Increased Glomerular Capillary Pressure Alters Glomerular Cytokine Expression

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Abstract Increased glomerular capillary hydrostatic pressure (PGC) is an important hemodynamic determinant of glomerular injury, but the molecular events responsible for this association are poorly understood. PGC is normal in spontaneously hypertensive rats (SHR), but uninephrectomy leads to an increase in PGC and accelerated glomerulosclerosis. Since recent studies have implicated transforming growth factor-\(\beta_1\) (TGF-\(\beta_1\)) and platelet-derived growth factor (PDGF) in the pathogenesis of glomerulosclerosis, we first sought to determine if uninephrectomy increased mRNA levels for TGF-\(\beta_1\) and PDGF in glomeruli of SHR. Since treatment with the angiotensin-converting enzyme (ACE) inhibitor enalapril lowers PGC and prevents glomerulosclerosis in uninephrectomized SHR, we also sought to determine if ACE inhibitor lowered mRNA levels for TGF-\(\beta_1\) and PDGF in the glomeruli of uninephrectomized SHR. PGC increased from 53±1 to 64±1 mm Hg 1 week after uninephrectomy in SHR (P<.05). The increase in PGC was associated with a sixfold rise in mRNA levels for TGF-\(\beta_1\) and a twofold rise in mRNA levels for PDGF in glomeruli. mRNA levels for PDGF returned to normal 2 weeks after nephrectomy, but the increase in mRNA levels for TGF-\(\beta_1\) was sustained. An increase in TGF-\(\beta_1\) immunostaining was detectable in glomeruli 4 weeks after nephrectomy. Treatment with ACE inhibitor normalized PGC (51±1 mm Hg) and prevented the rise in glomerular mRNA levels for TGF-\(\beta_1\) and PDGF. We conclude that an acute increase in PGC leads to increased TGF-\(\beta_1\) and PDGF expression in the glomerulus, thus linking changes in PGC to cytokine gene expression. (Circ Res. 1994;75:844-853.)

Key Words • glomerulus • transforming growth factor • platelet-derived growth factor

Micro puncture studies of renal-ablated rats and rats with experimental diabetes mellitus have shown that an increased single-nephron glomerular filtration rate is associated with progressive glomerular injury.\(^1,3\) The increase in single-nephron glomerular filtration is due to an increase in single-nephron plasma flow and an increase in transcapillary hydraulic pressure gradient (\(\Delta P\)). Further studies showed that treatment with the angiotensin-converting enzyme inhibitor enalapril normalized \(\Delta P\) and prevented glomerular injury in renal-ablated rats\(^4,5\) and diabetic rats\(^6\) while maintaining single-nephron plasma flow rate and hyperfiltration. Together, these observations led to the suggestion that an increase in \(\Delta P\) was the critical hemodynamic determinant of glomerular injury, but the molecular mechanisms responsible for this association have not been fully elucidated.

The cytokines transforming growth factor-\(\beta_1\) (TGF-\(\beta_1\)) and platelet-derived growth factor (PDGF) have been implicated in the development of glomerular injury.\(^7,8\) TGF-\(\beta_1\) increases synthesis of extracellular matrix proteins, including collagen, laminin, and fibronectin,\(^9,13\) by a variety of cells, and in vivo studies by Border and colleagues\(^14,15\) have implicated TGF-\(\beta_1\) in the pathogenesis of glomerular injury in anti-thyl.1 glomerulonephritis in the rat. PDGF expression transiently increases in the glomeruli of rats after renal ablation, and the increase precedes the development of glomerulosclerosis.\(^16\) Taken together, these studies suggest that changes in cytokine expression may be important early events in the pathogenesis of immune and nonimmune glomerular injury.

Uninephrectomy in the spontaneously hypertensive rat (SHR) leads to the development of accelerated glomerulosclerosis in the remaining kidney, and micro puncture studies by Dworkin and colleagues\(^4,17,19\) have shown that an increase in \(\Delta P\) or glomerular capillary hypertension precedes glomerular injury. Treatment with the angiotensin-converting enzyme inhibitor enalapril lowers glomerular capillary pressure and prevents the development of glomerulosclerosis.\(^3\) Accordingly, we first sought to determine whether acute glomerular capillary hypertension was associated with an increase in TGF-\(\beta_1\) and PDGF expression in the glomeruli of uninephrectomized SHR. To link changes in TGF-\(\beta_1\) and PDGF expression to the subsequent development of injury, we further sought to determine if enalapril treatment would alter TGF-\(\beta_1\) and PDGF expression in the glomeruli of uninephrectomized SHR.

Materials and Methods

Animal Model

Twelve to 14-week-old male SHR were used in all of the experimental protocols (Harlan Bioproducts for Science, Inc, Indianapolis, Ind). Awake systolic blood pressure (SBP) was measured by the tail-cuff method, and SHR were randomly assigned to undergo either a right nephrectomy or a sham nephrectomy under Brielatal anesthesia (50 mg/kg IP).

For Northern blot analysis, nephrectomized SHR (SHR-Unx) were divided into two groups. A first group (n=4) was anesthetized with Brielatal (50 mg/kg) 1 week after uninephrectomy, and the kidneys were rapidly removed and pooled for glomerular isolation and extraction of RNA. A second group of nephrectomized SHR (n=4) was anesthetized with Brielatal...
(50 mg/kg) 2 weeks after uninephrectomy, and the kidneys were removed and pooled for glomerular isolation and extraction of RNA. A third group of sham-operated SHR (n=4) served as the control group. All animals had unrestricted access to water and chow (Purina rat chow).

For semiquantitative reverse transcriptase (RT)-polymerase chain reaction (PCR) analysis, separate groups of SHR-Unix were divided into three groups. A first group (n=5) received no treatment. One week after uninephrectomy, the kidneys were removed and pooled for glomerular isolation and extraction of RNA. A second group of SHR-Unix (n=5) received the angiotensin-converting enzyme inhibitor enalapril (75 mg/L) in the drinking water (Merck Sharp & Dohme). One week after uninephrectomy, the kidneys were removed and pooled for glomerular isolation and extraction of RNA. A third group (n=3) also received no treatment. Four weeks after uninephrectomy, the kidneys were rapidly removed and pooled for glomerular isolation and extraction of RNA. Renal cortical tissue was also snap-frozen from this group for immunohistochemical analysis (n=3). A fourth group of sham-operated SHR (n=5) served as the control group. Renal cortical tissue from this group was also snap-frozen for immunohistochemical analysis (n=3). All animals had unrestricted access to water and chow (Purina rat chow).

Micropuncture studies were performed in sham-operated SHR, untreated SHR-Unix, and enalapril-treated SHR-Unix 1 week after surgery (n=6).

Micropuncture Studies
Rats were anesthetized with Inactin (100 mg/kg IP) and placed on a temperature-regulated micropuncture table. PE-50 tubing was inserted into the left femoral artery and used to monitor mean arterial pressure and to obtain arterial blood samples. The mean arterial pressure was monitored continuously with an electronic transducer connected to a dual-channel direct-writing recorder. After tracheostomy, PE-50 catheters were introduced into the right and left internal jugular veins for infusion of rat plasma, saline, and inulin. A PE-10 catheter was inserted into the left ureter. After insertion of the jugular catheters, 1% body weight plasma was infused over 35 to 45 minutes, followed by a plasma infusion of 0.50 mL/h. Saline was infused at a rate of 2.0 mL/h.

A continuous-recording servo-null micropipette transducer system (model V, Instrumentation for Physiology and Medicine) was used to obtain time-averaged hydraulic pressures in surface proximal tubules after blockade with an oil droplet and free-flowing proximal tubules. Hydraulic output from the servo-null system was transmitted through the second channel of the direct-writing recorder by means of a pressure transducer.

Glomerular Isolation
Rats were anesthetized with Bretil (50 mg/kg IP), and the kidneys were rapidly removed. Perirenal fat was trimmed off, the capsule was removed, and the cortex was separated from the medulla. The cortical tissue was placed in ice-cold phosphate-buffered saline (PBS) buffer (pH 7.4) and cut into 2-mm³ pieces, and glomeruli were isolated by the technique of differential sieving. The tissue was first passed through a 250-μm sieve and resuspended in ice-cold PBS before centrifugation at 2000 rpm for 10 minutes at 4°C. The pellet was resuspended in ice-cold PBS (pH 7.4) and drawn up through a 20-gauge needle, discharged three times, and then centrifuged at 2000 rpm for 10 minutes at 4°C. The tissue pellet was resuspended in ice-cold PBS (pH 7.4) and passed through a 106-μm sieve and then a 72-μm sieve. Glomeruli were collected on the 75-μm screen and finally resuspended in ice-cold PBS (pH 7.4). All solutions were treated with diethylpyrocarbonate (DEPC). The purity of the final suspension was determined by light microscopic examination. On average, there were fewer than five tubular fragments per 100 glomeruli. These suspensions of glomeruli were then used for RNA isolation.

RNA Isolation
Total RNA from both the glomerular and renal cortical tissue was extracted by the single-step method of Chomczynski and Sacchi.21 Two milliliters of a solution containing 4 mol/L guanidinium isothiocyanate and 0.1 mol/L 2-mercaptoethanol was added to the glomerular suspension and vortexed, and 0.2 mL 2 mol/L sodium acetate, 2.0 mL of DEPC-treated phenol, and 0.4 mL of chloroform were then added. The solution was placed on ice for 15 minutes and then centrifuged at 8000 rpm for 20 minutes. An equal volume of isopropanol was added to the aqueous phase and precipitated at −20°C for 60 minutes. Total RNA was pelleted by centrifugation at 8000 rpm for 20 minutes, resuspended in 300 μL of a solution containing 4 mol/L guanidinium isothiocyanate, 0.1 mol/L 2-mercaptoethanol, and 300 μL isopropanol, and again precipitated at −20°C for 60 minutes. After centrifugation, the RNA pellet was resuspended in 300 μL cold 70% ethanol and stored in DEPC-treated water at −70°C. Purity and concentration were determined by measuring the optical densities at 260 and 280 nm before use. The ratio of the optical densities at 260 nm and 280 nm ranged from 1.75 to 1.95.

Northern Blot Analysis
Twenty micrograms of total RNA was separated by electrophoresis on 1% agarose formaldehyde gels containing ethidium bromide, transferred to a nylon filter (Hybond N), and cross-linked by UV illumination. The cDNA probes used were rat TGF-β1 (a generous gift from Dr A. Eddy, University of Toronto),22 rat PDGF-B chain (a generous gift from Dr D. Bowen-Pope, University of Washington),16 and GAPDH (a generous gift from Dr P. Marsden, University of Toronto).23 cDNA probes were radiolabeled by random oligolabeling with DNA polymerase I (T7 Quickprime kit, Pharmacia) in the presence of [32P]dCTP (3000 Ci/mmol). [32P]dCTP-labeled cDNA probes were separated from unincorporated nucleotides by gel filtration with Sephadex G-50 (Nick columns, Pharmacia LKB Biotechnology). The membranes used were prehybridized for 15 minutes at 65°C in 10 mL QuikHyb TM rapid-hybridization solution (Stratagene). For hybridization, the radiolabeled probe (total counts, 10⁶ cpm) and 100 μL (50 mg/mL) of salmon sperm DNA were added to this solution for 1 hour at 65°C. The membranes were washed three times. The first wash was with 2× standard saline citrate (SSC)/0.1% sodium dodecyl sulfate (SDS) at room temperature, followed by 2× SSC/0.1% SDS at 40°C. The final wash was 1× SSC/0.1% SDS at 40°C. The radioactivity emitted by the filters was monitored during washing with a hand-held beta-emission counter. Filters were then exposed to x-ray film at −70°C with two intensifying screens (Fisher Scientific) for 24 to 72 hours. Filters were stripped with two sequential washes of boiling 1× SSC/0.1% SDS for 15 minutes for subsequent hybridization to the filter.

The autoradiograms were quantified with a GS 300 transmittance/reflectance scanning densitometer (Hoefer Scientific Instruments) by use of a Macintosh Classic II (System 7.0) and Dynamax HPLC Method Manager (version 1.2).

Semi quantitative RT-PCR
One microgram of total RNA (10-μL volume) was combined with 10 U of RNAsin and 300 pmol of random hexamers. The mixture was heated to 65°C for 5 minutes and cooled on ice. After addition of 100 U of Moloney murine leukemia virus RT, 10 U of RNAsin, and 4 μL of 5× RT buffer so that the final volume was 20 μL, the reaction mixture was incubated at 42°C for 2 hours. The reaction was stopped by heating at 95°C for 5 minutes. The RT products were then diluted to 100 μL with deionized water and stored at −70°C before subsequent amplification.
Primers for PCR were designed to flank at least one putative intron site. For TGF-β1, the sense primer corresponded to base pairs 1142 to 1168; the antisense primer, to base pairs 1520 to 1546.24 The two primers were designed to cross introns 5 and 6. For β-actin, the sense primer corresponded to base pairs 331 to 354; the antisense primer, to base pairs 550 to 571.26 The two primers were constructed to cross intron 2. For PDGF-A, the sense primer corresponded to base pairs 168 to 185; the antisense primer, to base pairs 675 to 699. The base pair sequences for the PDGF-A chain primers were chosen because they are homologous in mice and humans. The primer sequences were as follows: for rat TGF-β1, 5’ sequence, CGA GGT GAC CTG GGC ACC ATC CAT GAC; 3’ sequence, CTG CTC CAC CTT GGG CTT CGG ACC CAC; for murine PDGF-A, 5’ sequence, AGTCAGATCCACAGCAT; 3’ sequence, CTC TAA CCT CAC CTG GAC; and for rat β-actin, 5’ sequence, AAC CCT AAG GCC AAC CGT GAA AAG; 3’ sequence, TCA TGA GGT AGT CTG TCA GGT.

For amplification, 5 µL of the RT product was combined with 6.5 µL of PCR mix containing 0.1 µmol/L of each of the primer pairs and 2 U of Taq polymerase. Coamplification of β-actin was performed to standardize the amount of RNA subjected to reverse transcription for each time point. The sample was placed onto a Perkin-Elmer DNA thermal cycler (model 480) and heated to 94°C for 4 minutes, followed by 30 temperature cycles. Each temperature cycle consisted of three periods: (1) denature, 94°C for 3 minutes, (2) cool anneal, 60°C for 3 minutes, and (3) heat extend, 72°C for 3 minutes.

After amplification, the PCR products were separated by electrophoresis on a 1.0% agarose gel containing ethidium bromide, photographed, and transferred to a nylon membrane (Nitrin+; Schleicher & Schuell). The expected PCR product size for the primers was as follows: TGF-β1, 405 bp; β-actin, 240 bp; and PDGF-A chain, 411 bp. An antisense oligonucleotide, synthesized to serve as amplification product-specific probe for TGF-β1, spanned base pairs 1394 to 1424 (5′ GCT GTA CTG TGT GTC CAG GCT CCA AAT GTA 3′). An antisense oligonucleotide was synthesized to serve as amplification product-specific probe for β-actin and spanned base pairs 427 to 456 (5′ CAC AAT GCC AGT GGT AGC ACC AGA GCC ATA 3′). An additional antisense oligonucleotide was synthesized to serve as amplification product-specific probes for PDGF-A and spanned base pairs 354 to 383 (5′ CCG AGG TAT CTC GTA AAT GAC CGT CCT GCT G3′). By design, each oligonucleotide localized to a sequence that was inside the amplification primer sequences. The oligonucleotides were labeled with [32P]ATP (4500 Ci/mmol) using T4 DNA ligase for 5' end labeling. Nylon filters were hybridized overnight at 55°C, washed, and exposed to x-ray film at room temperature with an intensifying screen for 30 to 120 minutes.

The autoradiograms were quantified with a GS 300 transmittance/reflectance scanning densitometer (Hoefer Scientific Instruments) by use of a Macintosh Classic II (System 7.0) and Dynamax HPLC Method Manager (version 1.2).

Immunohistochemistry

After Brietal anesthesia (50 mg/kg), the kidneys were removed and the capsule was excised. Blocks of renal cortical tissue (8 mm3) were immersed in OCT compound (Miles Inc) and snap-frozen in liquid nitrogen. Cryostat sections (5 µm) were mounted on poly-L-lysine–coated slides. Immunoperoxidase staining was performed by the ABC method.26 In brief, sections were fixed in acetone (4°C) and air-dried. The sections were washed for 5 minutes in tap water, incubated at room temperature overnight with specific TGF-β1 antisera (R&D Systems), and diluted 1:100 in antibody-diluting buffer (Dimension Laboratories Ltd). After a PBS wash (Dimension Laboratories Ltd), tissue sections were incubated at room temperature for 30 minutes with an anti-rabbit IgG biotinylated antibody (Vectorstain Elite Kit, Vector Laboratories) and diluted 1:200 with PBS. Finally, sections were washed and then incubated for 30 minutes with an avidin-biotin-peroxidase complex (diluted 1:50 in PBS). Sites of peroxidase activity were visualized by incubation in DAB solution (Zymed Laboratories) for 2 minutes. Sections were counterstained with Harris modified hematoxylin with acetic acid (Fisher Scientific Ltd) for 5 seconds, washed with tap water, and covered with a glass coverslip. Negative control experiments were performed by either (1) replacing the primary antibody with antibody diluting buffer or (2) replacing the primary antibody with nonimmune rabbit antisem (1:4000).

Data Analysis

The statistical significance of differences among values of individual parameters was evaluated by ANOVA. Scheffé’s F test was used to assess the significance of differences between individual group means (P<.05). Values are expressed as mean±SEM.

Results

Animal Model

In SHR randomly assigned to uninephrectomy or sham operation, the mean value for body weight was 290±4 g, and the mean value for awake SBP was 206±2 mm Hg.

One week after nephrectomy, SBP averaged 211±4 mm Hg in SHR-Unix. As expected, treatment with enalapril lowered SBP in SHR-Unix to a mean value of 117±12 mm Hg 1 week after nephrectomy (P<.05 versus sham-operated and untreated SHR-Unix). The mean value for left kidney weight in sham-operated SHR was 1.21±0.05 g. One week after nephrectomy, the mean value for kidney weight was 1.35±0.05 g in untreated SHR-Unix. Treatment with enalapril lowered SBP, but the mean value for kidney weight 1 week after nephrectomy (1.38±0.05 g) was similar to that for untreated SHR-Unix.

Micropuncture Studies

Mean values for ΔP in sham-operated SHR, untreated SHR-Unix, and enalapril-treated SHR are illustrated in Fig 1. ΔP averaged 41±1 mm Hg in sham-operated SHR. One week after nephrectomy, there was a significant increase in ΔP, so that values averaged

![Fig 1. Bar graph showing transcapillary hydraulic pressure gradient (ΔP) in sham-operated spontaneously hypertensive rats (SHR) (C), untreated SHR 1 week after uninephrectomy (Nx), and enalapril-treated SHR 1 week after uninephrectomy (Nx-E). Values are mean±SEM. *P<.05 vs C; **P<.05 vs Nx.](http://circres.ahajournals.org/)

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53 ± 1 mm Hg (P < .05 versus sham-operated SHR). The increase in ΔP was due to an increase in glomerular capillary hydrostatic pressure. Mean values for glomerular capillary pressure were 56 ± 1 mm Hg in sham-operated SHR and 64 ± 1 mm Hg in SHR-Unix. Treatment with enalapril after uninephrectomy normalized ΔP (40 ± 1 mm Hg) (P < .05 versus SHR-Unix, 1 week after nephrectomy), and the decrease in ΔP was due to a decrease in glomerular capillary pressure (52 ± 1 mm Hg).

**Northern Blot Analysis**

The effect of increased glomerular capillary pressure on steady-state levels of mRNA for TGF-β1 and PDGF-B chain in glomeruli of SHR-Unix was first determined by Northern blot analysis. A representative Northern blot analysis of TGF-β1 mRNA levels is illustrated in Fig 2, left. A representative Northern blot analysis of PDGF-B chain mRNA levels is illustrated in Fig 2, middle. Northern blot analysis for the housekeeping gene, GAPDH, is shown in Fig 2, right. The ratios of the densitometry measures of glomerular TGF-β1 mRNA levels and GAPDH mRNA levels in sham-operated SHR and SHR-Unix 1 and 2 weeks after nephrectomy are shown in Fig 3. One week after uninephrectomy, there was a sixfold increase in mRNA levels for TGF-β1 in the glomeruli of uninephrectomized SHR compared with the glomeruli of sham-operated SHR. The increase in TGF-β1 mRNA levels was also evident 2 weeks after uninephrectomy. For this analysis, the glomeruli from four animals were pooled for RNA extraction. Thus, the densitometry ratio for each group represents a mean value obtained from four animals.

The ratios of the densitometry measures of glomerular PDGF-B chain mRNA levels and GAPDH mRNA levels are shown in Fig 4. One week after uninephrectomy, there was a twofold increase in mRNA levels for PDGF-B chain in the glomeruli of uninephrectomized SHR compared with the glomeruli of sham-operated SHR. In contrast to the observation for TGF-β1, the increase in PDGF-B chain mRNA levels was not sustained. Two weeks after nephrectomy, steady-state levels of mRNA for PDGF-B chain normalized. For this analysis, the glomeruli from four animals were pooled for RNA extraction. Thus, the densitometry ratio for each group represents a mean value obtained from four animals.

**Semiquantitative RT-PCR**

The effect of increased glomerular capillary pressure on steady-state levels of mRNA for TGF-β1 and PDGF-A chain in glomeruli of SHR-Unix was also determined by coamplification RT-PCR in separate groups of sham-operated SHR and uninephrectomized SHR. A representative photograph of the RT-PCR amplification products is illustrated Fig 5. One week after uninephrectomy there was a 10-fold increase in the ratio of RT-PCR products for TGF-β1 and β-actin in glomeruli of uninephrectomized SHR compared with glomeruli of sham-operated SHR (Figs 6, left, and 7). For this analysis, the glomeruli from five animals were pooled for RNA extraction. Thus, the densitometry ratio for the sham-operated SHR and uninephrectomized SHR represents a mean value obtained from five animals. There was a striking concordance between the results obtained with Northern blot analysis and the results obtained with RT-PCR for TGF-β1 expression.

mRNA levels for PDGF-A chain were undetectable by RT-PCR in glomeruli of sham-operated SHR but were detected in the glomeruli of SHR-Unix 1 week after uninephrectomy (Figs 6, right, and 8). Again, for this analysis, the glomeruli from five animals were pooled for RNA extraction. Thus, the densitometry ratio for the sham-operated SHR and uninephrectomized SHR represents a mean value obtained from five animals. The increase in mRNA levels for PDGF-A chain occurred at the same time point that we observed the increase in mRNA levels for PDGF-B chain.

Treatment with enalapril reduced steady-state levels of mRNA for TGF-β1 and PDGF-A chain in glomeruli of SHR-Unix so that no RT-PCR products were detected from glomerular samples by UV transillumination or Southern blot analysis with the oligonucleotide probe for TGF-β1 and PDGF-A chain. For this analysis, the glomeruli from five animals were pooled for RNA extraction. Thus, the densitometry ratio for the treated
group also represents a mean value obtained from five animals.

Four weeks after uninephrectomy the ratio of the densitometry measures of the RT-PCR products for glomerular TGF-β1 and β-actin remained 10-fold higher than the value obtained in sham-operated SHR (Figs 7 and 9). No RT-PCR product for PDGF-A chain was detected 4 weeks after uninephrectomy (Figs 8 and 9). In accord with the Northern blot analysis, the increase in mRNA levels for TGF-β1, as assessed by RT-PCR, was sustained in SHR-Unix. The increase in mRNA levels for PDGF-A chain, like PDGF-B chain, was not sustained. For this analysis, the glomeruli from three animals were pooled for RNA extraction. Thus, the densitometry ratio for this group of uninephrectomized SHR represents a mean value obtained from three animals.

**Immunohistochemistry**

The effect of uninephrectomy on immunostaining for TGF-β1 in the renal cortex of sham-operated SHR (n=3) and SHR-Unix (n=3) is illustrated in the six panels of Fig 10. Panels A and B show representative glomeruli in sections of renal cortex from sham-operated SHR and SHR-Unix, respectively, 4 weeks after uninephrectomy. The primary antibody was omitted from the immunostaining protocol, and there is no nonspecific staining present in the glomeruli or tubules of either group. Panels C and D illustrate the typical TGF-β1 immunostaining pattern of glomeruli from sham-operated SHR. Immunostaining for TGF-β1 is present in the glomerular capillary tufts. Panels E and F illustrate the typical TGF-β1 immunostaining pattern of glomeruli from SHR-Unix 4 weeks after uninephrectomy. In accord with the increase in mRNA levels for TGF-β1, glomerular TGF-β1 immunostaining also increased 4 weeks after nephrectomy.

**Discussion**

Micropuncture studies have shown that glomerular capillary hypertension is an important determinant of glomerular injury, but the mechanisms responsible for this association have not been elucidated. Recent studies by Yoshimura et al29 and Border et al30 have focused on two growth factors that play an important role in the immune-mediated glomerular injury induced by administration of anti-thyl.1: PDGF29 and TGF-β1.30

The first goal of the present study was to determine if an increase in glomerular capillary pressure led to an increase in mRNA levels for PDGF and TGF-β1 in glomeruli.

We found that uninephrectomy in hypertensive SHR led to an acute elevation in glomerular capillary hydrostatic pressure so that ΔP rose from 41±1 to 53±1 mm Hg. The increase in ΔP was accompanied by significant increases in glomerular mRNA levels for both TGF-β1 and PDGF-A and -B chains. When the rise in ΔP was prevented by treatment with the angiotensin-converting enzyme inhibitor enalapril, the rise in both TGF-β1 and PDGF mRNA levels was prevented. This is the first demonstration of an association between glomerular capillary hypertension and cytokine expression by the glomerulus in vivo. Since Dworkin and coworkers4,17 have shown that the increase in glomerular capillary hydrostatic pressure that follows uninephrectomy in the SHR is associated with the development of proteinuria and progressive glomerulosclerosis and maneuvers that lower ΔP, like treatment with enalapril,
attenuate glomerular injury, our observations suggest that the early increases in glomerular TGF-β1 and PDGF may be important events in the pathophysiology of glomerular injury.

To further implicate TGF-β1, we examined the early time course of TGF-β1 expression in the uninephrectomized SHR. The increase in TGF-β1 expression was sustained, and mRNA levels remained elevated 2 and 4 weeks after uninephrectomy compared with levels for sham-operated SHR. A sustained rise in TGF-β1 is consistent with the hypothesis that TGF-β1 may mediate glomerular injury in the uninephrectomized SHR. Many experimental observations support a central role for TGF-β1 in glomerular injury. TGF-β1 is expressed by glomerular endothelial, epithelial, and mesangial cells in vitro, and immunohistochemical studies and bioassay studies have shown that TGF-β1 is present in the normal glomerulus. One of the major cellular effects of TGF-β1 is to stimulate extracellular matrix protein production. In vitro, TGF-β1 increases collagen, laminin, and fibronectin synthesis by mesangial cells. In vivo, TGF-β1 expression increases in the glomeruli of rats with anti-thy-1.1 glomerulonephritis and correlates with an increase in extracellular matrix protein expression. Moreover, glomerular scarring is attenuated by anti-TGF-β1 antibody in these rats. TGF-β1 expression is also increased in the glomeruli of rats with experimental type 1 diabetes mellitus and in patients with type 1 diabetes mellitus. Taken together with our data, it is tempting to speculate that a glomerular capillary hypertension–induced increase in TGF-β1 expression could lead to an increase in extracellular matrix protein production, thus linking glomerular capillary hypertension and glomerulosclerosis.

The relation between mechanical stress and extracellular matrix protein production has also been studied in vitro. In 1976, Leung et al reported that cyclic stretching of vascular smooth muscle cells increased collagen and proteoglycan synthesis fourfold. More recently, Riser et al extended these observations by showing that cyclic stretching of cultured mesangial cells led to the increased synthesis of a number of extracellular matrix proteins, including type IV collagen, type I collagen, laminin, and fibronectin. Increased extracellular matrix protein synthesis was dependent on the degree of cell stretch, and the authors postulated that these mechanisms might be responsible for glomerulosclerosis. This group has gone on to show that cyclic stretching of mesangial cells also increases TGF-β1 production in vitro. The results of the present study suggest that this mechanism may also occur in vivo.

The mechanism(s) by which mechanical stretch or increased glomerular ΔP might lead to an increase in TGF-β1 expression by cells of the glomerular capillary tuft are unknown, but the TGF-β1 promoter has a 12-O-tetradecanoylphorbol-13-acetate–responsive site. In vitro, protein kinase C (PKC) activity increases in mesangial cells exposed to cyclical stretching. Therefore, it is tempting to speculate that stretch-mediated increases in PKC activity in mesangial cells might in-
increase TGF-β expression in vivo. Nollert et al.\(^2\) have reported that shear stress increases intracellular concentrations of inositol triphosphate in endothelial cells. An associated increase in 1,2-diacylglycerol could activate PKC,\(^4\) increase transcription of TGF-β₁, and thus link mechanical stress to cytokine expression. Further studies are necessary to determine if acute increases in glomerular capillary pressure increase glomerular PKC activity.

The observation that enalapril normalized ΔP and prevented the increase in TGF-β₁ mRNA levels in the glomeruli of uninephrectomized SHR is also compatible with the hypothesis that angiotensin II activity is increased in the kidneys of untreated uninephrectomized SHR. Lafayette et al.\(^1\) have reported that the nonpeptide angiotensin II antagonist losartan lowers glomerular capillary pressure and attenuates glomerular injury in rats with partial renal ablation, thus implicating angiotensin II in the pathogenesis of remnant glomerular injury. This mechanism of injury is further supported by the observation that chronic infusion of angiotensin II raises glomerular capillary pressure and leads to glomerulosclerosis in normal, intact rats.\(^1\)

More recently, Kagami et al.\(^4\) performed a series of studies to link angiotensin II and glomerular injury. They found that angiotensin II increased TGF-β₁ expression by mesangial cells in culture and led to an increase in the synthesis of the extracellular matrix proteins, fibronectin, and type I collagen. Angiotensin II blockade prevented all of these changes, and coinoculation with TGF-β₁-neutralizing antibodies prevented the rise in extracellular matrix synthesis. Of particular note, angiotensin II also led to an increase in the conversion of latent to active TGF-β₁. Finally, Kagami et al. showed that angiotensin II infusion into normal rats also increased TGF-β₁ and collagen type I mRNA levels in glomeruli. In the context of these studies, it is possible that glomerular capillary hypertension in the uninephrectomized SHR could have led to an increase in glomerular angiotensin II activity and that this effect is responsible for an increase in TGF-β₁ expression and bioactivity. We did not determine the effect of glomerular capillary hypertension on expression of the components of the renin-angiotensin system in the uninephrectomized SHR, so further studies will be necessary to address this hypothesis.

Previous studies have shown that mRNA levels for TGF-β₁ do not always correlate with protein levels.\(^1\) Therefore, the second major goal of the present study was to test the hypothesis that TGF-β₁ immunostaining would increase in glomeruli along with the increase in mRNA levels after the development of glomerular capillary hypertension. Four weeks after uninephrectomy, there was an increase in TGF-β₁ immunostaining in the glomeruli of uninephrectomized SHR compared with the sham-operated SHR, demonstrating that early changes in TGF-β₁ mRNA levels were associated with increases in protein expression. The increases in TGF-β₁ mRNA levels and immunoreactive protein are consistent with the hypothesis that TGF-β₁ plays a role in the pathophysiology of glomerular injury in the uninephrectomized SHR, but TGF-β₁ bioactivity was not measured.

Mackay et al.\(^4\) observed that there was a progressive decline in TGF-β₁ mRNA levels 1 and 2 weeks after uninephrectomy in Wistar rats, although measures of TGF-β₁ bioactivity were unchanged. Thus, increased glomerular TGF-β₁ expression does not accompany the compensatory glomerular hypertrophy that follows reduction in nephron number in normotensive rats. In the present study, kidney weight was similar in untreated SHR 1 week after uninephrectomy (1.35±0.05 g) and enalapril-treated SHR 1 week after uninephrectomy (1.38±0.05 g). Both values tended to be greater than the kidney weight in sham-operated SHR (1.21±0.05 g), although the differences did not attain statistical significance. Therefore, enalapril treatment lowered glomerular capillary pressure and attenuated the increase in mRNA levels for TGF-β₁ in uninephrectomized SHR but did not prevent early compensatory renal hypertrophy. This observation is consistent with the previous work of Dworkin and colleagues.\(^4,17\) In studies of uninephrectomized SHR, enalapril-treated rats had an average kidney weight of 2.56±0.09 g 42 weeks after nephrectomy.\(^4\) Intact SHR, with similar body weights, had an average kidney weight of 1.77±0.05 g.\(^17\) Thus, long-term enalapril treatment does not prevent compensatory renal hypertrophy, although it does prevent development of glomerulosclerosis.

We also examined the early time course of PDGF expression in the uninephrectomized SHR. Like TGF-β₁, mRNA levels for PDGF-B chain were increased 1 week after nephrectomy, but unlike the findings for TGF-β₁, PDGF-B chain mRNA levels normalized 2 weeks after nephrectomy. Similarly, mRNA levels for PDGF-A chain assessed by semiquantitative RT-PCR increased 1 week after nephrectomy, but the increase was not sustained. Levels were undetectable 4 weeks after nephrectomy. Interestingly, Floege et al.\(^4\) found a transient increase in PDGF expression in the glomeruli of rats subjected to subtotal ablation, and we have observed early, unsustained PDGF-A chain expression in the glomeruli of diabetic rats (unpublished observation). It is tempting to speculate that an early increase in PDGF expression may be a common feature of the glomerular response to a variety of stimuli.

PDGF is a cytokine consisting of two chains, an A chain and a B chain, that can form homodimers or heterodimers,\(^47\) and in vitro, mRNA for both PDGF-A chain and PDGF-B chain can be detected in mesangial cells.\(^48-50\) In vivo, PDGF has been implicated in the cellular proliferation that characterizes anti-thy1.1-induced glomerulonephritis in the rat, because mRNA levels for PDGF-B chain rise, and neutralizing antibodies to PDGF attenuate cell proliferation.\(^51\) Although PDGF induces cell prolifer-
ation, it has also been shown to increase the synthesis of other cytokines, including TGF-β. Although we did not examine time points earlier than 1 week, changes in PDGF expression could have preceded the changes in TGF-β and played a role in the early increase in TGF-β mRNA levels. In this regard, it is interesting to note that the promoter region for PDGF-B chain has a shear-stress-responsive element and that changes in glomerular hemodynamic function in vivo might be expected to increase transcription in endothelial cells and perhaps mesangial cells. In support of this hypothesis, elevated pressures in the pulmonary circulation have been shown to increase expression of the B chain of PDGF by vascular smooth muscle cells of the pulmonary artery. In vitro studies also show that cyclic mechanical strain increases secretion of PDGF-AA and PDGF-BB by vascular smooth muscle cells and that a similar mechanism may be operative in the glomerular microcirculation of SHR-Unix.

In vitro, Gibbons et al and Naftilan et al have shown that angiotensin II increases steady-state levels of mRNA for PDGF-A chain in vascular smooth muscle cells. Taken together, these studies suggest that changes in mRNA levels for TGF-β and PDGF-A and -B chains in glomeruli of SHR-Unix could have been due to a more proximate effect of glomerular capillary pressure on glomerular angiotensin II activity. Our observation that enalapril treatment reduced mRNA levels for PDGF is consistent with this hypothesis. However,
further studies will be necessary to determine if acute glomerular capillary hypertension leads to an increase in expression of the components of the renin-angiotensin system in the glomerulus.

In summary, we have shown that uninephrectomy leads to an increase in glomerular capillary hydraulic pressure and an increase in glomerular mRNA levels for TGF-β, and PDGF-A and -B chains in glomeruli. Normalization of AP with enalapril ablates this response. The increases in TGF-β, mRNA levels are sustained and associated with an increase in glomerular immunostaining for TGF-β. The increases in PDGF mRNA levels are transient. These observations link glomerular capillary hypertension and cytokine expression in the uninephrectomized SHR, events that may be responsible for subsequent glomerular injury.

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