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Chronic anti-Thy-1 nephritis is aggravated in the nonclipped but not in the clipped kidney of Goldblatt hypertensive rats

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Chronic anti-Thy-1 nephritis is aggravated in the nonclipped but not in the clipped kidney of Goldblatt hypertensive rats.

Background. We have previously shown that renovascular hypertension does not inhibit healing of the acute Thy-1 nephritis. To test whether a chronic model of the Thy-1 nephritis is more susceptible to high blood pressure, the repetitive hit model was evaluated in rats with 2-kidney, 1-clip Goldblatt hypertension.

Methods. Six weeks after initiation of 2-kidney, 1-clip hypertension, chronic Thy-1 glomerulonephritis was induced in hypertensive rats by four consecutive injections of rabbit antiserum in weekly intervals. Renal structure and function were examined two weeks after the last injection. Glomerular binding of rabbit IgG as well as expression of transforming growth factor-beta (TGF-β), α-smooth muscle actin (α-SMA) and cyclooxygenase (COX)-1 and -2 were evaluated by Western blotting.

Results. Similar glomerular deposition of rabbit IgG was detected in normotensive rats and in both kidneys of Goldblatt hypertensive rats indicating similar delivery and binding of the heterologous antibody. Induction of the repetitive Thy-1 model significantly enhanced glomerular damage in the nonclipped kidney and increased albuminuria. Surprisingly, no glomerular damage developed in the clipped kidney of nephritic hypertensive rats. In contrast, increased glomerular volume and increased expression of TGF-β, α-SMA as well as COX-1 and COX-2 were found in normotensive nephritic rats and in both kidneys of nephritic hypertensive rats.

Conclusion. Glomerular and tubulointerstitial damage of the chronic Thy-1 model is dramatically enhanced in the nonclipped kidneys of Goldblatt hypertensive rats. In contrast, the clipped kidney is completely protected from this immunological injury despite similar activation of glomerular cells, induction of TGF-β, COX-1 and COX-2 and glomerular hypertrophy.

Arterial hypertension is the most important factor determining progression to end-stage renal disease regardless of the underlying renal disease. The mechanisms by which hypertension aggravates progression of renal disease are not well understood. Hypertension may exert its adverse effects through vasoconstriction and intrarenal vascular sclerosis causing ischemia [1, 2]. Other studies suggest that hypertension may damage the glomerulus directly by transmission of elevated systemic pressure [3]. Glomerular hypertrophy may enhance this injury [4, 5]. In the model of 2-kidney, 1-clip Goldblatt hypertension, the effect of ischemia can be studied in the clipped kidney and the effect of elevated glomerular capillary pressure and glomerular hypertrophy in the nonclipped kidney. Nonhemodynamic trophic properties of angiotensin II promoting glomerular and tubular hypertrophy and accumulation of matrix can be studied under two different hemodynamic circumstances. A widely studied model of glomerulonephritis is the Thy-1 nephritis in which a single intravenous injection of an anti-Thy-1 antiserum induces complement dependent mesangiolysis. This lysis is subsequently followed by mesangial cell reappearance, proliferation and matrix deposition. The glomerular lesions of the Thy-1 nephritis resume and hypercellularity and extracellular matrix accumulation disappear within weeks [6, 7]. We recently described that renovascular hypertension enhances impairment of renal function and structure in the acute phase of Thy-1 nephritis. Decreased renal function and increased number of glomerular microaneurysms were found in the nonclipped kidney five days after induction of the nephritis [8]. However, complete healing of the nephritis was found in the clipped and nonclipped kidney of renovascular hypertensive rats six weeks after induction of the nephritis [7]. In rats injected with one dose of anti-Thy-1 antibody, the glomerular disease resolves [6, 7], but if multiple doses are administered in the repetitive hit model, a progressive and irreversible glomerular damage develops [9, 10]. The effects of hypertension and ischemia on this chronic process are unknown and the present study was therefore performed to gain insight whether hypertension has an impact on that process ei-

Key words: renovascular hypertension, glomerulosclerosis, glomerulonephritis, albuminuria, TGF-β, glomerular volume, cyclooxygenase.

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ther by enhancing the hypertension specific lesions or by modifying the course of the nephritic changes.

METHODS

Two-kidney, one-clip hypertension

Studies were performed in male Sprague-Dawley rats (Charles River, Kisslegg, Germany). In rats weighing 120 to 140 g, 2-kidney, 1-clip hypertension was induced as described previously [7, 8, 11, 12]. For this purpose a U-shaped silver clip (0.23 to 0.25 mm internal diameter) was placed around the right renal artery in 26 rats through a loin incision, while the animals were under ketamine/xylazin anesthesia (100/10 mg/kg IM; Parke-Davis/Bayer, Berlin/Leverkusen, Germany). Rats were studied six weeks after surgery. Only those rats with systolic blood pressure >160 mm Hg at six weeks were included in the protocol. One out of 26 clipped rats did not develop hypertension, 4 of 26 clipped rats died within six weeks after surgery due to severe hypertension. In a second study 18 rats were clipped. All rats developed arterial hypertension. The clip was removed six weeks after surgery under ketamine/xylazin anesthesia. Sham surgery of clip removal was performed in normotensive rats.

Induction of glomerulonephritis

Immune mediated mesangial cell injury was induced six weeks after initiation of 2-kidney, 1-clip hypertension in hypertensive and normotensive rats by four consecutive IV injection of 0.5 mL/100 g body weight of an anti-rat-thymocyte antiserum in weekly intervals. The antiserum was induced in rabbits by repeated immunization with thymocytes from Lewis rats as described [13]. Four groups of animals were studied: (*1*) normotensive control animals; (*2*) normotensive animals + nephritis (Thy-1); (3) 2-kidney, 1-clip hypertensive animals (2K1C); (4) 2-kidney, 1-clip hypertensive animals + nephritis (2K1C + Thy-1).

The study was carried out in two complete sets of experiments. Each set of experiments contained two to six animals in each group. Before injection of antiserum no significant difference was measured for systolic blood pressure in hypertensive controls (178 ± 10 mm Hg, $N = 9$) and in hypertensive rats receiving antiserum (193 ± 6 mm Hg, $N = 12$). Blood pressure was 120 ± 6 and 124 ± 5 mm Hg, respectively, in both normotensive groups before injection of the antiserum. In the second protocol clips were removed six weeks after surgery. Blood pressure was 179 ± 11 mm Hg in hypertensive rats and 190 ± 8 in hypertensive rats receiving antiserum before and 110 ± 7 mm Hg and 113 ± 5 mm Hg, respectively, one to two days after removal of the clip. Blood pressure was 106 ± 5 and 95 ± 11 mm Hg in both normotensive groups, respectively. Induction of the repetitive model of the nephritis was started two to three days after removal of the clip.

Systolic blood pressure and albuminuria

Systolic blood pressure was measured by tail cuff plethysmography in awake rats as previously described [7, 8, 11, 12]. Blood pressure data shown are the mean of two measurements performed on two consecutive days. The animals were placed in individual metabolic cages and 24-hour urine collections were made for determination of albuminuria. Albuminuria was measured with a commercial enzyme-linked immunosorbent assay (ELISA; WAK Chemie, Bad Soden, Germany) as recommended by the manufacturer.

Renal morphology

At the end of the experimental protocol kidneys were removed and wet weight determined. Kidney slices were fixed in 4% buffered formalin or Carnoy solution, paraffin embedded, cut into 3- to 4-μm thick sections and stained with periodic acid-Schiff (PAS). Glomerular damage was evaluated using a modification of the method of Raij [14] as described earlier by us [7, 8, 11, 12]. Grade 1 represents involvement of up to 25% of the glomerulus and grade 4 represents injury of 75 to 100% of the glomerulus. Thirty glomeruli were evaluated from each kidney. In addition, the amount of tubulointerstitial damage in the kidney (tubular atrophy and dilation, interstitial fibrosis, proteinaceous casts) was estimated by examining 20 fields (magnification ×100) and semiquantitatively grading the degree of damage in each field using a 0 to 3+ scale as described previously [7, 12]. Planimetric examinations of glomerular cross sectional area were performed by means of a Zeiss drawing tube in combination with a semiautomatic interactive image analysis system (Morphomat 30; Zeiss, Oberkochen, Germany) as described [7, 11, 12]. Using a serpentine movement from cortex to medulla and vice versa, the outlines of 30 consecutively encountered capillary tufts were traced manually and the mean glomerular random cross sectional area (AG) was determined. The average glomerular tuft volume (VG) was then calculated as $VG = (\beta/k)\,(AG)^{3/2}$, where $\beta = 1.38$ and $k = 1.1$ are shape and size distribution coefficients, respectively [15]. Tissues also were stained with antibodies against ED-1 (Chemikon International, Temecula, CA, USA). The tissue sections were developed with the APAAP complex using antibodies as described earlier [7, 8]. The number of interstitial ED-1 positive cells was counted per high power field (15 fields/kidney, magnification ×400).

Western blotting

Western blotting was performed as described recently by us [7, 13]. Glomeruli were isolated by differential sieving and resuspended in Laemmli buffer. Samples

Table 1. Body, kidney and heart weights of the animals in each study group

			Kidney weight		
		Body weight	Clipped	Nonclipped	Heart weight
	N		*g*		
Controls	5	552 ± 33	1.62 ± 0.14		1.27 ± 0.09
Thy-1	5	537 ± 31	1.57 ± 0.14		1.28 ± 0.09
2K1C	9	506 ± 17	1.09 ± 0.10[b]	1.77 ± 0.09	1.57 ± 0.08[a]
2K1C + Thy-1	12	496 ± 17	1.23 ± 0.07[a]	1.83 ± 0.11	1.68 ± 0.06[b]

[a] $P < 0.05$, [b] $P < 0.01$ vs. Controls

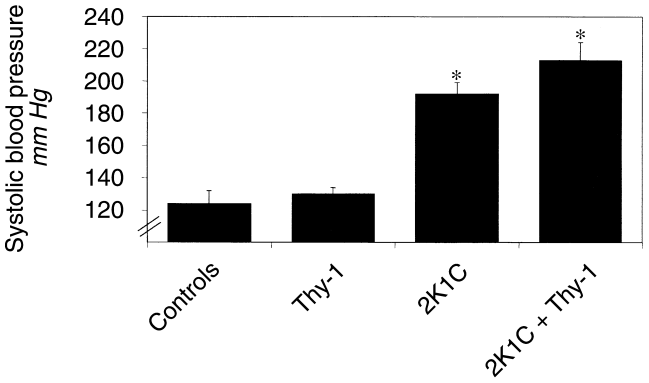

Fig. 1. Systolic blood pressure measured in conscious rats two weeks after the last injection of the antiserum. Abbreviations are: 2K1C, 2-kidney, 1-clip hypertensive animals; Thy-1, nephritis. *$P < 0.01$ vs. controls.

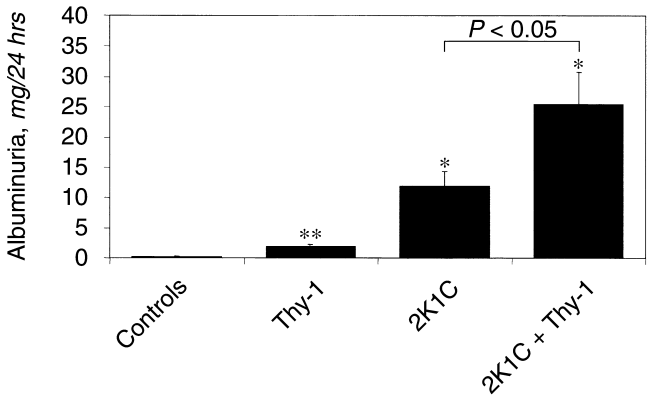

Fig. 2. Albuminuria in the various groups. Abbreviations are: 2K1C, 2-kidney, 1-clip hypertensive animals; Thy-1, nephritis. *$P < 0.01$, **$P < 0.001$ vs. controls.

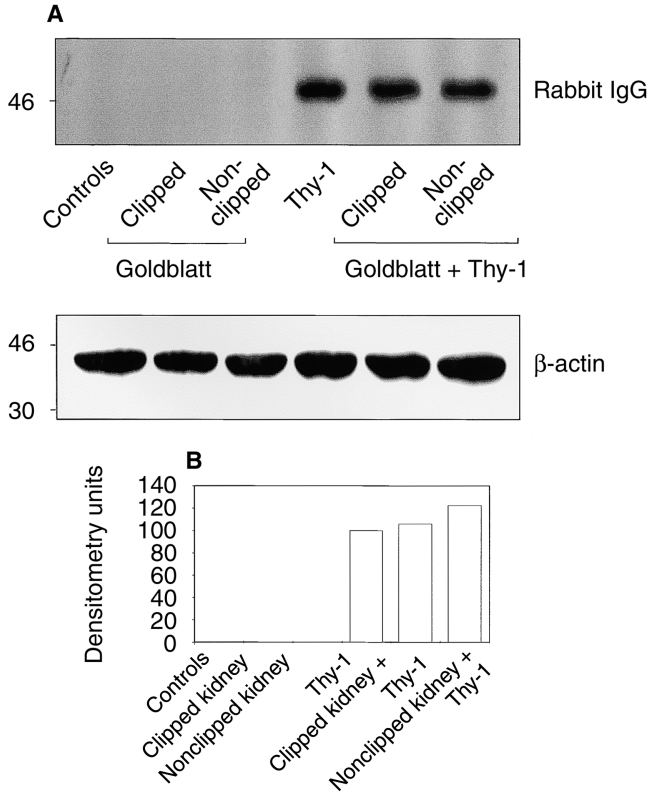

Fig. 3. Western blots for rabbit IgG from glomerular proteins. (*A*) Similar amounts of rabbit IgG were detected in glomeruli from normotensive nephritic and both kidneys of hypertensive nephritic rats. The blot was washed and re-incubated with an antibody against β-actin to proof similarity in protein loading and transfer. The glomerular lysate used in this Western blot was pooled from 3 to 6 kidneys in each group. A very similar pattern was found in an another Western blot from an independent second set of experiments again using pooled proteins from 2 to 6 kidneys. (*B*) Densitometric analysis of the IgG bands from both blots.

were boiled and centrifuged. Protein concentration was determined with the protein DC-assay (Bio-Rad, Muenchen, Germany). To equal amounts of protein (100 μg), Laemmli buffer 2 and 1/5 staining solution (42.5% glycerol, 0.5% bromphenol blue) were added. Samples were electrophoresed on SDS/polyacrylamide gels. Proteins were electroblotted onto nitrocellulose (Hybond-ECL; Amersham, Braunschweig, Germany). The membrane was blocked with 5% nonfat dry milk in washing buffer (1 × PBS 0.1% Tween 20). The following antibodies were used in a 1:1000 dilution: rabbit anti-porcine TGF-β antibody (R&D Systems, Minneapolis, MN, USA), mouse monoclonal anti-human alpha smooth muscle actin antibody (Sigma), cyclooxygenase (COX): goat anti-mouse COX-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and mouse anti-rat COX-2 antibody (Transduction Laboratories, Lexington, MA, USA). The second antibody was a rabbit anti-mouse or rabbit anti-goat or mouse anti-rabbit or mouse anti-porcine horseradish peroxidase-conjugated antibody in a 1:1000 dilution (Transduction Laboratories). Peroxidase labeling was detected with luminescence immunodetection (ECL; Amersham) according to the manufacturer's recommendations. Detection of rabbit IgG was performed by applying the mouse anti-rabbit IgG horseradish peroxidase-conjugated antibody without using a primary antibody (Transduction Laboratories). To control for small variations in protein loading and transfer, membranes were washed and re-incubated with a mouse monoclonal anti-beta-

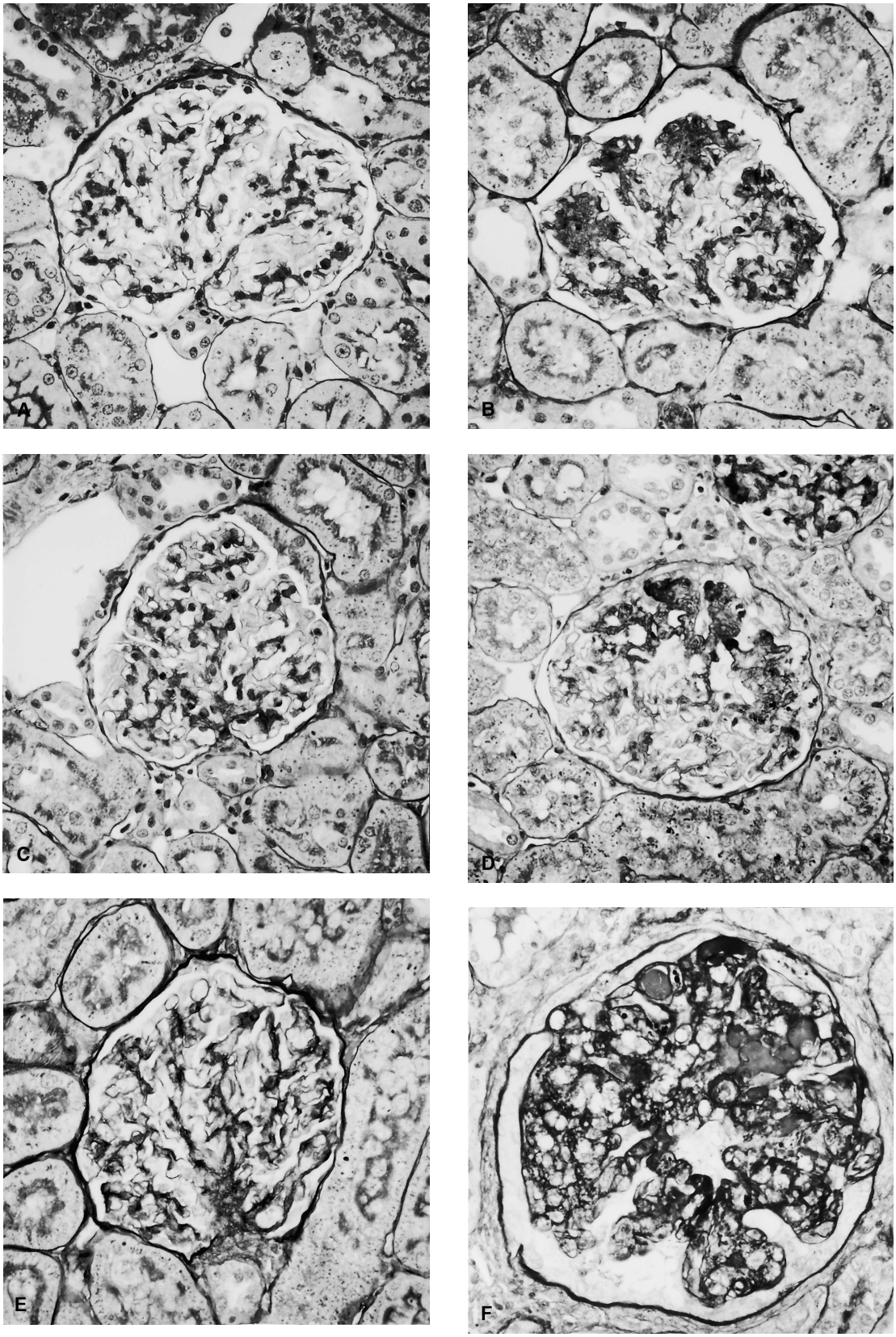

Fig. 4. See legend next page.

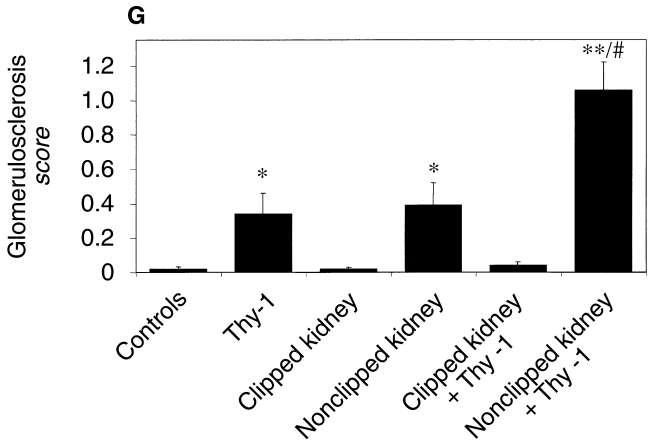

Fig. 4. Glomeruli from the different groups. Glomeruli from controls revealed a normal appearance (*A*). Chronic glomerulonephritis with matrix deposition, hypercellularity and increased glomerular scarring was found in normotensive nephritic rats (*B*). The glomeruli of the clipped kidney generally had a normal appearance (*C*). A light micrograph of glomeruli from the nonclipped kidney demonstrated segmental sclerosis (*D*). Surprisingly, light microscopy of glomeruli in the clipped kidney from the hypertensive and nephritic group revealed almost no increased damage (*E*). In contrast, a dramatic increase in glomerular damage was detected in the nonclipped kidneys from the same group (*F*; original magnification ×400). Scoring of the glomerular damage is shown in *G*. $**P < 0.001$, $*P < 0.01$ versus normotensive controls, $\#P < 0.05$ vs. nonclipped kidney of non-nephritic 2K1C.

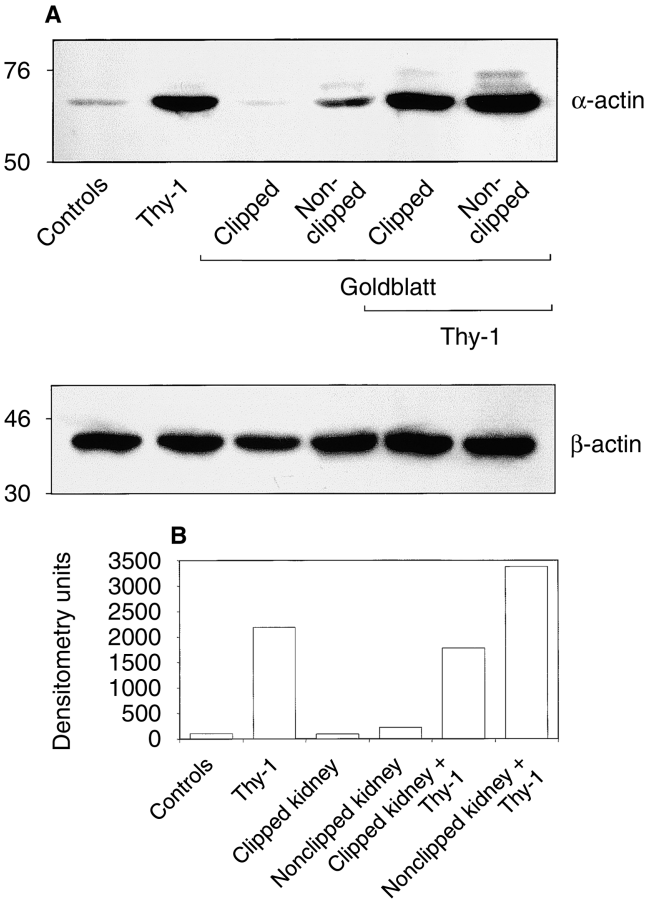

Fig. 5. Western blotting for α-smooth muscle actin from glomerular proteins. (*A*) α-Smooth muscle expression was heavily induced in glomeruli from normotensive nephritic rats as well as in the clipped and in the nonclipped kidney from nephritic 2K1C rats. The blot was washed and re-incubated with an antibody against β-actin. The glomerular lysate used in this Western blot was pooled from 3 to 6 kidneys in each group. A very similar pattern was found in an another Western blot from an independent second set of experiments using again pooled proteins from 2-6 kidneys. (*B*) Densitometric analysis of the actin bands from both blots.

actin antibody (Sigma). Exposed films were scanned with Fluor-STM Multi-Imager (Bio-Rad Laboratories, Hercules, CA, USA), and data were analyzed with the computer program Multi-Analyst™ from Bio-Rad.

The American Physiological Society's guidelines of experimental animal research were followed and approval was obtained from the University animal care committee. A detailed analysis of the fate and the number of animals studied in the present series is given in the **Methods** and **Results** sections, as recently suggested in a correspondence to the journal *Nature* to avoid poorly reported animal tests [16].

Statistical analysis

Results are expressed as means ± SEM unless stated otherwise. Statistical significance was defined as $P < 0.05$. To compare two distinct treatment groups, we applied the Student *t* test. For comparison of all groups one way analysis of variance was used as a screening test, if appropriate.

RESULTS

General characteristics

Table 1 shows body, kidney and heart weight of the animals studied. In hypertensive rats hypertrophy of the nonclipped kidney and atrophy of the clipped kidney occurred as determined by kidney weight. Induction of nephritis did not change this pattern. Heart weight was significantly increased in hypertensive animals versus normotensive. Survival after randomization was 100% in all four groups.

Blood pressure

The systolic blood pressure at the end of the study is demonstrated in Figure 1. Systolic blood pressure as measured by tail plethysmography was elevated in hypertensive rats compared with controls and was not sta-

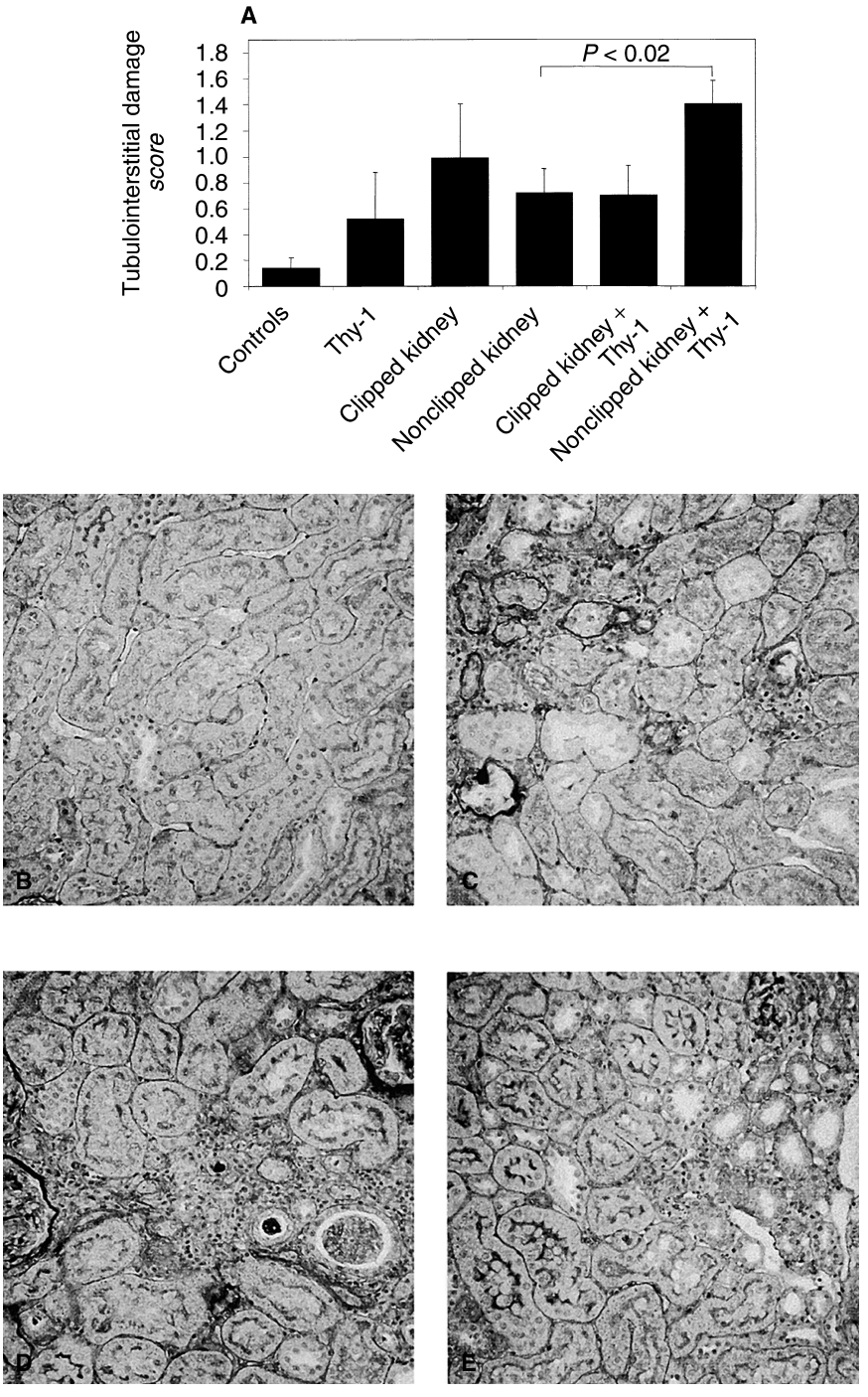

Fig. 6. Tubulointerstitial damage evaluated by scoring. (*A*) Tubulointerstitial damage was increased in the nonclipped kidney of hypertensive nephritic rats compared with the nonclipped kidney of 2K1C rats ($P < 0.02$). (*B–G*) Light micrographs of the tubulointerstitial changes are shown in (B) controls, (C) Thy-1, (D) clipped kidney, (E) nonclipped kidney.

tistically different between hypertensive rats and hypertensive rats with chronic Thy-1 nephritis.

Albuminuria

Albuminuria two weeks after the last injection of the Thy-1 antiserum is depicted in Figure 2. Albuminuria was significantly increased in normotensive nephritic rats compared with controls. In addition, urinary albumin excretion was significantly increased in 2K1C rats. Moreover, a further and significant increase was found in hypertensive nephritic rats.

Glomerular binding of the antiserum

Following the injection of rabbit anti-thymocyte-serum, selective binding of rabbit IgG to the mesangium can be detected [8]. Figure 3 shows a Western blot of glomerular proteins using an anti-rabbit IgG antibody. Bands of similar intensity were found in normotensive

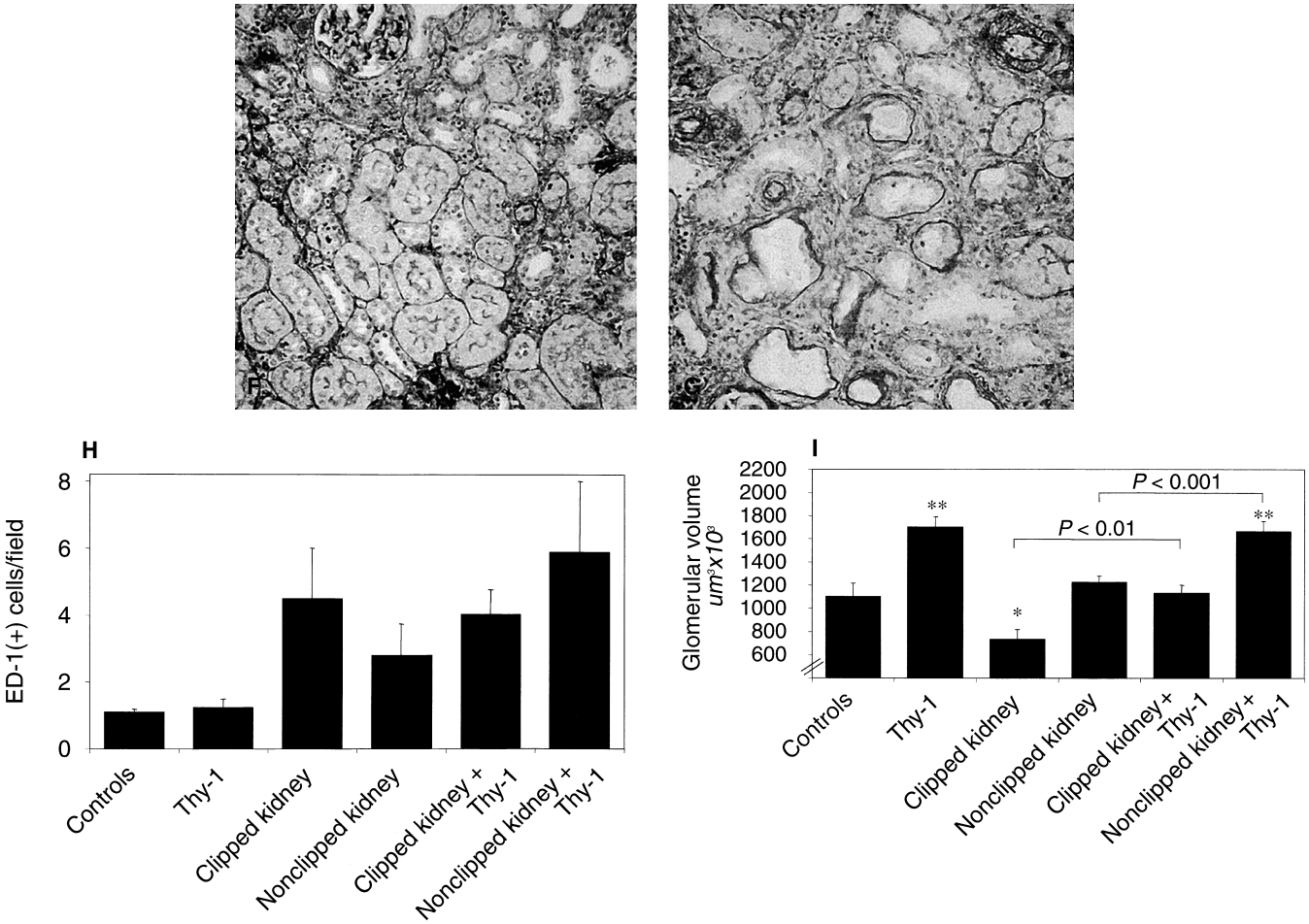

Fig. 6. (Continued). (F) clipped kidney + Thy-1, (G) nonclipped kidney + Thy-1 (original magnification ×200). (*H*) The number of interstitial ED-1 positive cells per high power field (15 fields/kidney). The number of interstitial monocyte/macrophage infiltration was increased in the clipped kidneys. The highest number was observed in the nonclipped kidney of nephritis 2K1C rats. However, due to the high variability the found differences were not statistically significant. (*I*) Glomerular volume measured by planimetry of glomerular cross sectional areas. All nephritic glomeruli revealed a significant increase in glomerular size over their respective controls. $*P < 0.05$, $**P < 0.01$ vs. normotensive controls.

nephritic rats and the clipped as well as the nonclipped kidney of nephritic 2K1C rats after four injections of the antiserum. This finding indicates no differences in delivery and binding of antiserum in normotensive and hypertensive rats [8].

Histologic finding

Quantitative evaluation of PAS stained sections obtained two weeks after the last of the four injections of the antiserum was performed. Intact glomeruli were found in normotensive controls (Fig. 4A). In contrast, chronic glomerulonephritis with matrix deposition, hypercellularity and scarring could be detected in normotensive nephritic rats (Fig. 4B). The clipped (Fig. 4C) as well as the nonclipped kidney (Fig. 4D) showed changes consistent with earlier reports by us and others of kidney morphology in rats with renovascular hypertension [7, 8, 11, 12, 17, 18]. However, no chronic glomerulonephritis could be detected in the clipped kidney after four injections of antiserum (Fig. 4E). In contrast, dramatically enhanced glomerular damage was found in the nonclipped nephritic kidney (Fig. 4F). Scoring of the described changes is summarized in Figure 4G.

Alpha-smooth muscle actin (α-SMA) is a very sensitive marker of mesangial cell activation and injury [19]. To further explore the changes in glomerular injury detected by light microscopy we measured glomerular levels of alpha-smooth muscle actin by Western blotting. As shown in Figure 5, α-SMA expression was up-regulated in normotensive nephritic rats as well as in the clipped and nonclipped kidney of hypertensive nephritic rats indicating activation of the glomerular cells in both kidneys of 2K1C nephritic rats.

Tubulointerstitial damage and glomerular volume

It is known that tubulointerstitial damage precedes glomerular injury in the nonclipped kidney of Goldblatt hypertensive rats, suggesting a dominant pathophysio-

logic role for the tubulointerstitial damage in nephrosclerosis [17, 18]. We therefore evaluated the pattern of tubulointerstitial injury. In accordance with the glomerular data, tubulointerstitial damage was increased in the nonclipped kidney of hypertensive nephritic rats compared with the nonclipped kidney of 2K1C rats ($P < 0.02$). Tubulointerstitial damage was increased in the clipped kidney. However, no significant difference could be detected between the clipped kidneys of hypertensive rats with and without nephritis (Fig. 6A). Light micrographs of the tubulointerstitial changes found in the different kidneys are shown in Figure 6 B-G. Intact tubuli were found in normotensive controls (Fig. 6B), while tubulointerstitial damage could be detected in normotensive nephritic rats (Fig. 6C). The clipped (Fig. 6D) as well as the nonclipped kidney (Fig. 6E) showed changes consistent with earlier reports by us [7, 8, 11, 12] and others [17, 18] of kidney morphology in rats with renovascular hypertension. Tubulointerstitial injury could be detected in the clipped kidney after four injections of antiserum (Fig. 6F). In contrast, dramatically enhanced tubulointerstitial damage was found in the nonclipped nephritic kidney (Fig. 6G).

Monocyte/macrophages are important mediators of renal injury. We therefore examined the interstitial infiltration of monocytes. As shown in Figure 6H, the number of monocytes was closely associated with the degree of tubulointerstitial injury. An increased number was found in the clipped kidney of nephritic and nonnephritic hypertensive rats. The highest number was detected in the nonclipped kidney of nephritic 2K1C rats. Glomerular hypertrophy is another important parameter contributing to the progression of chronic renal disease [4, 5]. At least to our knowledge, no data are available on the changes of glomerular size in the repetitive model of the Thy-1 nephritis. As shown in Figure 6I glomerular hypertrophy occurred in the nonclipped kidney and a decrease of glomerular size was noted in the clipped kidney compared with controls. Interestingly, glomerular size was significantly increased in normotensive nephritic rats as well as in the clipped and nonclipped kidney of hypertensive nephritic rats compared with nonnephritic rats.

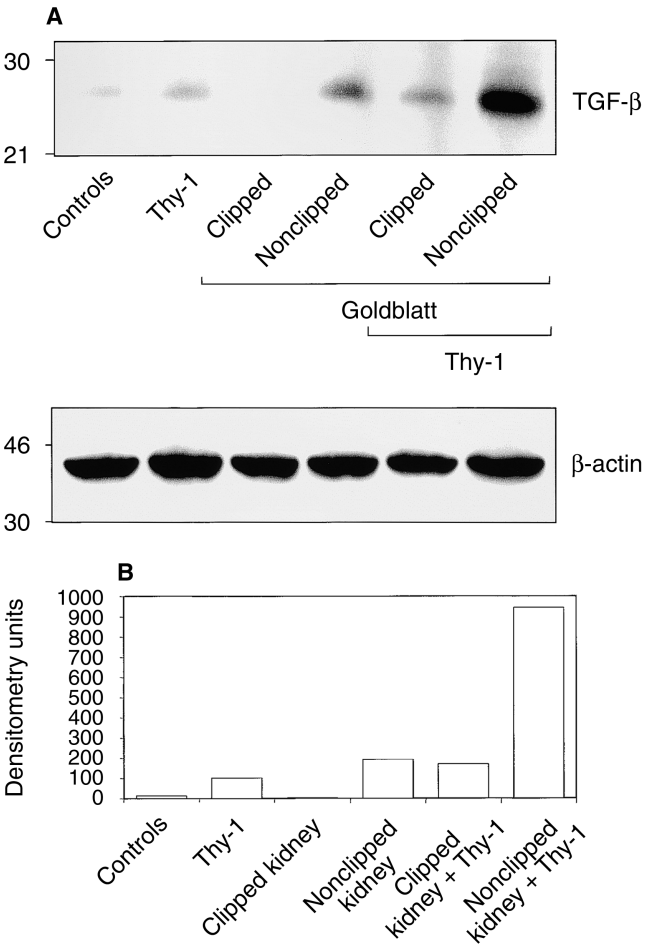

Fig. 7. Expression of glomerular transforming growth factor-β (TGF-β) protein levels. (*A*) The 21- and 30-kD molecular marker is shown. A single band of around 25 kD is visible reflecting the TGF-β dimer. Increased expression was visible in normotensive nephritic rats. Hypertensive rats showed increased levels of TGF-β in the nonclipped kidney compared with the clipped kidney and controls. The expression of TGF-β in the clipped and nonclipped kidney of hypertensive nephritic rats was increased. The blot was washed and re-incubated with an antibody against β-actin. The glomerular lysate used in this Western blot was pooled from three to six kidneys in each group. A very similar pattern was found in an another Western blot from an independent second set of experiments using again pooled proteins from two to six kidneys in each group. (*B*) Densitometric analysis of the TGF-β bands from both blots.

TGF-β

We and others have demonstrated that TGF-β plays a pivotal role in the Thy-1 nephritis [20, 21] as well as in Goldblatt hypertension [7, 22]. To further quantify glomerular TGF-β levels Western blotting of glomerular lysates was performed. Similar to our previous finding in rats with short- and long-term 2K1C hypertension [7, 22], hypertensive rats in the present study 12 weeks after clipping showed increased levels of TGF-β in the nonclipped kidney compared with the clipped kidney and controls (Fig. 7). Glomerular expression of TGF-β was modestly increased in normotensive nephritic rats and the clipped kidney of nephritic 2K1C rats. However, a strong increase of TGF-β abundance was found in the nonclipped kidney of nephritic 2K1C rats matching the increased glomerular damage found in these kidneys.

COX-1 and COX-2

Recent evidence from models of inflammatory kidney disease revealed that COX products are important in inflammation and might participate in the healing process [23]. Moreover, we have demonstrated different glo-

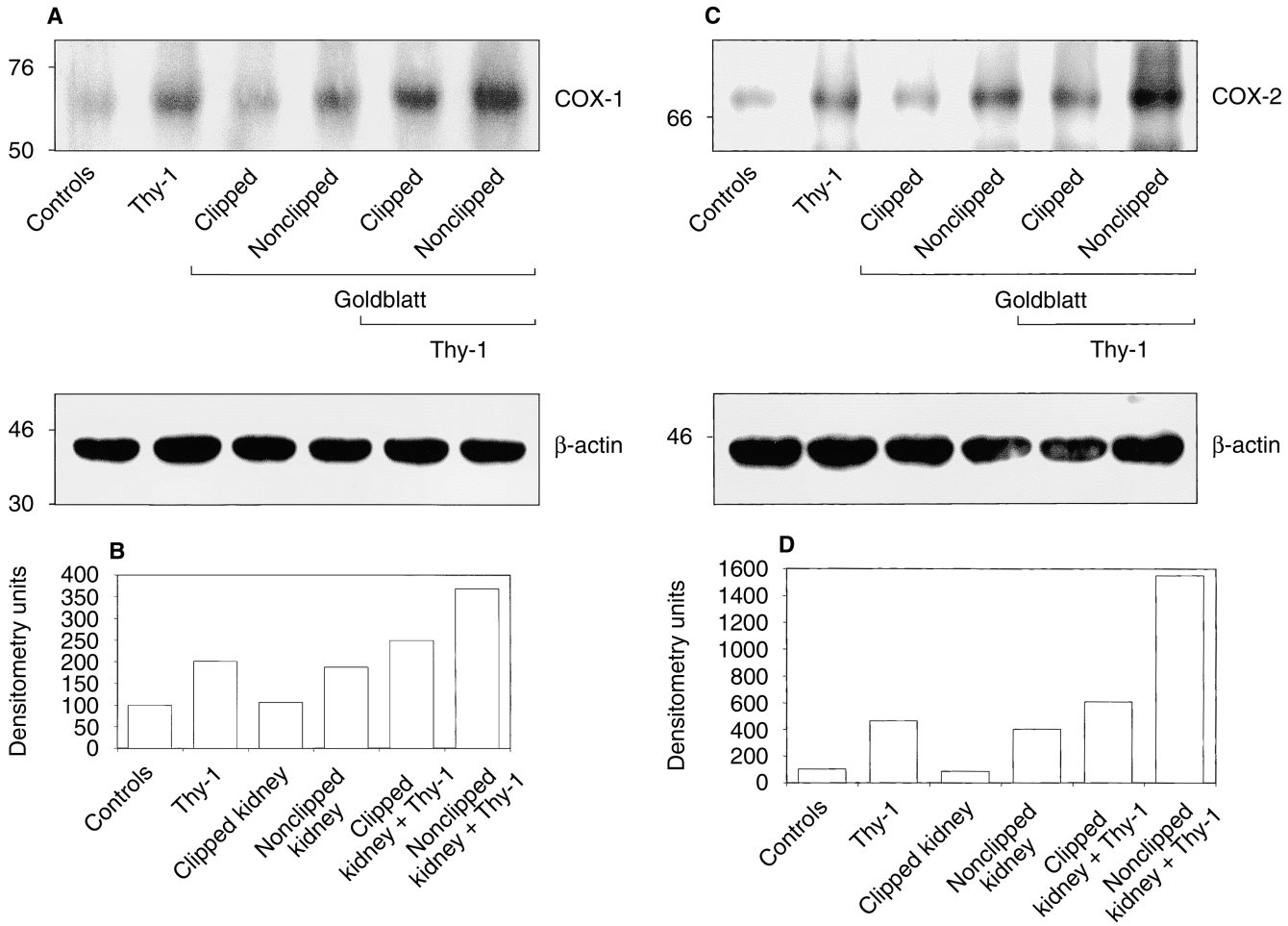

Fig. 8. Western blotting for COX-1 (*A*) and COX-2 (*C*) from glomerular proteins. Both enzymes were induced in glomeruli from normotensive nephritic rats as well as in the clipped and in the nonclipped kidney from nephritic 2K1C rats. The blots were washed and re-incubated with an antibody against β-actin. The glomerular lysate used in both Western blots was pooled from 3 to 6 kidneys in each group. A very similar pattern was found in two Western blots from an independent second set of experiments using again pooled proteins from 2 to 6 kidneys in each group. (*B* and *D*) Densitometric analyses of the COX bands from both blots are shown below.

merular levels of prostaglandins in rats with 2K1C hypertension [24]. Therefore, a differential induction of COX-1 and COX-2 might cause the different development of damage in the Goldblatt rats. The glomerular abundance of COX-1 and COX-2 was evaluated by Western blotting as an initial screening test. Modestly increased levels of COX-1 and COX-2 were found in the nonclipped kidney compared with the clipped kidney and controls (Fig. 8 A, C). Glomerular expression of both proteins was further increased in normotensive nephritic rats, in the clipped kidney and in the nonclipped kidney of nephritic 2K1C rats.

Removal of the clip

We next asked whether the aggravation of glomerular damage in the nonclipped and sparing of the clipped kidney depends on the presence of the stenosis and high blood pressure or is due to irreversible structural changes in both kidneys at the time when repetitive injections of the Thy-1 antiserum started. Therefore, nephritis was induced in rats immediately after removal of the clip. Systolic blood pressure was normotensive six weeks after removal of the clip in all four groups studied [controls 133 ± 4, Thy-1 108 ± 6, 2K1C(ex) 131 ± 5, 2K1C(ex) + Thy-1 135 ± 5 mm Hg]. Survival was 100% in all four groups. As shown in Figure 9 glomerular damage increased in all nephritic kidneys and no significant difference could be detected between the formerly clipped kidney and the nonclipped kidney in nephritic 2K1C rats. In addition, albuminuria was not significant different between 2K1C(ex) (1.38 ± 0.38 mg/24 h) and 2K1C(ex) + Thy-1 (5.29 ± 2.15 mg/24 h). Albuminuria was 0.26 ± 0.10 mg/24 h in controls and 2.72 ± 0.88 mg/24 h in normotensive Thy-1 rats. As shown in Figure 10, glomerular binding of IgG and induction of TGF-β occurred to a similar extent in normotensive nephritic rats and both kidneys of the unclipped nephritic rats.

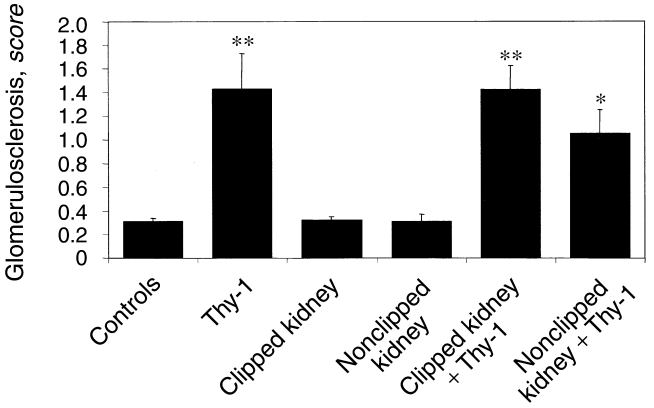

Fig. 9. Glomerular damage evaluated by scoring. No more damage was found in the formerly nonclipped kidneys 6 weeks after removal of the clip. Glomerular damage was significantly increased in normotensive nephritic rats and in the formerly clipped and nonclipped nephritic kidneys. $*P < 0.05$, $**P < 0.01$ vs. normotensive controls.

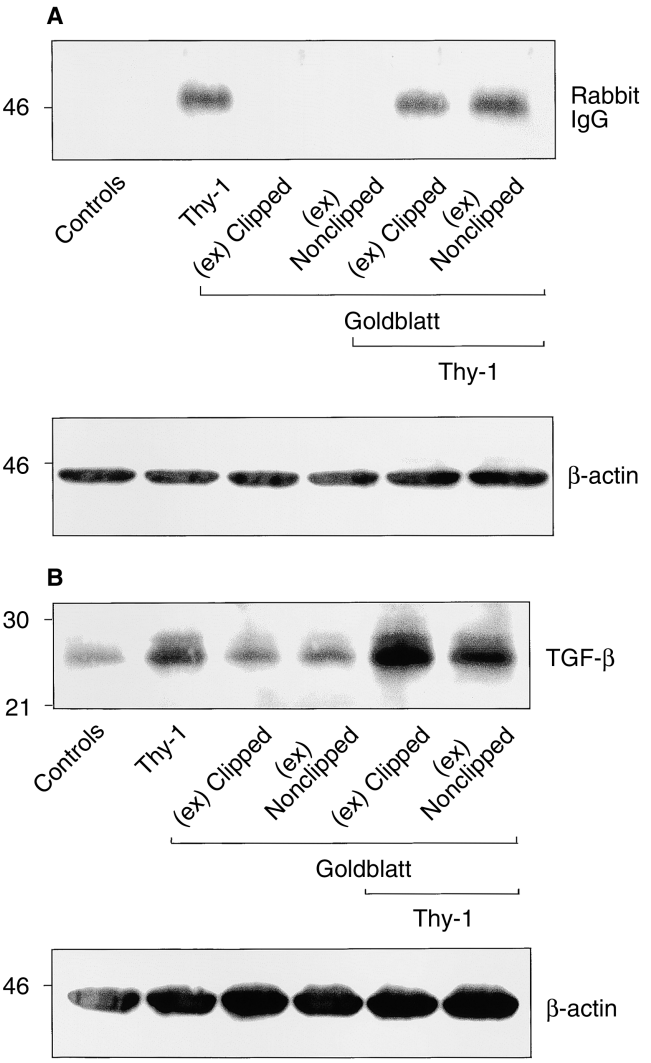

Fig. 10. Western blotting from glomerular proteins for rabbit IgG (*A*) and TGF-β (*B*) is shown. The glomerular lysate used in the Western blot was pooled from 4 rats in controls and Thy-1, respectively, and 9 rats in both clipped (ex) groups, respectively.

DISCUSSION

Systemic hypertension is a major risk factor that determines the rate of progression of renal disease. We and others have recently studied and characterized the model of anti-thymocyte serum induced glomerulonephritis [6–9, 13, 20, 21, 23, 25]. The Thy-1 nephritis is a model of immune mediated glomerular injury, in which hypertension normally does not occur. This makes it a good model to study the effects of superinduced hypertension on glomerular and tubulointerstitial injury because hypertension and nephritis can be experimentally separated. In agreement with the detrimental effect of high blood pressure on renal disease, we described aggravation of the glomerular lesion five days after induction of the Thy-1 nephritis in rats with 2-kidney, 1-clip hypertension [8]. In contrast, we found complete healing of the glomerular lesions of the Thy-1 nephritis in renovascular hypertensive rats after six weeks, indicating that neither glomerular hypertension and hypertrophy nor ischemia inhibits healing of this form of glomerulonephritis [7].

The mechanisms by which hypertension damages the kidney in renal disease are incompletely understood. Our present studies document accelerated development of glomerular lesions in the kidney exposed to hypertension compared with the clipped kidney in the same rat or to kidneys of normotensive nephritic animals. Furthermore, the clipped kidneys were almost completely protected from sclerotic nephritic lesions. The most straightforward explanation for these findings is nephron hemodynamics. Normal to high glomerular pressure is an absolute requirement for injury [3]. Elevated glomerular capillary pressure and glomerular blood flow as well as increased single nephron glomerular filtration rate have been found in the nonclipped kidney of Goldblatt hypertensive rats [26, 27]. Thus, the advanced glomerular damage in the nonclipped kidney might be caused by altered glomerular hemodynamics. Although single nephron hemodynamics are less well documented in the clipped kidney [26], it is reasonable to assume that glomerular hemodynamics in these kidneys may be altered in a direction that protects the glomerulus from the chronic immunological damage. Angiotensin-converting enzyme (ACE) inhibition lowers glomerular pressure, which is beneficial in several models of renal disease. Therefore, the protection against glomerular damage may be explainable by the altered hemodynamics in the clipped kidney that somehow mimic the hemodynamic effects of ACE inhibition. Additional experiments will be clearly required to address these issues. However, in a previous study we did not find a protective effect of the clipped kidney on the early phase of the

Thy-1 nephritis, since proliferation and matrix deposition were not different between kidneys of normotensive nephritic rats and the clipped kidney of Goldblatt hypertensive nephritic rats [8]. Goldblatt hypertension is at least in part an angiotensin II-dependent model of hypertension [28]. Especially the clipped kidney is rich in renin [29]. Although the harmful effects of angiotensin are well established [30], angiotensin II is also a potent inducer of prostaglandins in isolated glomeruli [31]. We have recently demonstrated that administration of prostaglandin E2 reduces collagen deposition in rats with Thy-1 nephritis [32]. Therefore, it is reasonable to speculate that increased production of prostaglandins or other antifibrogenic substances in the clipped kidney might have contributed to the reduced glomerular damage. We evaluated glomerular expression of COX-1 and COX-2 as a screening test to determine whether different induction of prostaglandins may influence glomerular damage. However, the pattern of induction of COX-1 and COX-2 was similar in the clipped as well as in the nonclipped kidney of nephritic 2K1C rats, making this possibility unlikely. In addition, angiotensin II receptors are down-regulated in the clipped kidney [29]. Therefore, the increased glomerular and tubulointerstitial damage in the nonclipped kidney and complete protection of the clipped kidney could be at least in part explained by the fact that the angiotensin II receptors are down-regulated in the clipped kidney. However, the function of the clipped kidney depends on angiotensin II, since prolonged ACE-inhibition induces severe tubulointerstitial damage in the clipped kidney as shown by us and others [11, 33].

A significant increase of albuminuria was found in hypertensive nephritic rats in the present study. We have not separately assessed albuminuria of the clipped and nonclipped kidney because we know from our previous work in 2-kidney, 1-clip hypertensive rats that most of the proteinuria is derived from the nonclipped kidney [12]. Moreover, 1-kidney, 1-clip hypertensive rats do not develop significant albuminuria (unpublished data). From these observations we believe that the increased albuminuria in the nephritic 2K1C rats originates from the nonclipped kidney.

We have recently studied in detail the increased glomerular size of the nonclipped kidney in rats with 2-kidney, 1-clip hypertension [7, 11, 12]. According to the law of Laplace an increased glomerular size causes an increased wall tension. The increased glomerular size in the nonclipped kidney may therefore enhance the damage caused by the immunologic events of the nephritis. Very similarly, the increased glomerular size in the normotensive nephritic rats may have facilitated the development of glomerular damage. However, glomerular hypertrophy by itself is not sufficient to induce sclerosis since the increased glomerular size of the clipped kidney in nephritic 2K1C rats did not enhance glomerular damage.

It has been described that tubulointerstitial changes precede glomerular damage in the nonclipped kidney of Goldblatt hypertensive rats [17, 18]. Glomerular and tubulointerstitial damage in the nonclipped kidney correlates well in the present study; however, a dissociation was found in the clipped kidney. Whereas the glomerular structure is well preserved, tubulointerstitial damage occurs in all studied groups. This dissociation clearly needs to be investigated further. We are currently attempting to understand the properties by studying separately tubular and glomerular expression of potential modulators of damage in Goldblatt hypertensive rats (abstract; Wenzel et al, *J Am Soc Nephrol* 11:449, 2000).

One of the most intensively studied profibrogenic cytokines is TGF-β [34]. We have recently described up-regulation of TGF-β in the nonclipped kidney of rats with 2K1C hypertension [7, 22]. In the present study there was a modest up-regulation of TGF-β in the clipped kidney and in normotensive rats after induction of chronic nephritis. Interestingly, a strong overexpression of TGF-β was found in the nonclipped kidney after induction of the nephritis that matched the increased glomerular damage and matrix deposition in this kidney. However, despite increased levels of TGF-β no glomerular damage was found in the clipped kidney of hypertensive nephritic rats, indicating that increased levels of TGF-β itself are not sufficient to induce glomerular damage. Glomerular damage and induction of TGF-β of similar magnitude were found in the clipped and nonclipped kidneys after removal of the clip, indicating that the clip as well as arterial hypertension are necessary for the different induction of damage in both kidneys. It shows that the enhanced damage in the nonclipped kidney and the protection of the clipped kidney are not caused by the structural changes like glomerular hypertrophy or atrophy six weeks after clipping of the renal artery.

To our knowledge, the present study is the first demonstrating enhanced glomerular damage in the nonclipped kidney and sparing of the glomeruli in the clipped kidney of Goldblatt hypertensive rats with the repetitive hit Thy-1 nephritis. Our data are in line with the finding of Mauer and colleagues, who described less diabetic lesions in the glomeruli of the clipped kidney of diabetic rats with Goldblatt hypertension [35]. Moreover, there is an observation in a patient with unilateral renal artery stenosis and diabetes that the stenotic kidney had only ischemic changes while the nonclipped kidney demonstrated advanced diabetic glomerulopathy [36]. Palmer, Eversole and Stamey also reported unilateral nephritis in patients with renal artery stenosis [37]. In addition, Dikman et al described in a clinical report a patient with unilateral glomerulonephritis, in whom a hydronephrotic kidney was spared from nephritic damage [38]. Our present

observation and these clinical reports are in line with a growing body of literature that certain models of renal disease induce a cross tolerance to a subsequent injury. It was recently shown that the remnant kidney is protected against ischemia/reperfusion injury [39]. In addition, endotoxin-induced cross tolerance to subsequent renal ischemia/reperfusion injury has been described [40]. We even found that angiotensin II infusion surprisingly ameliorated the early phase of the Thy-1 nephritis [41]. Although it is most likely that reduced glomerular pressure in the clipped kidney explains that no glomerular damage developed in this kidney, another possibility is that mild ischemia in the clipped kidney induced cross tolerance against the inflammatory injury of the chronic Thy-1 nephritis.

Three major conclusions can be drawn from these data and our previous studies [7, 8]. First, renovascular hypertension aggravates the glomerular damage in the early phase of the Thy-1 glomerulonephritis. Second, whereas renovascular hypertension does not prevent healing of the one shot Thy-1 nephritis, an increased glomerular damage is found in the nonclipped kidney after induction of the repetitive hit model of this nephritis. The third and particularly interesting observation is that despite activation of glomerular cells and an increased expression of TGF-β and COX-1 and -2 as well as an increased glomerular volume, no glomerular damage develops in the clipped kidney of nephritic 2K1C rats. The mechanisms protecting the clipped kidney remain to be determined.

In summary, induction of the repetitive hit model of the Thy-1 nephritis in rats with 2-kidney, 1-clip hypertension induces severe glomerular damage in the nonclipped kidney. In contrast, no glomerular damage develops in the clipped kidney after induction of this chronic glomerulonephritis.

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