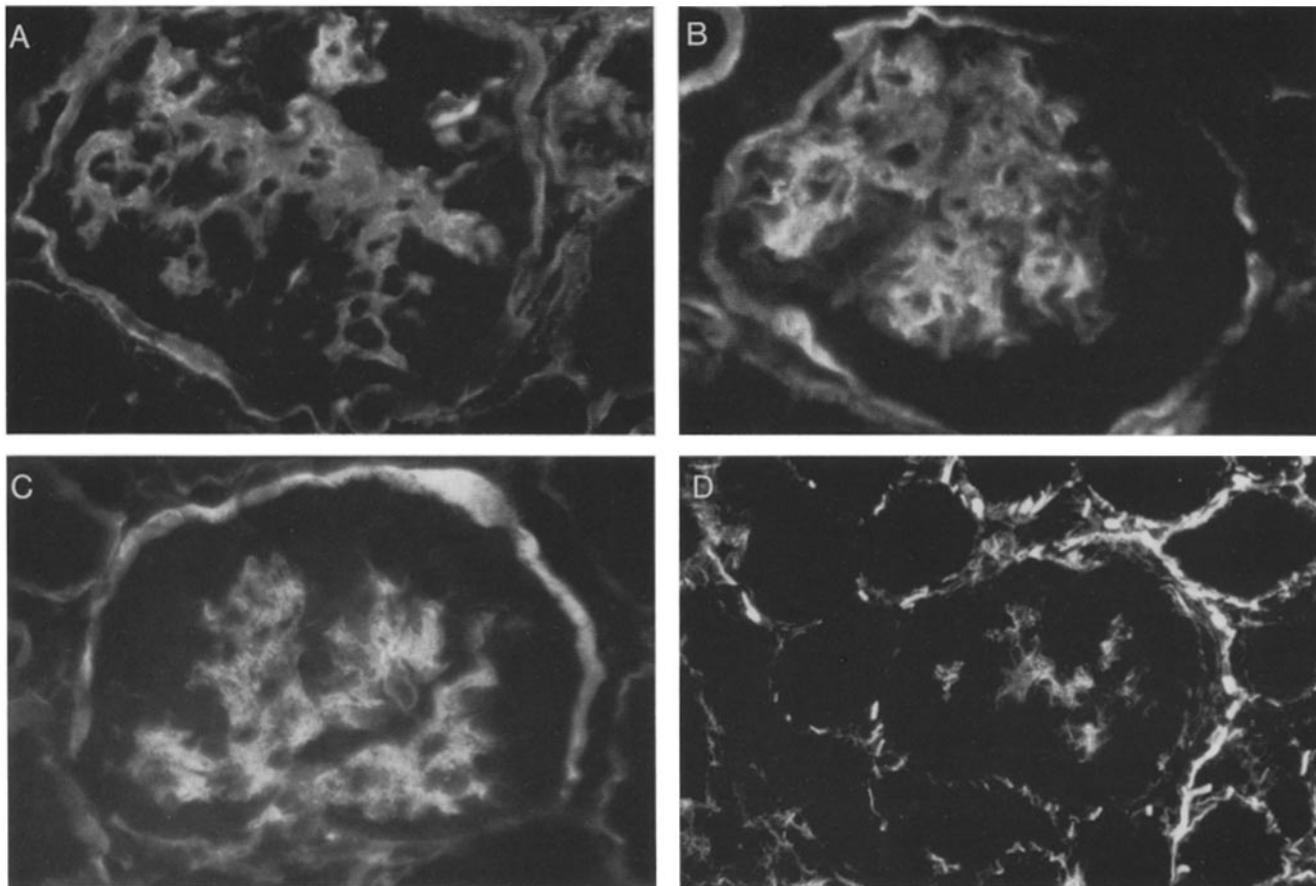


Figure 1. Immunofluorescence of ECM in GH mice (19 wk old). (A) Type IV collagen ($\times 1,300$); (B) laminin ($\times 1,300$); (C) HSPG ($\times 1,300$); (D) type I collagen ($\times 810$).

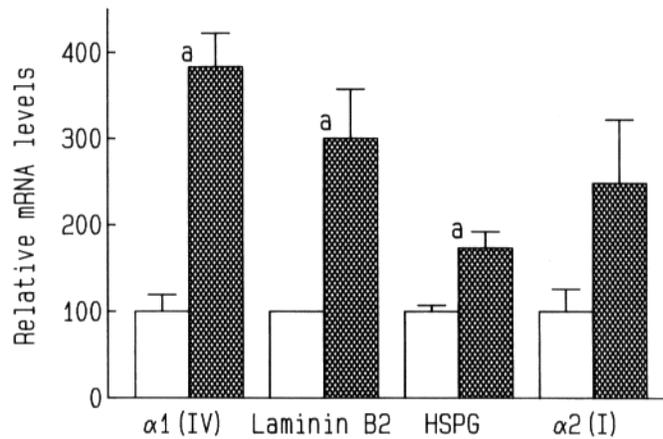
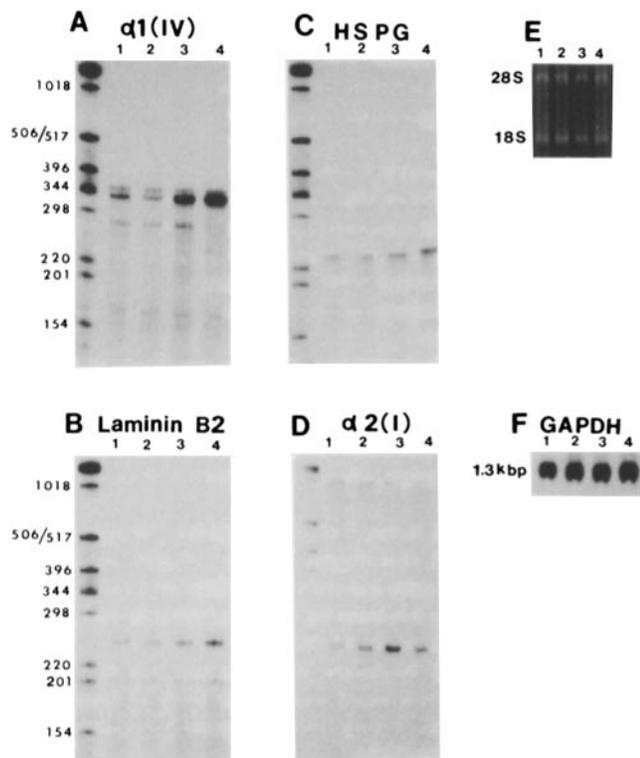


Figure 3. Densitometric analysis of mRNA for ECM. Data are expressed as the mean \pm SE. (\square) WT mice; (\blacksquare) GH mice; (a) $p < 0.05$.

Figure 2. ECM mRNA in the kidney. (A) $\alpha 1$ (IV); (B) laminin B2; (C) HSPG; (D) $\alpha 2$ (I). (E) 18S and 28S bands were stained with ethidium bromide. Approximately equal amounts of RNA were applied to each lane and no significant degradation occurred. (F) GAPDH mRNA. (Lanes 1 and 2) WT mice; (lanes 3 and 4) GH mice.

nephritis in which there was an increased level of type IV collagen mRNA association with an increase in the synthesis and deposition of collagen in the glomeruli (17). Others have examined total kidney for laminin B1, fibronectin, and type IV collagen mRNA in hyperglycemic rats. They found that the laminin B1 and fibronectin were increased while that for type IV collagen was decreased (18). The fact that hyperglycemic rats do not develop severe mesangial lesions may explain this discrepancy.

The model of GH-induced glomerulosclerosis provides the

first evidence that a growth factor, in excess, produces an increase in the mRNA coding for ECM components as well as an increase in the amount of extracellular matrix. This suggests that increased ECM synthesis plays a major role in the pathogenesis of glomerulosclerosis. Finally, the increase in mRNA coding for ECM persisted in the presence of advanced sclerosis. This unexpected finding suggests that the accumulation of matrix is an ongoing process and that late remodeling may be possible even if the lesions are advanced and appear densely sclerotic on histological sections.

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