Renal Expression of Fibrotic Matrix Proteins and of
Transforming Growth Factor-β (TGF-β) Isoforms in TGF-β
Transgenic Mice

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Abstract. Renal pathology in mice that are transgenic for the
murine albumin enhancer/promoter linked to a full-length por-
cine transforming growth factor-β1 (TGF-β1) gene has been
described previously. In these mice, transgene expression is
limited to the liver and the plasma level of TGF-β is increased.
The earliest renal pathologic change is glomerulosclerosis, at 3
wk of age, and this is followed by tubulointerstitial fibrosis. In
this study, it was hypothesized that circulating TGF-β increases
renal extracellular matrix accumulation and activates
local TGF-β gene expression. Immunostaining at 5 wk re-
vealed increased amounts of collagen I and III within the
mesangium, glomerular capillary loops, and interstitium, while
the amount of collagen IV was normal. Similarly, Northern
analysis showed increased expression of mRNA encoding col-
gen I and III, as well as biglycan and decorin, while the
expression of collagen IV was unchanged. These changes
began as early as 1 wk of age, a time before the appearance of
glomerulosclerosis. To evaluate matrix degradation, colla-
genase IV activity was evaluated by gelatin zymography and an
increase in matrix metalloproteinase-2 was found. Finally, the
production of tissue inhibitors of metalloproteinase was eval-
uated. Tissue inhibitor of metalloproteinase-1 (TIMP-1) mRN
was increased 18-fold, while TIMP-2 and TIMP-3 were un-
changed. In 2-wk-old transgenic kidney, local expression of
TGF-β1, β2, and β3 protein was similar to wild-type mice. In 5-wk-old transgenic mice, TGF-β1 and β2 protein
was in increased amounts within glomeruli, and renal
TGF-β1 mRNA was increased threefold. It is concluded that
eliminated levels of circulating TGF-β1 may act on the kidney to
increase matrix protein production and decrease matrix remod-
eling. Only after glomerulosclerosis is established does local
glomerular overproduction of TGF-β become manifest.

Transforming growth factor-β1 (TGF-β1) is a pleiotropic cy-
tokine that participates in development, wound repair, and a
variety of physiologic and pathogenetic processes (1,2). In the
human kidney, local accumulation of TGF-β protein has been
implicated in several renal diseases (3–5). Investigation of
renal disease in experimental animal models has demonstrated
an increase in TGF-β mRNA expression by both intrinsic renal
cells and infiltrating cells, particularly macrophages (6–10).
Furthermore, in vivo transfection of the TGF-β1 gene (11) has
been associated with glomerulosclerosis. These findings sug-
gest that local renal production of TGF-β may mediate extra-
cellular matrix accumulation in these models. Recently, it has
become apparent that pathologically elevated plasma concen-
trations of TGF-β may also contribute to fibrotic diseases in
experimental animals with diabetes (12) and in human patients
with breast cancer (13) and with thrombotic thrombocytopenic
purpura (14). Similarly, in experimental animals, systemic
administration of TGF-β (15) has been associated with glo-
merulosclerosis.

We have recently described glomerulosclerosis in mice
transgenic for TGF-β1 under the control of the murine albumin
promoter and enhancer (16,17). These mice express the trans-
gene exclusively in the liver and have elevated circulating
levels of TGF-β1. We have noted marked glomerular disease
in these mice, with deposits of Ig and extracellular matrix
material. This model provides a unique system to evaluate the
renal effects of chronic exposure to elevated circulating concen-
trations of active TGF-β1. In the present study, we have
characterized the pattern of extracellular matrix protein accu-
mulation in the kidney of these transgenic mice and evaluated
local renal expression of TGF-β isoforms and TGF-β recep-
tors.

Materials and Methods

Transgenic Mice

Alb/TGF-β1 mice were established as described previously (16).
The transgene construct consists of the murine albumin promoter and
enhancer, a mutated porcine TGF-β1 gene in which cysteine 223 and
225 were mutated to serine to destabilize the latent TGF-β1 molecule and promote secretion of active TGF-β1, and human growth hormone 3’ untranslated sequences including the polyadenylation signal. Of multiple lines prepared from the same DNA construct, line 25 mice have the highest circulating levels of TGF-β1 at 3 wk of age and the most severe renal disease. The present investigations were carried out exclusively in mice from this line. Glomerular disease appears in 100% of mice, can be first reliably detected by light microscopy at 3 wk of age, and becomes severe by 5 wk of age (Figure 1B). Among line 25 mice, we have described two phenotypes: Mice with proteinuria at 5 wk of age (approximately 25 to 40% of mice) have a severe phenotype and die with edema and uremia by 14 wk of age, whereas mice without proteinuria have a mild phenotype and live longer, although with glomerulosclerosis. For the present studies, we have identified mild phenotype mice as those without edema at 5 wk of age and severe phenotype as those with edema at 5 wk of age.

**Immunoperoxidase Staining for Collagen**

Kidney tissue was fixed in methanol Carnoy’s solution overnight at room temperature and embedded in paraffin. Four-micrometer sections were deparaffinized and rehydrated, and endogenous peroxidase was blocked with methanolic hydrogen peroxide. The sections were digested with 1% Pronase (Proteinase E; Sigma, St. Louis, MO) in 10 mM sodium acetate, pH 7.8 for 6 min at room temperature. Biotinylated goat antibodies raised against bovine collagen I, III, and V (Southern Biotechnology Associates, Birmingham, AL) were used. These antibodies cross-react with mouse collagen and are isoform-specific when tested against bovine collagen I-VI in enzyme-linked immunosorbent assay (information supplied by the manufacturer). These antibodies were diluted to 5 μg/ml in phosphate-buffered saline and applied for 60 min at 37°C in a humidified chamber. Streptavidin-peroxidase complex (Zymed, Burlingame, CA) and the substrate amino-ethyl carbazole were used according to the manufacturer’s instructions. The sections were counterstained with hematoxylin. For collagen IV, rabbit antiserum against EHS tumor-derived collagen IV (Dr. H. Kleinman, National Institute on Dental Research [NIDR], National Institutes of Health [NIH]) was diluted 1:300 with phosphate-buffered saline, and biotinylated goat anti-mouse IgG (Zymed) was applied for 10 min at room temperature.

**Immunoperoxidase Staining for TGF-β**

For TGF-β immunostaining, formalin-fixed, paraffin-embedded tissue sections were prepared, and endogenous peroxidase was blocked with methanolic hydrogen peroxide. The tissue was digested with 1% hyaluronidase (Sigma) and blocked with 5% normal goat serum. Sections were incubated overnight at 4°C with the following rabbit polyclonal antibodies to TGF-β1 (Promega, Madison, WI), anti-TGF-β1 (LC, which is directed against residues 1 to 30 of mature TGF-β1 and which recognizes intracellular TGF-β1) (18), anti-TGF-β2 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-TGF-β3 (which is directed against residues 50 to 60 of mature TGF-β3 and recognizes intracellular TGF-β3) (19), and anti-TGF-β1/β3 (CC, which is directed against residues 1 to 30 of mature TGF-β1 and recognizes extracellular TGF-β1 and TGF-β3) (18), and normal rabbit Ig. After extensive washing, the sections were incubated with biotinylated goat anti-rabbit IgG (Zymed), and the sections were then processed using the Vector Elite kit according to the manufacturer’s directions. The intensity of staining of the glomeruli and tubules were separately scored by review of the entire tissue section, using a semiquantitative scale: 0, no staining; 0.5, trace staining; 1, light staining; 2, moderate staining; 3, intense staining.

**Northern Analysis**

Kidney tissues were snap-frozen in liquid nitrogen and stored at −80°C until use. Kidneys were homogenized in TRIZol (Life Technologies, Gaithersburg, MD), extracted with phenol-chloroform, and the RNA was precipitated with isopropanol. Total RNA (15 μg) was electrophoresed through a 1% formaldehyde-agarose gel and transferred to a Nitran membrane (Schleicher & Schuell, Keene, NH). The membrane was ultraviolet-crosslinked, baked at 80°C for 2 h, and prehybridized for 1 h with Hybrisol I (Oncor, Gaithersburg, MD). The following cDNA were used: rat TGF-β1, murine TGF-β1, and mouse TGF-β3 (all from S. W. Qian, National Cancer Institute, NIH); TGF-β receptor type I (C. H. Heldin, Uppsala, Sweden), TGF-β receptor type II (J. Massague, Memorial Sloan Kettering), mouse collagen I (B. de Crombrugghe, University of Texas), human collagen III and mouse collagen IV (p1234, Y. Yamada, NIDR, NIH), mouse decorin and mouse biglycan (S. Suzuki, Doheny Eye Institute, Los Angeles, CA), murine TIMP-1, (plasmid no. 63196, American Type Culture Collection, Rockville MD), murine TIMP-2 and murine TIMP-3 (D. R. Edwards, Calgary, Alberta), and, as a housekeeping gene, human S14 ribosomal protein (plasmid no. 59247, American Type Culture Collection). The cDNA inserts were labeled with 32P-dCTP (Amersham, Arlington Heights, IL), using the Prime It II random prime labeling kit (Stratagene, La Jolla, CA). The membranes were hybridized overnight at 42°C, and washed with PES buffer (40 mM sodium phosphate, pH 7.4, 1 mM ethylenediaminetetra-acetic acid, 0.5% sodium dodecyl sulfate) twice at room temperature for 15 min and once at 55°C for 15 min. The membranes were exposed to x-ray film and in addition the signal was quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**Gelatin Zymography of Total Kidney Extracts**

Kidney tissue was homogenized in 50 mM sodium acetate, 200 mM sodium chloride, pH 8.5, with protease inhibitors (0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin A, and 200 μM phenylmethylsulfonyl fluoride). Protein concentration was measured with a kit based on the Lowry method (BioRad, Hercules, CA). Samples with equal protein concentration were loaded onto 10% sodium dodecyl sulfate-polyacrylamide precast minigels containing 1% gelatin (Novex, Encinitas, CA). After electrophoresis, the gels were incubated in 1% Triton X-100 for 1 h at room temperature and then in a collagenase buffer (50 mM Tris, 150 mM NaCl, 10 mM CaCl2, 0.2% Brij-35, pH 7.5) for 42 h at 37°C. The gels were stained with Coomassie blue, destained, and dried. As a positive control, we used conditioned media from mouse mesangial cell cultures.

**Statistical Analyses**

Statistical analysis of TGF-β immunostaining was carried out using the Mann–Whitney U test, and Northern analysis was carried out using ANOVA with post-test comparisons using Bonferroni’s test (Instat, GraphPad, San Diego, CA). Data are reported as mean ± SD.

**Results**

**Renal Collagen Protein and mRNA Expression**

In Alb/TGF-β1 transgenic mice, glomerulosclerosis first appears at 3 wk of age and becomes severe by 5 wk of age (Figure 1B). Therefore, we chose 5 wk as the time point to characterize the nature of extracellular matrix accumulation in these mice. Kidney tissue from wild-type mice exhibited collagen I focally distributed within the interstitium, particularly around blood vessels, but absent from glomeruli (Figure 1C).
Collagen III was present in trace amounts throughout the interstitium and in Bowman's capsule, but was absent from the glomerulus (Figure 1E). Collagen IV was present within glomerular capillaries, the mesangium, and tubular basement membrane (Figure 1G).

Kidneys taken from Alb/TGF-β1 transgenic mice at 5 wk of age.
age showed abundant glomerular collagen I, located within peripheral glomerular capillary loops, the mesangium, and Bowman’s capsule (Figure 1D). There was some variability among glomeruli as to the pattern of expression. Deposits of collagen I were also focally present in the interstitium. In transgenic kidneys, collagen III expression was considerably increased within the glomerular mesangium and to a lesser extent within glomerular capillary loops (Figure 1F). There was a dramatic increase in collagen III within the interstitium, with deposits in the space between tubular basement membranes and peritubular capillaries. In transgenic kidneys, collagen IV expression was similar in intensity to that of wild-type mice, but with some increased diffuse mesangial staining (Figure 1H). Collagen V was essentially absent from normal mouse kidney, while trace amounts were present within the mesangium from diseased transgenic kidneys (data not shown).

We next evaluated collagen gene expression by Northern analysis. Collagen α1(I) mRNA declined with age in both normal and transgenic mice; however, the steady-state levels were elevated in the transgenic mice compared to normal mice by 3.0 ± 0.74-fold ($P < 0.001$) at 1 wk, by 3.0-fold at 2 wk, by 3.1 ± 0.41-fold ($P < 0.01$) at 3 wk, and by 2.6 ± 0.19-fold ($P < 0.01$) at 5 wk (Figure 2). Collagen α1(III) mRNA levels were stable with age in the normal mice; in the transgenic mice, the mRNA levels were increased compared with normal mice by 2.8 ± 0.18-fold ($P < 0.001$) at 1 wk, 2.2 ± 0.1-fold ($P < 0.05$) at 3 wk, and 1.7 ± 0.1-fold ($P = NS$) at 5 wk (Figure 3).

At 5 wk of age, there was no significant difference in the collagen (α1)I and (α1)III mRNA expression level between the transgenic animals with mild or severe phenotype. In contrast to the interstitial collagens, expression of the basement membrane component collagen (α1)IV mRNA was similar in normal and transgenic mice (Figure 4).

**Expression of Proteoglycan Genes**

The level of decorin mRNA expression declined with age in the normal mice, but increased significantly in transgenic mice compared to normal mice, by 2.1 ± 0.73-fold ($P < 0.05$) at 1 wk, by 2.7 ± 0.57-fold ($P = NS$) at 3 wk, and by 4.6 ± 0.8-fold ($P < 0.01$) at 5 wk (Figure 5). Furthermore, the expression of decorin mRNA was significantly increased in 5-wk-old Alb/TGF-β transgenic mice with severe phenotype (7.7 ± 0.06) compared to the animals with mild phenotype (3.1 ± 0.13, $P < 0.001$). Biglycan mRNA expression was relatively stable with age in normal mice, but increased significantly in transgenic mice compared to normal mice, by 2.5 ± 0.92-fold ($P = NS$) at 1 wk, by 2.4 ± 1.1-fold ($P < 0.001$) at 3 wk, and by 2.5 ± 1.2-fold ($P < 0.001$) at 5 wk (Figure 6). The expression of biglycan mRNA was significantly increased in animals with severe phenotype (3.3 ± 0.32) compared to the animals with mild phenotype (2.09 ± 0.53, $P < 0.001$).

**Expression of Proteinase and Proteinase Inhibitor Protein and mRNA**

Since TGF-β is known to alter the matrix-degrading pathways, we evaluated kidney expression of matrix-degrading enzymes, using gelatin zymography (Figure 7). As a standard, we included mouse mesangial cell supernatant which contained gelatinase activity, identified as matrix metalloproteinase-9 (MMP-9; running as a doublet at approximately 92 kD) and MMP-2 (running as a doublet at approximately 68 kD, composed of the proenzyme and a higher molecular weight complex characteristic of rodent MMP-2) (20). In the mouse kidney samples, MMP-2 appeared as a triplet, including the higher molecular weight complex, the proenzyme, and, at a slightly smaller size, the active enzyme. In the wild-type mice, the expression of MMP-2 and MMP-9 was highest at 1 wk of age and fell progressively at 3 and 5 wk of age. In the Alb/TGF-β transgenic mice, the expression of MMP-2 was similar to the normal mice at 1 and 3 wk of age. At 5 wk of age, however, MMP-2 was increased in transgenic compared to wild-type mice.

Northern analysis was insufficiently sensitive to detect
mRNA from total kidney for MMP-2 (72-kD type IV collagenase), MMP-3 (stromelysin), and MMP-9 (92-kD type IV collagenase) in both wild-type and Alb/TGF-β1 transgenic mice (data not shown). There was, however, a striking change in renal tissue inhibitor of metalloproteinase-1 (TIMP-1) mRNA expression (Figure 8). The expression of TIMP-1 mRNA, normalized to expression of S14 gene, was 1.00 ± 0.39 in wild-type mice, 2.8 ± 0.97 (P < 0.001) in mild phenotype transgenic mice, and 18.2 ± 2.75 (P < NS) at 5 wk. Collagen α1 (III) mRNA is approximately 4.8 kb.

Renal TGF-β Protein and mRNA Expression

Hepatic transgene expression, assessed by Northern blotting, is highest at 1 wk of age and declines thereafter. Similarly, plasma TGF-β1 levels are highest at the earliest time point that could be measured, 2 wk of age, and decline thereafter (16).

We therefore chose to examine TGF-β protein in kidneys at 2 wk of age, when plasma TGF-β1 levels are highest, and at 5 wk, when severe glomerulosclerosis is present. In 2-wk-old kidneys, TGF-β1 was localized with two antibodies (LC and Promega) and both demonstrated similar expression of TGF-β1 in wild-type mice and transgenic mice, predominantly in cortical tubules and to a lesser extent in glomeruli (data not shown). Staining with antibodies directed against TGF-β2 and -β3 showed similar protein expression in wild-type and transgenic mice. Transgenic kidneys immunostained with CC antibody, which recognizes both TGF-β1 and β3 present within the extracellular matrix, showed slightly but nonsignificantly more immunoreactivity within the interstitium (1.0 ± 0.0 versus 2.4 ± 0.9, P = 0.09) and no activity within the glomeruli.

At 5 wk of age, transgenic mice exhibited slightly increased TGF-β1 staining within cortical tubules when using either the LC antibody or the Promega antibody, although there was more heterogeneity among transgenic tubules (data not shown). By contrast, glomerular TGF-β1 was absent from
The expression of decorin mRNA declined with age in the normal mice, but the steady-state levels of decorin mRNA were increased in the transgenic mice, by $2.1 \pm 0.73$-fold ($P < 0.05$) at 1 wk, $2.7 \pm 0.57$-fold ($P = \text{NS}$) at 3 wk, and $4.6 \pm 0.8$-fold ($P < 0.01$) at 5 wk. Decorin mRNA size is approximately 1.3 kb.

Within glomeruli, TGF-$\beta_1$ was localized to cells consistent with parietal and visceral epithelial cells, as well as cells within the glomerular tuft that could not be conclusively identified. TGF-$\beta_2$ was present in cortical tubules in wild-type mice and Alb/TGF-$\beta_1$ transgenic mice to a similar extent (Figure 9, E and F, and Figure 10). TGF-$\beta_2$ was absent from wild-type glomeruli and present in Alb/TGF-$\beta_1$ glomeruli, in a distribution quite similar to that of TGF-$\beta_1$. TGF-$\beta_3$ was present in cortical tubules, in some cases in a basolateral distribution, to an equal extent in Alb/TGF-$\beta_1$ transgenic mice and wild-type mice. TGF-$\beta_3$ was absent from normal glomeruli and variably present in transgenic glomeruli (data not shown). Finally, the CC antibody showed increased activity in the interstitium of transgenic mice compared with wild-type mice (Figure 9, G and H, and Figure 10). Immunostaining with normal rabbit Ig gave no reaction product (data not shown).

In summary, Alb/TGF-$\beta_1$ transgenic mice at 2 wk of age had similar intracellular staining for TGF-$\beta_1$, $\beta_2$, and $\beta_3$, and slightly increased staining for extracellular TGF-$\beta_1$/$\beta_3$, whereas at 5 wk the transgenic mice exhibited increased glomerular staining for TGF-$\beta_1$ and $\beta_2$ and increased extracellular staining for TGF-$\beta_1$/$\beta_3$ within the interstitium.

Next, we investigated whether this increase in renal TGF-$\beta$ protein expression was associated with increased renal TGF-$\beta$ gene expression. At 2 wk of age, renal expression of TGF-$\beta_1$ mRNA was similar in the transgenic (0.76 $\pm$ 0.07) and wild-type mice (1.00 $\pm$ 0.14; data not shown). At 5 wk of age, however, renal TGF-$\beta_1$ expression was increased in transgenic mice (0.76 $\pm$ 0.07) and wild-type mice (1.00 $\pm$ 0.14; data not shown). At 5 wk of age, renal expression of TGF-$\beta_1$ mRNA was similar in the transgenic (0.76 $\pm$ 0.07) and wild-type mice (1.00 $\pm$ 0.14; data not shown).

In the present study, we have found evidence that elevated circulating levels of TGF-$\beta_1$ are accompanied by evidence of
a shift toward the accumulation of fibrotic matrix constituents and toward the degradation of the normal constituents of renal basement membranes and mesangial matrix. Renal expression of the interstitial collagens I and III mRNA and protein were increased, as were mRNA for biglycan and decorin. By contrast, renal expression of collagen IV and laminin \(\beta_1\) mRNA were unchanged, and collagen IV protein accumulation was only slightly altered, with more diffuse mesangial distribution.

*In vitro* studies have shown that TGF-\(\beta\) modulates the phenotype of multiple renal cell types, including mesangial cells, glomerular epithelial cells, and tubular epithelial cells. In general, TGF-\(\beta\) suppresses proliferation and increases matrix production. In mesangial cells, TGF-\(\beta\) is a bifunctional growth regulator and stimulates gene expression of collagens I and IV and fibronectin (21,22). In glomerular epithelial cells, TGF-\(\beta\) increases the production of collagen IV, laminin, and biglycan (23). In tubular epithelial cells, TGF-\(\beta\) increases collagen synthesis, primarily types I, III, and V (24).

Transgenic kidney tissue showed increased quantities of MMP-2, a proteinase that degrades collagen IV and laminin, compared to wild-type mice. Interestingly, mRNA for TIMP-1, which inhibits all MMP family members, was greatly increased. TGF-\(\beta\) has been shown in gingival fibroblasts to decrease MMP-1 (interstitial collagenase) and increase MMP-2 and TIMP-1 expression (25), which are similar changes to what we observed *in vivo*. Furthermore, TGF-\(\beta\) increases MMP-2 expression in cultured human glomerular mesangial cells (26). The net effect of a simultaneous increase in MMP-2 and TIMP-1 is difficult to determine *in vivo*. Furthermore, in the present work we have not localized the expression of these proteins. Nevertheless, it is possible that basement membrane constituents (collagen IV, laminin) are subject to proteolytic attack, which would explain the apparent mesangiolysis that occurs in some glomeruli. Although we did not assess the

![Figure 7. Gelatin zymography of type IV collagenase from mouse kidney. Paired kidney homogenates derived from two animals each, aged 1, 3, and 5 wk, and from wild-type and Alb/TGF-\(\beta\)-IV transgenic mice were subjected to gelatin zymography. As a positive control (first and last lanes), conditioned medium from mouse mesangial cultures (MC) showed matrix metalloproteinase-9 (MMP-9) activity (doublet at approximately 92 kD) and MMP-2 activity (two bands at approximately 68 kD). In both wild-type and transgenic mice, the expression of MMP-2 and MMP-9 was highest at 1 wk of age and fell progressively with age. At 5 wk of age, expression of MMP-2 was increased in the transgenic mouse kidneys. Lanes 2 and 3, normal mouse kidney at 1 wk of age; lanes 4 and 5, transgenic mouse kidney at 1 wk of age; lanes 6 and 7, normal mouse kidney at 3 wk of age; lanes 8 and 9, transgenic mouse kidney at 3 wk of age; lanes 10 and 11, normal mouse kidney at 5 wk of age; lanes 12 and 13, transgenic mouse kidney at 5 wk of age. Upper arrowhead denotes the position of the MMP-9 doublet; lower two arrowheads denote the position of the MMP-2 proenzyme and active enzyme.](image)

![Figure 8. Northern analysis of renal tissue inhibitor of metalloproteinase-1 (TIMP-1) mRNA expression at 5 wk of age. Total kidney RNA was harvested from normal (N) and Alb/TGF-\(\beta\)-1 transgenic (TG) mice with mild and severe phenotype. The expression of TIMP-1 mRNA normalized to expression of S14 gene was 1.0 ± 0.39 in the three normal animals, 2.87 ± 0.97 (\(P > 0.05\)) in three mild phenotype transgenic mice, and 18.2 ± 0.275 (\(P < 0.001\)) in three severe phenotype transgenic mice. Mouse TIMP-1 mRNA size is approximately 0.9 kb.](image)
Figure 9. Immunolocalization of TGF-β isoforms. Normal mouse cortex shows tubular staining for TGF-β1 using the LC antibody, while glomerular staining is absent (A). Alb/TGF-β1 transgenic mouse kidney of the mild phenotype shows comparable TGF-β1 staining within tubules and also shows glomerular staining (B). Similarly, normal mouse cortex shows uniform tubular staining for TGF-β1 using the Promega antibody, while glomerular staining is absent (C). Transgenic kidney of the severe phenotype shows TGF-β1 in tubular epithelial cells, within the glomerular tuft and cells located within a small extraglomerular crescent (D). Normal mouse proximal tubules exhibit strong, uniform staining for TGF-β2, while glomerular staining is absent (E). Transgenic kidney of the severe phenotype exhibits both tubular and glomerular staining; TGF-β2 appears to be localized to visceral and parietal epithelial cells, as well as possibly to other cell types (F). Normal mouse cortex shows focal perivascular staining extracellular TGF-β1/β3 detected with the CC antibody (G); transgenic mouse kidney of the severe phenotype shows increased expression of TGF-β1 focally within the interstitium (H). All tissue was obtained from 5-wk-old mice. Magnification: ×160 in A through F; ×40 in G and H.
activity of interstitial collagenase, which initiates degradation of the fibrillar collagens I and III, this enzyme is known to be suppressed by TGF-β. The combination of reduced MMP-1 and increased TIMP-1 would potently greatly increase accumulation of fibrillar collagen, which is what we observed.

These changes in matrix protein expression were accompanied by increased renal expression of TGF-β1 mRNA and protein, and TGF-β2 protein. Particularly notable was glomerular expression of TGF-β1 and -β2 proteins, which were not detected in wild types and were induced in the Alb/TGF-β1 transgenic mice. Renal expression of TGF-β receptor I and II mRNA was unchanged. In vitro, TGF-β has been shown to induce its own gene transcription and so circulating TGF-β1 might be responsible for the increased renal TGF-β1 mRNA and protein (27). On the other hand, TGF-β1 does not stimulate, and indeed may repress, transcription of the TGF-β2 and -β3 genes. The observation that the increased expression of TGF-β1 and β2 protein was detected at 5 wk but not at 2 wk, while plasma TGF-β1 levels peak at 2 wk of age in this model, suggests that circulating TGF-β1 is unlikely to be directly responsible for increased local renal expression. Instead, it appears that circulating TGF-β initiates a process of glomerular injury which, in its later stages, involves upregulation of local TGF-β expression.

Recently, it has been recognized that TGF-β may act not only as an autocrine and paracrine factor, but also as an endocrine factor. Diseases linked to altered levels of circulating TGF-β have included hepatic and pulmonary fibrosis (13), atherosclerosis (28), and diabetic nephropathy in rodents (12). Administration of 125I-labeled TGF-β1 to rats has shown that the endothelium of kidney and liver are particularly rich in TGF-β binding sites, which may explain the localization of disease in the Alb/TGF-β mice (29). Intravenous administration of TGF-β to rats for 14 d was associated, at the highest dose level, with the development of glomerulosclerosis (15). In the present transgenic mouse, we have previously shown that plasma TGF-β1 levels are elevated as early as 2 wk of age and that glomerular disease is first noted at 3 wk of age. Thus, a relatively brief period of exposure of the kidney to elevated concentrations of active TGF-β is sufficient to induce profound renal fibrosis. The findings from the present study suggest that this occurs by heightened expression of fibrotic matrix proteins and inhibition of matrix degradative pathways. Furthermore, it appears that stimulation of local TGF-β, particularly within the glomerulus, may contribute.

In conclusion, we have found that Alb/TGF-β transgenic mice with elevated circulating levels of TGF-β1 have increased deposition of fibrotic matrix constituents (collagen I and III, biglycan, and decorin), increased expression of basement membrane degrading protease MMP-2 and MMP-antagonist TIMP-1, and increased renal expression of TGF-β1 and β2. The net effect of these alterations is to induce the rapid and profound fibrosis that affects both the glomerulus and the interstitium. This model presents a powerful tool to dissect the significance and pathogenetic mechanisms induced by circulating TGF-β in the kidney.
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