Interaction of Angiotensin II and TGF-β1 in the Rat Remnant Kidney

ASAD JUNAID,* THOMAS H. HOSTETTER,† and MARK E. ROSENBERG‡
*University of Manitoba, Winnipeg, Manitoba, Canada; and †University of Minnesota, Minneapolis, Minnesota.

Abstract. An interaction between angiotensin (Ang) II and transforming growth factor (TGF)-β1 is gaining increasing recognition. Ang II has been implicated in the progression of renal disease, and TGF-β1 is a potent fibrosis-promoting cytokine. We sought to determine whether the beneficial effects of renin-angiotensin system blockade on remnant kidney function were associated with a reduction in renal TGF-β1 in this model of chronic renal failure. After subtotal renal ablation, rats fed a 40% protein diet and treated with losartan not only had a reduction in systolic BP (96 ± 8 versus 130 ± 8 mmHg, \( P < 0.05 \), losartan versus control) and proteinuria (4 ± 5 versus 23 ± 20 g/d, \( P < 0.05 \), losartan versus control), but also exhibited a reduction in renal TGF-β1 mRNA (194 ± 64 versus 411 ± 101 optical density units, \( P < 0.05 \), losartan versus control) and TGF-β1 protein levels (9.8 ± 2.5 versus 18.6 ± 5.8 ng/g of renal tissue, \( P < 0.05 \), losartan versus control). The elevation of TGF-β1 in the remnant kidney was most pronounced in the scar region (22.9 ± 13.1 versus 5.8 ± 3.7 ng/g, \( P < 0.05 \), scar versus nonscar). A combination of reserpine, hydralazine, and hydrochlorothiazide, although effective in lowering systemic BP in this model of chronic renal failure, was not associated with a reduction in proteinuria or TGF-β1. We conclude that in this model of progressive renal injury, Ang II antagonism may exert a beneficial effect in part by its negative influence on TGF-β1. (J Am Soc Nephrol 8: 1732–1738, 1997)

The renin-angiotensin system has been strongly implicated in the progression of renal injury in both experimental models and various forms of progressive renal injury in humans (1). Previously, this influence has been largely attributed to direct angiotensin (Ang) II-mediated increases in glomerular capillary pressure in experimental models and by extrapolation in humans (2). Recent in vitro data and an increasing volume of in vivo data suggest that not only is Ang II a potent vasoconstrictor, but it also may participate in the regulation and expression of potentially damaging cytokines, such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β (3,4). The remnant kidney model of progressive renal injury is characterized by localized areas of increased renal renin activity, systemic and glomerular capillary hypertension, and proteinuria (1). As disease progresses, glomerular sclerosis and tubulointerstitial fibrosis figure prominently in the histopathological profile of this model (5,6). Intensive study of the forces governing the composition and dynamics of extracellular matrix in response to disease has provided evidence for a pre-eminent role for TGF-β1 in these processes (7). By using the remnant kidney model, we chose to study whether the beneficial effect of renin-angiotensin system blockade on the progression of renal injury was associated with an alteration of renal TGF-β1. We purposely examined an early phase of this model to assess the more primary processes and in recognition of the well established efficacy of such blockade on the chronic course of the model. As expected, Ang II antagonism with losartan was associated with a reduction in proteinuria and BP. To control for the antihypertensive effects, a combination of reserpine, hydralazine, and hydrochlorothiazide was compared with losartan; although effective in lowering systemic BP, as shown previously, this combination of agents had no appreciable effect on proteinuria.

Materials and Methods

Animal Preparation and Study Design

Male Sprague-Dawley rats weighing 250 to 275 g were placed on an ad libitum, 6% protein diet for 3 d before surgery. On the day of surgery, the right kidney was removed under pentobarbital (65 mg/kg, intraperitoneally) anesthesia, and a branch of the left renal artery was ligated, leaving each animal with approximately one-third of its original renal mass. The rats were maintained on a 6% protein diet for an additional 3 d to minimize compensatory renal growth before initiation of the interventions. Serum creatinine determination was performed on a tail vein blood sample with a creatinine analyzer (Beckman Instruments, Brea, CA), and the animals were stratified by serum creatinine values, pair-fed a 40% protein diet, and subjected to the manipulations described below. The duration of the experimental protocols was 8 d (experiment IV animals were followed for 14 d). The following experimental protocols were evaluated.

Experiment I

Group \( I_A \) \((n = 6)\) received losartan (180 mg/L) in their drinking water, and group \( I_B \) \((n = 6)\) received water alone. Before sacrifice, a 24-h urine collection for determination of protein excretion by the Coomassie blue method (Bio-Rad Laboratories, Richmond, CA) was performed. On the day of sacrifice, conscious systolic BP was mea-
sured under passive restraint by the tail-cuff method, using a physiograph (model MK-IV, Narco Bio-systems, Houston, TX). In addition, these animals had determinations of serum creatinine from a tail vein blood sample and were weighed before sacrifice. Remnant kidneys were weighed after removal, and the renal capsules were removed before slicing the tissue with a parallel array of razor blades (interblade distance, 1 mm), followed by immersion in Zenker’s fixative. The fixed tissue slices were embedded in paraffin and subjected to morphometric analysis.

**Experiments II and III**

Group II_A (losartan, n = 4), group II_B (vehicle, n = 4), group III_A (losartan, n = 7), group III_B (water, n = 7) and group III_C (sham, water, n = 7) animals were prepared as above. At the end of the study period, whole kidney RNA was analyzed for TGF-β1 mRNA from group II_A and II_B animals. Remnant kidneys from groups III_A and III_B were removed, divided into scar and nonscar portions, and immediately frozen in liquid nitrogen, followed by storage at -70°C until analysis for TGF-β1 messenger RNA. Whole kidney mRNA for TGF-β1 was determined for group III_C.

**Experiment IV**

Group IV_A (losartan, n = 4) and group IV_B (water, n = 4) animals were subjected to the above protocol, with the exception that after removal, the right native kidney was placed in liquid nitrogen at the time of renal ablative surgery and then stored at -70°C until analysis. After sacrifice, remnant kidneys were divided coronally into two halves, each containing scar and nonscar tissue, and one of these portions was frozen until analysis. The other half was further divided into scar and nonscar portions before freezing. Remnant kidney tissue sections from groups IV_A and IV_B were compared with each other and with six of the previously frozen right kidneys for TGF-β1 content as measured by antibody sandwich enzyme-linked immunosorbent assay (ELISA).

**Experiment V**

Three groups of four rats each underwent subtotal renal ablation, stratification, and were pair-fed in the manner described above. Groups V_A, V_B, and V_C received, respectively, losartan (180 mg/L); a combination (“triple therapy”) of reserpine (3 mg/L), hydralazine (40 mg/L), and hydrochlorothiazide (13 mg/L) in drinking water; and water alone. Before sacrifice at the end of the 14 observation period, urinary protein excretion, systolic BP, and serum creatinine determinations were done. Remnant kidney tissue was analyzed for TGF-β1 content by ELISA.

**Composition of Diets**

Diets were obtained from Teklad Corp. and consisted of the following [in g/kg]: (1) 6% protein: casein [69], corn starch [200], sucrose [572], and corn oil [54]; and (2) 40% protein: casein [460], corn starch [200], sucrose [232], and corn oil [50]. In addition, 8.8 g of NaCl per kilogram of chow (1%) was added to avoid hypotension during losartan administration.

**Morphometry**

Morphometry was performed in a blinded manner on hematoxylin and eosin-stained sections of paraffin-embedded tissue from group I animals. Analysis was based on the method of point counting as described by Nath et al. (6). Grids drawn on transparency film containing a tessellation of course and fine points were used (8). The number of coarse points counted was multiplied by the inverse of the ratio of coarse-to-fine points to obtain the number of fine points falling on the whole kidney profile. A projection microscope (Nikon Inc., Garden City, NY) was used to project the slide of kidney tissue onto a screen. The grid was placed in the center of the screen, and the slide was systematically scanned to obtain counts of 100 profiles/kidney.

**Measurement of Mean Glomerular Volume.** A grid containing a tessellation of points 0.02 mm apart was used. The mean glomerular volume was defined as follows:

\[
MGV = \left( \frac{P \times A}{2} \right) \times \frac{\beta}{K}
\]

where \(P\) is the average number of points per profile, \(A\) is the area in square micrometers represented by each point, \(\beta\) is 1.38 and represents a correction factor that assumes glomeruli are spherical, and \(K\) is 1.01 and represents a correction factor that assumes the variation in glomerular volume has a coefficient of variation of 10%.

**Measurement of Percent Tubular Epithelial Volume.** Grid tessellations were 0.15 mm apart. The course-to-fine point ratio was 1/2. Percent tubular epithelial volume was equal to the sum of fine points falling on tubular epithelial cells divided by the sum of fine points falling on whole kidney profile.

**Evaluation of Glomerular Pathology**

Glomeruli were evaluated by light microscopy for degree of mesangial cellularity and sclerosis, which were defined as a clustering of three or more mesangial nuclei and capillary loop closures.

**Antibody Sandwich ELISA**

Tissue that had been kept at -70°C was homogenized in acid-ethanol solution at 4°C and extracted overnight by gentle rocking at 4°C. After centrifugation, supernatants were extensively dialyzed (3 x 100 volumes) against 4 mM HCl, and Triton X-100 was added to a concentration of 1%. Samples were further clarified by centrifugation.

ELISA was performed by the method of Danielpour (9). Wells were coated with either mouse monoclonal anti-TGF-β antibody (recognizes TGF-β1 and -2, Genzyme Inc., Cambridge, MA) or control mouse IgG1 (Chemicon International, Temecula, CA), washed with phosphate-buffered saline containing 0.05% Tween 20, blocked with 1% crystalline bovine serum albumin in Tris-buffered saline, and washed again before the addition of samples. Concentrations of recombinant human TGF-β1 (R&D Systems, Minneapolis, MN) in wells used to generate the standard curve (triplicate) were 100, 50, 25, 12.5, and 6.25 pmol. Samples were incubated for 1 h at room temperature, washed, and incubated with chicken anti-TGF-β1 antibody (<5% cross-reactivity with TGF-β2 and -3; R&D Systems) for 1 h at room temperature. Wells were washed again before addition of and incubation with phosphatase-linked goat anti-chicken antibody for 1 h. Plates were washed and incubated overnight at 4°C with phosphatase substrate in ethanolamine buffer. The difference in optical density between 405 and 450 nm was measured by a Vmax microplate reader (Molecular Devices, Menlo Park, CA). Unknown TGF-β1 concentrations were determined by a four-parameter regression equation with Molecular Device’s Softmax program, which accounted for background absorbance (20 to 30%) from the wells coated with mouse IgG1. This assay measured total TGF-β1 due to activation of latent TGF-β1 during the acid-ethanol extraction step.
RNA Isolation and Northern Hybridization

RNA isolation was performed by the method of Chomczynski and Sacchi (10). After extraction, total RNA was dissolved in sterile water and the concentration was determined by absorbance readings at 260 nm. Twenty micrograms per lane were loaded onto a 1% agarose gel containing 20 mM 4-morpholinepropanesulfonic acid, 1 mM ethylenediamine tetra-acetic acid, 5 mM sodium acetate, pH 7.0, and 2.2 M formaldehyde. After separation by electrophoresis, RNA was transferred to nylon membranes (Duralon UV, Stratagene, La Jolla, CA) and fixed by ultraviolet cross-linking (Stratalinker, Stratagene). Prehybridization was performed at 60°C for 4 h in 5X SSC, 5X Denhardt's reagent, 50 mM Tris-hydrochloride, pH 7.5, 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate (SDS), 200 μg/ml denatured salmon testes DNA, and 100 μg/ml yeast transfer RNA.

Complementary DNA (cDNA) probe for rat TGF-β1 (985-bp fragment corresponding to major coding region of rat TGF-beta 1 precursor) was generously provided by Su Wen Qian (Laboratory of Chemoprevention, National Institutes of Health, Bethesda, MD). This probe was labeled with 32P by the random-priming method (Promega, Madison, WI). After hybridization at 42°C for 16 to 18 h in buffer containing 50% deionized formamide, 1% SDS, 1X Denhardt's reagent, 50 mM Tris-hydrochloride, pH 7.5, 0.1% sodium pyrophosphate, 1% SDS, 100 μg/ml salmon testes DNA, and 100 μg/ml yeast tRNA, and washing in 2X SSC and 0.1% SDS, membranes were subjected to autoradiography (Kodak XAR-5 film). Hybridized TGF-β1 mRNA was quantified by computer-assisted video densitometry, with the density of the 18S RNA (quantified from intensity of ethidium bromide staining) serving as a control for variations in loading. All values are reported in optical density units (O.D.) factored for variations in loading of total RNA.

Statistical Analyses

All results are expressed as means ± SD. Unpaired t test with Bonferroni correction for multiple comparisons was used for the statistical comparisons between groups, and results were considered significant at the P < 0.05 level.

Results

Experiment I

Dietary modifications and losartan administration were well tolerated and reflected by lack of a significant difference in food intake or body weight between the losartan and vehicle-treated groups. As expected, losartan-treated animals had a lower systolic BP compared with vehicle-treated rats on the day of sacrifice. At this early stage of renal injury, a significant difference in serum creatinine levels was not apparent, with both groups averaging 0.6 ± 0.1 mg/dl. However, losartan administration was associated with a reduction in urinary protein excretion rate (Table 1).

Lack of a significant difference in kidney weight was in agreement with the morphometric findings. Specifically, there were no significant differences in mean glomerular volume, percent cortical tubular epithelial volume, or percent cortical interstitial volume (Table 1). In addition, the degree of glomerular sclerosis was not affected by Ang II blockade in this early period after subtotal renal ablation (5 ± 1% versus 4 ± 1%, vehicle versus losartan, P = NS).

Experiments II and III

Losartan administration was associated with a reduction in remnant kidney TGF-β1 mRNA compared with vehicle-treated animals (Figure 1). To approximate the relative contribution of scar and nonscar areas to remnant kidney TGF-β1 mRNA, remnant kidney tissue from groups IIIA and IIIB was divided into scar and nonscar portions, and mRNA for TGF-β1 from these sections was factored for the weight of these sections and expressed as optical density (O.D.) units per gram of tissue. Although not statistically different, in comparison to sham-operated animals, TGF-β1 mRNA levels tended to be higher in the scar region of vehicle-treated rats (Table 2). Losartan administration resulted in a reduction in TGF-β1 mRNA in both the scarred and unscarred renal parenchyma. It is of interest that therapy with losartan resulted in a significant difference between scar and nonscar regions of the remnant kidney and tended to suppress nonscar TGF-β1 mRNA levels below those of sham-operated animals.

Experiment IV

There was a significant increase in renal TGF-β1 content in the left remnant kidney compared with the right kidney (18.6 ± 5.8 versus 6.7 ± 3.3 ng/g, P < 0.01), which had been removed at the time of subtotal renal ablation (Figure 2). The rise in renal TGF-β1 in the remnant kidney was attenuated by losartan compared with administration of vehicle (9.8 ± 2.5 versus 18.6 ± 5.8 ng/g, P < 0.05) and was not significantly different from the normal right kidney (6.7 ± 3.3 ng/g). In addition, the scar region of the remnant kidney had a much higher TGF-β1 content than the nonscar portion (22.9 ± 13.1 versus 5.8 ± 3.7 ng/g, P < 0.05).

Table 1. Physiologic and morphometric profiles of losartan-treated (group 1A) versus vehicle-treated (group 1B) animals

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>BP (mmHg)</th>
<th>Uprot V (mg/24 h)</th>
<th>KW (g)</th>
<th>Vp (×10⁶ μm²)</th>
<th>VpTE (%)</th>
<th>VpI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A (n = 6)</td>
<td>262 ± 10</td>
<td>96 ± 8b</td>
<td>4 ± 5b</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>73 ± 8</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>1B (n = 6)</td>
<td>255 ± 8</td>
<td>130 ± 8</td>
<td>23 ± 20</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>70 ± 6</td>
<td>12 ± 1</td>
</tr>
</tbody>
</table>

* BW, body weight; BP, conscious systolic BP; Uprot V, 24-h urinary protein excretion; KW, remnant kidney weight; Vp, mean glomerular volume; VpTE, percent cortical tubular epithelial volume; VpI, percent cortical interstitial volume. Values are expressed as mean ± SD.

b P < 0.05 versus vehicle-treated animals.
Transforming growth factor (TGF)-β1 mRNA from remnant kidneys of group IIₐ (losartan, n = 4) and group IIₐ (vehicle, n = 4). Values are expressed as mean ± SD. *P < 0.05 versus vehicle.

**Experiment V**

There were no significant differences in body weight, food intake, or remnant kidney weight at the end of the 2-wk study period among rats administered losartan, triple therapy, or vehicle alone (data not shown). Similar to the results of experiment I, there was no significant difference in serum creatinine values between the groups 14 d after subtotal renal ablation (Table 3). Although statistically significant differences in systolic BP were not noted between the losartan and triple therapy groups, the losartan-treated animals tended to have a lower systolic BP. Losartan-treated animals had a reduction in urinary protein excretion when compared with vehicle-treated animals. This reduction in proteinuria was associated with a reduction in remnant kidney tissue TGF-β1 (Table 3). Thus, lowering of systolic BP in the face of reductions in renal mass without Ang II antagonism is not associated with an improvement in proteinuria and does not reduce renal TGF-β1.

**Discussion**

Progressive renal injury is characterized by systemic hypertension, proteinuria, and eventual glomerulosclerosis and interstitial fibrosis. The remnant kidney model of chronic renal failure exhibits an early onset of proteinuria followed by structural deterioration in both the glomerular and interstitial compartments as disease progresses. Recent investigations into the mechanisms of progressive renal fibrosis have identified TGF-β1 as a potent mediator of this process (7). In a chronic model of anti-thymocyte, serum-induced glomerulonephritis, Yamamoto et al. found that TGF-β1 expression closely correlated with the degree of proteinuria and glomerular, as well as interstitial, injury (11). Isaka et al. have shown that gene transfer of TGF-β1 into the kidneys of normal rats results in the development of glomerulosclerosis (12). Our finding of an elevation of TGF-β1 in the remnant kidney suggests that this cytokine may also participate in the progression of injury in this model.

The remnant kidney model is characterized by altered sites of renin activity, and dietary protein serves to accelerate its progression, in part by further enhancing renin-angiotensin system activity (13). In this study, we successfully accelerated the early phase of the remnant kidney model by high-protein feeding and found that before the presence of gross structural alterations, glomerular functional integrity was compromised, as evidenced by the presence of a significant degree of proteinuria in vehicle-treated rats. In addition, systemic hypertension was manifest 11 d after subtotal renal ablation. Losartan administration reduced systolic BP and proteinuria in this early phase of injury.

Although the beneficial effects of interruption of the renin-angiotensin system in the progression of renal injury have been directly attributed to reductions in glomerular capillary pressure, recent evidence suggests that glomerular capillary pressure may also influence the expression of cytokines, which have an impact on structural modifications within the kidney as renal failure progresses (14). Shankland et al. found that an increase in glomerular capillary pressure in uninephrectomized, spontaneously hypertensive rats was associated with an elevation of renal TGF-β1 mRNA expression (15). Indirect support for a relationship between glomerular capillary pressure and TGF-β1 was provided by the finding that increases in fluid shear stress promoted elaboration of active TGF-β1 by vascular endothelial cells (16). Also, cyclic stretching of mesangial cells in culture, which simulates the influence of glomerular hypertension on these cells, increases TGF-β1 and β3 mRNA expression and protein, and the enhancement of these TGF-β correlates with the degree of distending force that is applied (17). In addition to its effects on hemodynamic parameters, renin-angiotensin system blockade may also directly influence the expression of TGF-β1. When rats with deoxycorticosterone acetate-induced hypertension were administered TGF-β1...
an Ang II antagonist, renal injury and proteinuria were diminished, despite the persistence of systemic hypertension (18). Our finding of a lack of reduction in remnant kidney TGF-β1 mRNA or protein with nonspecific control of systemic hypertension by a triple drug regimen supports the notion that alterations in glomerular capillary pressure, renal Ang II activity, or both, are responsible for elevations of TGF-β1 in the remnant kidney. It is possible that the tendency toward a lower systolic BP in losartan-treated rats compared with those that received triple therapy may have accounted, in part, for the lack of the latter intervention’s effect on remnant kidney TGF-β1. Whether a critical threshold for systemic BP is required to increase remnant kidney TGF-β1 remains to be determined. However, the persistence of a similar degree of proteinuria despite significant reductions in systemic BP of the triple therapy group compared with vehicle-treated rats is in keeping with previous studies that have reported on this model and make this possibility less likely (14,19).

It has been postulated that proteinuria itself may enhance tubulointerstitial TGF-β1 elaboration and that renal interstitial cells, including infiltrating macrophages, may be responsible for this elevation (20,21). The possibility that decreased mRNA expression and protein levels for TGF-β1 with Ang II antagonism may have been related to reductions in the glomerular filtration and subsequent delivery of proteins to the tubulointerstitium cannot be excluded by our findings. It is also possible that TGF-β1, which has been elaborated by the glomerular compartment, may filter to the tubulointerstitium and be activated in this region. Dickson and colleagues have recently shown that exogenously administered TGF-β1 localizes to the glomerulus and is subsequently filtered into the proximal tubule (22). In addition, the cell types responsible for enhanced TGF-β1 production in the remnant kidney remain to be evaluated. Candidates for this role include glomerular endothelial, mesangial, and infiltrating macrophage cells. Using in situ reverse transcription-PCR, Lee et al. found increased RNA staining for TGF-β1 in glomerular endothelial cells shortly after subtotal renal ablation (23). Mesangial cells have been shown to elaborate TGF-β1 after exposure to Ang II in culture (4). Other disease models have characterized infiltrating macrophages as the cells responsible for TGF-β1 elaboration in the kidney as fibrosis proceeds (11). It is of interest that at the time point of analysis in this study (11 d post subtotal renal ablation), marked macrophage infiltration into the remnant kidney has not occurred, suggesting that resident, rather than infiltrating, cells may be the source of TGF-β1 (24).

TGF-β1 inhibits the proliferation of glomerular endothelial and epithelial cells in vitro, but its effect on mesangial cells appears to be more complex and seems to depend on the degree of confluence (25). Ang II promotes growth of proximal tubular cells and may have this effect through TGF-β1. Wolf et al. have shown that the hypertrophic response of cultured murine proximal tubular cells to Ang II is inhibited by antagonizing TGF-β (26). Subtotal renal ablation is associated with compensatory hypertrophy of the remnant kidney. However, we found no significant difference in mean glomerular volume or percent cortical tubular epithelial cell volume 11 d after subtotal renal ablation between losartan- and vehicle-treated animals. When others examined TGF-β in the uninephrectomy model, they noted a decrease in glomerular TGF-β1 mRNA, but not protein levels in the remaining kidney (27). Kanda et al. described a gradual decrease in TGF-β1 protein in the remaining kidney; however, this was associated with increased immunostaining for TGF-β in proximal tubules (28). Those authors postulated that TGF-β may function as a “brake” for renal growth after reductions in functioning renal mass. Similarly, Walton et al. noted an increase in TGF-β1 mRNA and a decrease in epidermal growth factor mRNA transcripts in obstructed kidneys, and speculated that TGF-β1 may facilitate apoptosis in this model (29).

Our finding of suppression of TGF-β1 mRNA levels in the nonscar portion of remnant kidneys treated with losartan to values below those for sham-operated rats suggests that AT1

**Table 3. Data from experiment V**

<table>
<thead>
<tr>
<th>Group</th>
<th>BP (mmHg)</th>
<th>$U_{\text{proteins}}$ (mg/d)</th>
<th>Cr (mg/dl)</th>
<th>TGF-β1 (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losartan (n = 5)</td>
<td>104 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 ± 0.01</td>
<td>13 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triple therapy (n = 5)</td>
<td>123 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88 ± 8</td>
<td>0.6 ± 0.01</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>Vehicle (n = 5)</td>
<td>172 ± 25</td>
<td>78 ± 6</td>
<td>0.7 ± 0.01</td>
<td>25 ± 5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Cr, serum creatinine. Other abbreviations as in Tables 1 and 2. Values are expressed as mean ± SD.

<sup>b</sup>P < 0.05 versus vehicle-treated animals.
receptor blockade may reduce the stimuli for TGF-β1 production in this region. The finding of lack of a decrease in TGF-β1 protein levels below those of sham-operated levels in the nonscar portion of losartan-treated animals may be partially accounted for by the fact that total (latent and active) TGF-β1 was measured. It is possible that the level of activated TGF-β1 in this region may have actually decreased. The finding of lack of elevation of TGF-β1 in the nonscar portion of the remnant kidney may in part reflect that the point of evaluation was relatively early in the course of renal failure for this model. Whether this region exhibits increases in TGF-β1 as disease progresses remains to be evaluated. Given a lack of difference in the morphometric parameters evaluated, it also remains to be determined whether AT1 blockade in the remnant kidney is associated with an increase in renal hyperplasia relative to hypertrophy. Sharma et al. noted that anti-TGF-β antibody treatment of mice with streptozotocin-induced diabetes resulted in a significant decrease in the wet weight of kidneys 9 d after the induction of diabetes (30). However, the influence of this intervention on other growth factors that may play a role in renal growth was not evaluated in this study. TGF-β has been shown to enhance the expression of PDGF B-chain and its receptor in rat mesangial cells in culture (31). Interestingly, pretreatment of these cells with anti-PDGF BB antibody was not able to diminish the enhanced DNA synthesis induced by TGF-β1, but suramin, which inhibits the interaction of PDGF with its receptors in intracellular compartments, was able to abrogate mitogenic responses to TGF-β1. Given these findings and the lack of lessening of compensatory growth despite reductions in remnant kidney TGF-β1 levels as evaluated by the present study, it is likely that remnant kidney growth is not solely driven by TGF-β1, but involves other factors, such as PDGF (24,32).

In this study, we noted that the scar region of the remnant kidney possess more TGF-β1 than zones farther from the infarct region. Correa-Rotter et al. have shown previously that the scar-adjacent area of the remnant kidney has an increased renin content, suggesting enhanced renin-angiotensin system activity in this location (33). Meyer and colleagues have proposed that in this model, glomeruli may be the source of enhanced renin production (34). This heightened level of renin activity could lead to elaboration of Ang II in surrounding glomerular structures (14). It is possible that the enhanced production of Ang II in this region may be at least in part responsible for the increase in TGF-β1, which we localized to this region in this study. Our findings are compatible with the hypothesis that Ang II enhances TGF-β1 elaboration by injured or ischemic glomeruli, or both. This TGF-β1 is filtered to the tubulointerstitial compartment, where it promotes fibrosis of the tubulointerstitium and peritubular capillaries of uninjured glomeruli, and by these actions may lead to failure of filtration in these otherwise nonischemic glomeruli. Our finding that losartan not only reduced TGF-β1 levels in the infarcted region, but also in the noninfarcted region, is in keeping with this hypothesis.

Aldosterone levels are elevated in the remnant kidney model and lowered by pharmacologic blockade with enalapril and losartan (35). This hormone appears to contribute to renal injury, and preliminary data suggest that it provokes, directly or indirectly, renal TGF-β1 message accumulation (36). Because Ang II can directly stimulate TGF-β1 synthesis by cells in vitro, the possibility arises that both of these elements of the renin cascade, aldosterone and Ang II, are contributors to damage, at least in part, through the agency of TGF-β1 (7).

In summary, Ang II antagonism with losartan is associated with a reduction in proteinuria and TGF-β1 in the remnant kidney. These effects are independent of the improvement in systemic hypertension associated with this maneuver. The influence of renin-angiotensin system interruption on remnant kidney TGF-β1 appears to be most pronounced in the scar and scar-adjacent areas. Additional studies will serve to further define this interaction in this model of progressive renal injury.

Acknowledgments
We thank Dr. D. Danielpour for his advice regarding the ELISA for TGF-β1. This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-31437, DK-13083, and DK-43075. Dr. Junaid was supported by grants from the National Kidney Foundation Fellowship and Kidney Foundation of Canada (Manitoba).

References


