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-IIIIM) were obtained from Biomesure 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-Aval 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 (NCI) of the mouse α1 (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.) (pGMPGI). An 850-bp XhoI genomic DNA fragment from pAZ1002 of the mouse α2(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 Apal (pGM101), PVUII (p1234), HindIII.
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-I 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(1) 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 (×1,300); (B) laminin (×1,300); (C) HSPG (×1,300); (D) type I collagen (×810).

Figure 2. ECM mRNA in the kidney. (A) α1(IV); (B) laminin B2; (C) HSPG; (D) α2(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.

Figure 3. Densitometric analysis of mRNA for ECM. Data are expressed as the mean ± SE. (□) WT mice; (■) GH mice; (a) p < 0.05.
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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Address correspondence to Toshio Doi, Renal Cell Biology Section, Building 10, Room 3N110, National Institutes of Health, Bethesda, MD 20892.

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References


