Antihypertensive therapy induces compartment-specific chemokine expression and a Th1 immune response in the clipped kidney of Goldblatt hypertensive rats

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CHEMOKINE EXPRESSION AND FIBROSIS IN HYPERTENSIVE RATS

The American Physiological Society’s guidelines of experimental animal research were followed, and approval was obtained from the University Animal Care Committee.

Systolic blood pressure. Systolic blood pressure was measured by tail cuff plethysmography using a semiautomatic device (TSE-Systems, Bad Homburg, Germany) in awake rats as described (25). The animals were placed in individual metabolic cages, and 24-h urine collections were made for determination of creatinine.

Removal of the kidney. At the end of the experimental protocol, blood was drawn from the aorta, and the stenosed kidney was perfused with ice-cold phosphate-buffered saline until the kidney blanched. The kidneys were removed, and slices were fixed in 4% buffered formalin for histology or frozen on dry ice and covered in Tissue Tek for in situ hybridization and laser microdissection (16). Plasma and urinary creatinine was measured using an autoanalyzer (Hitachi 717; Roche, Mannheim, Germany).

Preparation of total RNA and real-time RT-PCR. Total RNA from the cortex of the clipped kidney was prepared by phenol-chloroform extraction after direct lysis of the glomeruli in 4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7, 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol as described previously (16). RNA from microdissected tissues was isolated using the RNA Nano prep kit (PALM, Bernried, Germany), as we previously described (16). Real-time RT-PCR was performed with ABI Prism 5700 using SYBR green as dye, as previously described (16). Quantitation was performed using 18S as an internal control to correct for small variations in RNA quantity and cDNA synthesis, essentially as described by ABI Prism.

The following rat-specific PCR-primers were used in this study: primer, rMCP-1, forward (Fw) 5’-CTG TCT CAG CCA GAT GCA GTT AA 3’; reverse (Rev) 5’-TGG GAT CAT CTT GCC AGT GA 3’; rIP10, Fw 5’-GGG CCA TAG GAA AAC TTG AAA TC 3’; Rev 5’-CAT TGT GGC AAT GAT CAC AAC AT 3’; rMig, Fw 5’-AAT CCC TAA ACC ACC ACA AAG AGT 3’; Rev 5’-GCC GAA GTG ATC TTC GGT CCT C 3’; rCXCR3, Fw 5’-TGG GCT CGT GTC TCT GCT GCA A 3’; Rev 5’-GCT TAT ACA GGC CAG CAG GAA 3’; rCAM-1, Fw 5’-TGA GAG GTG GAT GGG AA AAG TT 3’; Rev 5’-GTC CCT CAA GCA GTC CTT CTT C 3’; osteopontin, Fw 5’-CGA TGA GCC GCT TCA AAC GAT C 3’; Rev 5’-CCC GCT CCT GCT GTC CTG ATC A 3’; rBNF-1, Fw 5’-GCC AAC TGG TAC GGT AAC AAC AAC 3’; Rev 5’-TAT ATT CTG ATG ACA GGT GGT GAA 3’; plasminogen activator inhibitor-1 (PAI-1), Fw 5’-CAA GTC TGG TAG CAT CAC CAT CTC 3’; Rev 5’-CCG GAG TGG TGG ACG TAC CAG TGT 3’; rCollagen a1 I, Fw 5’-CCG GCC CCT GCT CCT GCT CTT A 3’; Rev 5’-AGG GAC CCT TAC GGC ATT GT 3’; rMMP-2, Fw 5’-CCG ATG AAG CCT GCT TGT TTA CCA 3’; Rev 5’-TGG AGG CCG AAC GGA ACT 3’.

The presented results are means of four independent RT-PCRs, each performed with different RNAs pooled from two to four rats.

In situ hybridization. In situ hybridization procedures were performed as described previously (16, 20). The probes for rat IP10, Mig, MCP-1, and PAI-1 used for in situ hybridization were prepared by in vitro transcription of subcloned cDNA. The IP10 probe corresponds to nucleotides 125–431 of sequence NM_139089, the Mig probe corresponds to nucleotides 74–428 of sequence BC087594, the MCP-1 probe corresponds to nucleotides 278–631 of sequence M57441, and the PAI-1 probe corresponds to nucleotides 1121–1384 of sequence M24067. Antisense and sense RNA transcripts were labeled with 35S-UTP (3000 Ci/mmol; Amersham) and served as hybridization probe and control, respectively. Free nucleotides were separated with a Sephadex G-50 column (quick-spin columns, Roche). In situ hybridization was performed on 12-μm cryosections of rat renal tissue using 5 ng of the 35S-labeled antisense and sense RNA probes, respectively. Sections were exposed overnight to Kodak Biomax MR X-ray films. Subsequently, they were treated with Kodak NTB-3 nuclear track emulsion and exposed for 3 wk, followed by development in Kodak D19 and fixation with Kodak Unifix. Finally, sections were stained with Mayer’s hemalum.

Tissue laser microdissection. Microdissection was carried out on 8-μm-thick cryosections of rat kidney tissue using the PALM MicroBeam IP 230V Z microscope for laser pressure catapulting (PALM), as we have described (16). Previous to the dissection procedure, tissue sections were stained with a short alcohol-based cresyl violet acetate protocol; cryosections were air dried for 1 min and subsequently incubated for 2 min in precooled 75% ethanol. For staining, sections were dipped for 20 s in 1% cresyl violet acetate dissolved in ethanol. Finally, slides were washed in 75 and 100% ethanol for 30 s each and allowed 10 min to air dry. Two different tissue types were selectively cut out: 1) areas with inflammatory cell clusters and 2) regions of the same kidneys without inflammatory cell clusters. RNA from microdissected tissue was prepared using the PALM RNA extraction kit according to the manufacturer’s protocol.

Histology. For histocytochemistry, 4% Formalin-fixed kidney slices were paraffin embedded, cut into 1- to 3-μm-thick sections, and stained with periodic acid Schiff (PAS, Sigma). For immunohistochemistry, 1- to 3-μm paraffin sections were deparaffinized and rehydrated. Staining occurred after antigen retrieval with protease-24 (15 min, 5 mg/ml; Sigma) or by microwave treatment with Tris base saline Tween-EDTA, pH 9.0, or citrate buffer, pH 6.1, for 25 min. After blocking of unspecific binding with 5% normal horse or goat serum (Vector) for 30 min at room temperature, sections were stained with either an antibody directed against the monocyte-specific marker ED-1 (1:300, 1.5 h at room temperature; Serotec) to evaluate renal monocyte/macrophage (M/M) infiltration, an antibody against the T-cell marker CD3 (1:400, overnight at 4°C, Clone 14F; Biozol, Eching, Germany), an anti-proliferating cell nuclear antigen antibody (1:200, overnight at 4°C; Ab-1; Calbiochem), or a B cell-specific antibody against CD79 (1:600, 1 h at room temperature, clone HM57; Dakopatts). Secondary antibodies (biotinylated affinity-purified donkey IgG) were incubated for 30 min at room temperature at a 1:400 dilution (all secondary antibodies were purchased from Jackson Immunoresearch Laboratories). Signal amplification was performed with the ABC-AP kit from Vector according to the manufacturer’s instructions. Color development was done with Neufuchsin as a substrate. Nuclei were counterstained with Mayer’s hemalum for 1 min and differentiated with HCl-ethanol. Smooth muscle cell actin (SMA) staining and quantitation were performed as we recently described (8).

Matrix metalloproteinases. Gelatin zymography of matrix metalloproteinases (MMPs) was performed as we have described (27). Renal cortex tissue was homogenized in dissociation buffer (1) and shaken overnight at 4°C, and supernatant was used for zymographic analysis. Equal amounts of protein were loaded onto the gel, and the lytic band intensity was scanned by a Bio-Rad gel scanner. MMP-2 was identified by size and positive controls.

Statistical analysis. Results are expressed as means ± SE unless stated otherwise. Statistical significance was defined as P < 0.05. For multiple comparisons, we used the Kruskal Wallis test with post hoc analysis according to Mann-Whitney U-test. P values were corrected for multiple testing.

RESULTS

General parameters. The systolic blood pressure, body weight, and relative weight of the clipped kidney are shown in Table 1. The blood pressure reduction with 50 mg/kg dosing was not significant compared with untreated Goldblatt rats. Treatment with 150 and 450 mg/kg vasopressidase inhibitor lowered blood pressure dose dependently. Intensified antihypertensive therapy decreased body weight significantly compared with untreated Goldblatt rats. The relative weight of the clipped kidney of treated rats decreased dose dependently compared with Goldblatt rats; however, this was not statistically significant. Treatment of normotensive rats with 450 mg/kg vasopressidase inhibitor decreased systolic blood press-
ure significantly compared with normotensive controls (85 ± 4 vs. 118 ± 3 mmHg, \( P < 0.05 \)).

**Renal morphologic changes.** Light micrographs of the changes in the clipped kidney are shown in Fig. 1. No tubulointerstitial abnormalities were found in normotensive controls (Fig. 1A). The interstitium was modestly increased, and occasionally focal tubular damage was found in the clipped kidney of hypertensive rats (Fig. 1B). In contrast, interstitial widening, severe tubular atrophy, and dilatation were found in clipped kidneys of treated rats, and the abnormalities worsened with the intensity of blood pressure reduction (Fig. 1, C–E). In addition to a diffuse interstitial infiltration of mononuclear cells, dense inflammatory cell clusters were found throughout the tubulointerstitial compartment in rats with blood pressure reduction by vasopeptidase inhibition (Fig. 1F). Glomeruli appeared normal in all animal groups. Treatment of normotensive animals with 450 mg/kg vasopeptidase inhibitor did not induce any renal injury as shown in Fig. 1H compared with untreated normotensive controls (Fig. 1G).

**Immunohistochemical characterization of intrarenal infiltrates.** Immunohistochemistry of serial sections identified mainly CD3-positive T cells and ED-1-positive M/M in the clusters as shown in Fig. 2, A and B. Only a few proliferating cells were found in the clusters, suggesting infiltration rather than local proliferation inside the kidney (Fig. 2C). Moreover, the clusters surprisingly contained a substantial number of B cells (Fig. 2D). Inflammatory cell clusters were almost only found in relevant numbers in kidneys of animals with intensified blood pressure reduction as shown in Fig. 2E.

The diffuse interstitial infiltrate consisted of almost exclusively ED-1-positive M/M and some CD3-positive T cells. Assessment of intrarenal leukocytes revealed a dose-dependent infiltration of ED-1-positive M/M, as shown in Fig. 2F, and some CD3-positive T cells. Infiltration of M/M into the clipped kidney has been described previously (6, 7). No relevant glomerular infiltration of immune cells could be seen. These strict dose-dependent correlations argue for a functional role of blood pressure lowering for infiltration of inflammatory cells into the stenosed kidneys as well as cluster formation.

**Intrarenal Th1-type chemokine expression.** Given the massive infiltration of T cells and M/M into the kidneys of anti-hypertensively treated rats, we performed real-time RT-PCR analyses of mRNA from whole kidney cortex of treated animals and controls to determine whether the immune reaction was consistent with a Th1 response. Anti-hypertensively treated rats demonstrated a Th1 phenotypic pattern of chemokines in their kidneys as manifested by increased mRNA expression of the Th1 chemokines IP-10 (Fig. 3A) and Mig (Fig. 3B) as well as the Th1 cytokine IFN-\( \gamma \) (Fig. 3C). In addition, the chemokine MCP-1 increased up to 240-fold in the clipped kidney of antihypertensively treated rats (Fig. 3D), while regulated on activation, normal T expressed and secreted (RANTES) was only marginally expressed. All these Th1-associated molecules were upregulated dose dependently with intensification of blood pressure reduction (Fig. 3, A–D). In addition, PCR analysis revealed increased expression of the cell surface adhesion molecule (intercellular adhesion molecule) ICAM-1 and of the chemoattractant protein osteopontin in the clipped kidney participating probably in adhesion and subsequent penetration of leukocytes into the tissue. However, the induction was not statistically significant. Our data resemble earlier reports by Haller et al. (7) and Gauer et al. (6). Haller et al. detected ICAM-1 in the clipped kidney by immunohistochemistry, and Gauer et al. found increased expression of osteopontin by Northern blot analysis. Interestingly, similar to our treated group with moderate blood pressure reduction, expression of osteopontin was not upregulated by mild blood pressure reduction in their study, despite enhanced infiltration of ED-1-positive cells.

Real-time RT-PCR analyses of mRNA from whole kidney cortex of antihypertensively treated normotensive rats revealed no change in expression of the chemokines IP-10, Mig, and MCP-1 as well as IFN-\( \gamma \) (treated vs. untreated rats: IP-10 1.6-fold, Mig 1.1-fold, MCP-1 0.7-fold, IFN-\( \gamma \) 1.2-fold; PCR analysis performed in duplicate with pooled RNA from 4 animals), indicating that the observed effects are not due to toxic or unspecific effects of the vasopeptidase inhibitor or blood pressure reduction by itself.

**Compartment-specific chemokine mRNA expression.** To analyze the cellular origin of the mRNA expression of the upregulated chemokines, we performed in situ hybridization studies. Controls and untreated hypertensive rats revealed almost no chemokine expression. A strong expression of IP10 and Mig mRNA was detected almost exclusively in the inflammatory cell clusters in the intensively treated animals (Fig. 4, A and B). This chemokine expression pattern overlaps with the pattern of infiltrating T cells. Interestingly, expression of the most upregulated chemokine, MCP-1, was found in the clusters (Fig. 4C). In addition, a diffuse (Fig. 4D) and tubular staining pattern (Fig. 4E) was seen. Infiltration of M/M was detected in the same anatomical regions as MCP-1 mRNA expression, namely, focally in the clusters and scattered throughout the cortex (see above). The sense probes revealed only background signals (data not shown).

**Laser microdissection of renal tissue.** To verify the compartment-specific expression pattern of IP10 and Mig mRNA, laser microdissection of the cell cluster was performed as shown in Fig. 5, A and B. Real-time PCR analyses of mRNA from

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### Table 1. Systolic blood pressure, body weight, and relative weight of clipped kidney

<table>
<thead>
<tr>
<th></th>
<th>Systolic Blood Pressure, mmHg</th>
<th>Body Weight, g</th>
<th>Relative Weight of Clipped Kidney, g/100 g</th>
</tr>
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<tbody>
<tr>
<td>Controls</td>
<td>12</td>
<td>121 ± 2</td>
<td>526 ± 23</td>
</tr>
<tr>
<td>Goldblatt</td>
<td>12</td>
<td>176 ± 48</td>
<td>499 ± 17</td>
</tr>
<tr>
<td>Moderate blood pressure reduction</td>
<td>10</td>
<td>168 ± 8</td>
<td>503 ± 19</td>
</tr>
<tr>
<td>Intermediate blood pressure reduction</td>
<td>11</td>
<td>133 ± 77†</td>
<td>463 ± 17</td>
</tr>
<tr>
<td>Intensified blood pressure reduction</td>
<td>9</td>
<td>94 ± 4†</td>
<td>437 ± 14*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *\( P < 0.0125 \) and †\( P < 0.001 \) vs. Goldblatt. ‡\( P < 0.0125 \) and §\( P < 0.001 \) vs. controls.
microdissected tissue of kidneys with intensified blood pressure reduction showed a strong expression of IP10 (39.8-fold), Mig (53.8-fold), and their corresponding Th1 receptor CXCR3 (3.2-fold) in the cell clusters compared with interstitial non-cluster tissue obtained from the same kidney sections. MCP-1 mRNA, even though expressed in both renal compartments, was found more abundantly in the cell clusters (31.4-fold of noncluster tissue). RANTES mRNA was generally only

Fig. 1. Kidney histological changes. A–E: light micrographs of the tubulointerstitial changes in the kidneys. Well-preserved tubuli were found in controls (A). Modest tubulointerstitial damage was seen in the clipped kidney of Goldblatt rats (B). Tubulointerstitial injury increased dramatically with moderate (C), intermediate (D), and intensified blood pressure reduction (E). Dense inflammatory cell clusters were found in rats with blood pressure reduction. Treatment of normotensive animals with 450 mg/kg vasopeptidase inhibitor did not induce any renal injury (H) compared with untreated controls (G). Magnification: ×100 (A–E) and ×200 (E–H).
slightly expressed, confirming the real-time results from whole kidney cortex. Within the clusters, the expression was a little stronger than in noncluster tissue (1.8-fold). Therefore, the results from microdissection with subsequent semiquantitative real-time PCR analysis strongly support the in situ hybridization data.

Quantification of renal injury. Myofibroblasts appear within the interstitium and function as the primary site of increased matrix synthesis. α-Smooth muscle cell staining is a marker of interstitial myofibroblast and can be used as a marker of interstitial injury. α-Smooth muscle cell-positive area in the clipped kidney increased progressively with blood pressure lowering (Fig. 6A). Lowering of blood pressure also progressively increased interstitial volume measured by point counting, confirming the interstitial injury with another methodical approach (Fig. 6B).

Fibrogenic marker. To evaluate the mechanisms leading to the progressive tubulointerstitial damage in the clipped kidney,
the expression of the profibrotic factor PAI-1 was evaluated. Lowering of blood pressure increased expression of PAI-1 in the clipped kidney (Fig. 7A). To analyze the localization of mRNA expression, we performed in situ hybridization for PAI-1. As shown in the overview autoradiogram (Fig. 7B) and in more detail in Fig. 7, C and D, PAI-1 expression was found diffusely in the whole cortex with tubular and periglomerular staining pattern. Finally, we examined expression of the interstitial collagen I. Lowering blood pressure led to increasing α1-collagen I mRNA expression in whole cortex, cumulating to a 600-fold upregulation with intensified blood pressure reduction (Fig. 7E).

MMPs. Matrix deposition is regulated by a balance of matrix synthesis and breakdown by MMPs. Surprisingly, MMP-2 was

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dramatically upregulated after lowering of blood pressure as evaluated by RT-PCR of mRNA and zymography (Fig. 8, A and B). Densitometry of the zymographs is shown in Fig. 8C. This clearly suggests that MMP-2 is a major modulator of the tubulointerstitial damage in the clipped kidney.

Similar to the chemokine data, PCR analyses of mRNA from whole kidney cortex of antihypertensively treated normotensive rats revealed no change in expression of PAI-1 and MMP-2 compared with untreated controls (treated vs. untreated rats: PAI-1 1.0-fold, collagen I 2.1-fold, MMP-2 1.2-fold), again indicating that the observed effects are not due to toxic or unspecific effects of the vasopeptidase inhibitor or blood pressure reduction by itself.

Renal function. To assess overall renal function, plasma creatinine and creatinine clearance were measured. Antihypertensive therapy decreased renal function, as measured by
creatinine clearance, dose dependently, reaching statistical significance in rats with intensified blood pressure reduction (Table 2). A reason might be the loss of function of the clipped kidney.

**DISCUSSION**

Unwanted deleterious effects of RAS blockade can occur in patients with reduced renal perfusion because of stenosis
Fig. 7. Fibrogenic response. RT-PCR analyses of mRNA from whole cortex revealed increased plasminogen activator inhibitor-1 (PAI-1) mRNA expression with blood pressure reduction (A). In situ hybridization showed widespread expression in the cortical interstitium (B), some diseased tubuli (C), and Bowman’s capsules (D). α1-Collagen I mRNA increased >600-fold in the clipped kidney of rats with intensified blood pressure reduction (E). *P < 0.05 vs. Goldblatt.
of the renal artery or arteriosclerotic stenoses of intrarenal small vessels (21). To gain more insight into the pathophysiology of acute renal failure induced by blood pressure reduction, we chose the two-kidney, one-clip rat model of Goldblatt hypertension. It is well known that blood pressure reduction increases survival and decreases renal injury in the nonclipped kidney (25, 23), and we have reported the beneficial effect of different levels of blood pressure reduction on the nonclipped kidney in abstract form previously (18). The tubulointerstitial damage that occurs in the clipped kidney has been described by us and others (9, 17, 25). Still, little is known about the mechanisms and mediators respon-

![Graph showing relative RNA expression of MMP-2](image)

**Fig. 8.** Matrix metalloproteinases (MMPs). RT-PCR analyses of mRNA from whole cortex revealed increased MMP-2 mRNA expression with blood pressure reduction (A). Zymography showed that the increased mRNA expression was accompanied by enhanced activity. The densitometry data from 4 zymographs are shown in C. *P < 0.05 vs. Goldblatt.
sible for the pronounced inflammation and fibrosis in the clipped kidney.

In the present study, we lowered blood pressure with a vasopeptidase inhibitor. Vasopeptidase inhibitors are a class of drugs comprising single molecules that simultaneously inhibit both ACE and neutral endopeptidase. It has been shown that vasopeptidase inhibitors exhibit greater antihypertensive effects than those elicited by ACE inhibitors (25). The use of a vasopeptidase inhibitor avoids introduction of covariables, as when using combination therapy to lower blood pressure, effectively in this model of hypertension. It would be of interest to compare the blood pressure reduction by the vasopeptidase inhibitor with an anti-hypertensive therapy, which does not inhibit the RAS, to see whether blood pressure reduction by itself or blood pressure inhibition due to ACE inhibition causes cluster formation and fibrotic response. However, as we have shown previously, we are unable to lower blood pressure sufficiently in our model by triple therapy without ACE inhibition (23, 24).

Many inflammatory renal diseases, such as acute renal transplant rejection (15, 20) and glomerulonephritis (19), are to a significant part driven by a Th1 immune response. The chemokine receptors characteristically found on the surface of Th1-type T cells are CXCR3 and CCR5 interacting with their corresponding chemokine ligands IP10, Mig, ITAC and RANTES, MIP-1α and MIP-1β, respectively. The first new finding of the present study was that, in addition to diffuse interstitial leukocyte infiltration, dense clusters of inflammatory cells were present in the kidneys of antihypertensively treated rats. Staining for the proliferation marker PCNA was negative, suggesting infiltration of the immune cells into the kidney rather than local expansion. Furthermore, we found that the kidneys had dose dependently elevated levels of MCP-1 and the Th1-type chemokines IP10 and Mig. By immunohistochemistry, we found a diffuse interstitial infiltration with ED-1-positive monocytes, whereas in the clusters, we found predominantly CD3-positive T cells in addition to monocytes. The T cells are likely of a Th1 phenotype, as the kidneys had a marked induction of mRNA expression of the Th1 chemokines IP10 and Mig as well as the Th1 cytokine IFN-γ. By in situ hybridization, we found intense staining for IP10 and Mig exclusively in the clusters. MCP-1 positivity, on the other hand, was found not only in the clusters but also diffusely in the whole interstitium, thereby matching the pattern of ED-1-positive cell infiltration. The in situ data were confirmed and extended by laser tissue microdissection, demonstrating selective upregulation of IP10, Mig, CXCR3, and MCP-1 mRNA in the inflammatory clusters. In the present investigation, therefore, we demonstrated for the first time formation of leukocyte cell clusters associated with a compartment-specific chemokine expression of the Th1 type in the clipped kidney of antihypertensively treated Goldblatt rats. Although their functional role in the tubulointerstitial injury is unclear at present, one might speculate the following. The observed cell clusters might serve as regions of monocyte activation, as they ensure numerous and repeated direct T cell-monocyte contacts, which are considered to be the most potent stimuli for the activation of monocytes. This local activation of inflammatory cells inside the kidney might be supported further by interaction of T cells with antigen presenting B cell aggregates that are also occasionally found in the cluster. Generally, the clusters might therefore serve as a pathological shortcut for the activation of a rapid and stable immune response independently of the regional lymph nodes.

In the present study, a Th1 response with subsequent upregulation of profibrotic mediators was induced by the vasopeptidase inhibitor, which inhibits the ACE. It was recently demonstrated that the renal injury in AT-1 receptor knockout mice is also associated with a T cell and monocyte response with activation of inflammatory mediators. It is likely that the mechanisms in both models are similar. Furthermore, the absence of a functional RAS has been associated with the development of tubulointerstitial inflammation (13).

The second new observation was that infiltration of inflammatory cells was accompanied by the massive induction of profibrotic mediators in the clipped kidneys after blood pressure lowering. Blood pressure lowering in our model dose dependently increased the accumulation of interstitial myofibroblasts. They may be resident, bone marrow derived, or tubular epithelial cells transformed in myofibroblasts (3, 7, 10, 20). MMPs are a prerequisite for epithelial mesenchymal transformation (EMT) (10), since the tubular cells after EMT are migrating through breaks in the tubular basement membrane that are produced by proteases like MMP-2. It is generally believed that the excessive matrix accumulation seen in fibrotic kidneys results from both overproduction of matrix components and defects in its degradation, i.e., by downregulation of MMPs. However, previous assumptions about matrix proteases in renal fibrogenesis appear to be overly simplistic (11). In a line of mice genetically engineered to overproduce MMP-2 in proximal tubules, these cells undergo spontaneous EMT and develop peritubular fibrosis (2). Moreover, leukocytes themselves use MMPs to pave their way through the different layers of the vascular wall when invading tissues (14). Thus our findings suggest that MMP-2 has additional functions in inflammation and fibrosis. More recently, the attention has focused on another fibrosis-associated protein, called PAL-1, which has a potential role in matrix regulation (5). It is now quite clear that PAL-1 promotes renal fibrosis (3). RT-PCR and in situ hybridization analysis revealed a strong and dose-dependent induction of PAL-1 in antihypertensively treated rats.

One may argue that the fibrotic response could be triggered by hypoxia or ischemia, independent of the inflammation. However, the observed inflammation with a robust compartment-specific expression of chemokines and their specific receptor in combination with a diffuse infiltration of mononuclear cells and circumspect mononuclear cell cluster formation described in our study is quite different from the changes observed in the ischemia/reperfusion model (4). The final proof that CXCR3-bearing leukocytes cause the inflammation in the

### Table 2. Plasma creatinine and creatinine clearance

<table>
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<th>Plasma Creatinine, mg/dl</th>
<th>Creatinine Clearance, ml/min</th>
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<tr>
<td>Control</td>
<td>0.35 ± 0.01</td>
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<tr>
<td>Goldblatt</td>
<td>0.41 ± 0.03</td>
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<tr>
<td>Moderate blood pressure reduction</td>
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<tr>
<td>Intensified blood pressure reduction</td>
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<td>1.8 ± 0.3*</td>
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Values are means ± SE. *P 0.0125 vs. Goldblatt.
clipped kidney and trigger fibrosis will be the induction of Goldblatt hypertension in CXCR3 knockout mice.

From the present data, we suggest that the tubulointerstitial injury induced by blood pressure lowering in the clipped kidney can be arbitrarily divided into sequential or overlapping pathogenetic mechanisms (12). Blood pressure reduction induces 1) an initial renal injury with 2) upregulation of the chemokines IP10, Mig, and MCP-1, which triggers 3) a cellular response phase characterized by inflammatory cell cluster formation with a Th1 response and a more diffuse monocyte infiltration. 4) The infiltration may cause synthesis of fibrogenic growth factors like PAI-1 and MMPs with appearance of myofibroblasts and EMT, which induces 5) excessive accumulation of extracellular matrix proteins. 6) This finally leads to progressive nephron destruction as interstitial capillaries and tubules are obliterated.

In conclusion, we present the first evidence for the compartment-specific expression of Th1-type chemokines and their targeted leukocytes with lymphoid tissue-like cluster formation in the clipped kidney of Goldblatt hypertensive rats due to blood pressure reduction. Finally, we provide evidence demonstrating the matrix metalloproteinase MMP-2 to be a critical mediator of renal fibrosis in the clipped kidney. These findings clearly suggest that interventions targeting Th1 chemokines like IP10 and Mig and/or their receptor CXCR3 or inhibiting MMP-2 merit further evaluation for the prevention of renal failure in ischemic nephropathy.

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