Renal allograft protection with losartan in Fisher→Lewis rats: Hemodynamics, macrophages, and cytokines

FARZAD ZIAI, HIROAKI NAGANO, MAMORU KUSAKA, ANA J. COITO, JULIA L. TROY, KARI C. NADEAU, HELMUT G. RENNKE, NICHOLAS L. TILNEY, BARRY M. BRENNER, and HARALD S. MACKENZIE

Renal Division, Department of Medicine and Department of Pathology, Surgical Research Laboratories, Harvard Medical School and Brigham and Women’s Hospital, Boston, Massachusetts, USA

Renal allograft protection with losartan in Fisher→Lewis rats: Hemodynamics, macrophages, and cytokines.

Background. We sought to assess the effects of angiotensin receptor blockade on glomerular hypertension, macrophage recruitment, and cytokine expression, all of which contribute to the development of chronic graft injury in this model.

Methods. The effects of treatment with the specific angiotensin II type 1 (AT1) receptor antagonist, losartan, were assessed over 24 weeks in F344→LEW rats (LOS, N = 9) versus vehicle-treated F344→LEW controls (CON, N = 9).

Results. U\textsubscript{\text{ProtV}} rose progressively in CON (from 7.0 ± 2.9 to 41 ± 17 mg/day at 24 wk) but remained at baseline in LOS (4.2 ± 0.6 to 9.4 ± 1.3 mg/day, P < 0.05 vs. CON). Glomerular capillary pressure (P\textsubscript{GC}) was increased in CON (71 ± 1 mm Hg at week 20), but remained within the normal range in LOS rats (54 ± 2 mm Hg, P < 0.05). Glomerulosclerosis averaged 0.3 ± 0.2% in LOS versus 4 ± 2% in CON rats (P < 0.05). Tubulointerstitial injury was minimal in both LOS and CON rats (+). The overexpression of renal cortical cytokine mRNA levels for the monocyte chemoattractants, monocyte chemoattractant protein-1 (MCP-1) and RANTES, as well as interleukin-1, inducible nitric oxide synthase, and transforming growth factor-β, assessed by competitive reverse transcription-polymerase chain reaction, was suppressed in LOS versus CON rats at 20 weeks. Macrophage and T-cell numbers were decreased, and MCP-1, RANTES, and intercellular adhesion molecule-1 staining in the graft, identified by immunohistochemistry, were attenuated in LOS versus CON rats.

Conclusions. The renoprotective effects of losartan in F344→LEW rats were associated with lowered P\textsubscript{GC}, inhibition of macrophage chemoattractants and recruitment, and suppression of macrophage-associated cytokines at 20 weeks. These findings suggest that chronic allograft injury in F344→LEW rats is, to a large extent, mediated by angiotensin II-dependent mechanisms and that these involve glomerular hemodynamics, macrophages, and macrophage-associated cytokines.

Recent experimental and clinical evidence emphasizes contributions from both antigen-independent and antigen-dependent factors in the pathogenesis of chronic renal allograft failure [1–6]. A growing body of data also implicates injury processes associated with extensive nephron loss as a significant determinant of progressive graft injury in the F344→LEW rat model of late renal allograft failure. Increased glomerular capillary hydraulic pressure (P\textsubscript{GC}) [7–9] and glomerular hyperfiltration [8, 9] have been observed in F344→LEW rats, suggesting that chronic glomerular injury in this model is driven, at least in part, by glomerular hemodynamic factors. Evidence supporting this view came from studies conducted in our laboratories showing that restoring total recipient kidney mass essentially normalized P\textsubscript{GC} and markedly reduced chronic allograft injury [4, 9].

In a variety of experimental models of chronic renal disease (CRD), elevated P\textsubscript{GC} is maintained, in part, by increased local angiotensin II (Ang II) activity. Pharmacological interruption of the renin-angiotensin system (RAS) either by angiotensin-converting enzyme inhibitors (ACEI) [10] or angiotensin II type 1 (AT\textsubscript{1}) receptor antagonists (AT\textsubscript{1}RAs) [11, 12] has been shown to be highly effective in reducing renal injury in experimental models of CRD. Agents that inhibit the RAS have also been shown to limit chronic renal allograft injury in F344→LEW rats [13–15], although sometimes at levels of systemic blood pressure below the autoregulatory range [13]. However, in addition to its hemodynamic effects, Ang II is also known to stimulate the synthesis of extracellular matrix proteins and cytokines by renal tubule cells in vitro, and may also induce the expression of the...
fibrogenic growth factor transforming growth factor-β (TGF-β) in cultured renal cells [16] and overexpression of cytokines and chemoattractant molecules such as monocyte chemoattractant protein-1 (MCP-1) [17, 18]. Chronic infusion of Ang II at suppressor doses also leads to perivascular fibrosis of arterioles [19]. Proinflammatory cytokines and profibrotic growth factors are thought to play a major role in the pathogenesis of chronic renal allograft injury [20–22]. Several are involved in macrophage recruitment; others are characteristic products of activated macrophage but may be synthesized by renal cells under certain circumstances. We therefore hypothesized that chronic treatment of F344→LEW rats with the AT₁RA losartan would lower glomerular capillary pressures (P_{GC}), ameliorate proteinuria, reduce macrophage recruitment, and retard the development of chronic renal allograft injury. We further hypothesized that the identification of those cytokines in which the expression is modified by losartan would help reveal key molecular mediators of chronic allograft injury in this model.

METHODS

Kidney transplantation was carried out between male Fisher 344 donors and male Lewis recipients under ether anesthesia. The left kidney of each donor was isolated, excised, cooled, and then positioned orthotopically in the left renal fossa of Lewis recipients of similar age and body weight. Vascular and ureteric anastomoses were fashioned end-to-end using 10-0 prolene sutures and without stenting. The recipients underwent ipsilateral (native) uninephrectomy at the time of transplantation; the contralateral native kidney was removed on the seventh postoperative day. All rats received low dose cyclosporine A (5 mg/kg/day IM) until the 10th postoperative day to suppress an early episode of acute rejection; they then went on to survive for prolonged periods but with progressive evidence of chronic rejection [20, 21]. From week 3 onward, recipient animals received losartan (60 mg/L) in the drinking water (LOS, N = 9) or no treatment (CON, N = 9). All rats were housed under standard conditions, receiving standard rodent chow (Purina 2001) and water ad libitum, and were treated in accordance with National Institutes of Health guidelines for the care and use of laboratory animals.

Chronic studies

At three-week intervals, body weight, systolic blood pressure (SBP), and urinary protein excretion rates (U_{prot} V) were determined. SBP was assessed using the tail-cuff method, and U_{prot} V was determined by the sulfosalicylic acid method on 24-hour urine samples collected from metabolic cages. At the conclusion of the chronic studies, all rats were anesthetized with pentobarbital (50 mg/kg IP), and the left kidney was perfusion fixed at the measured arterial pressure of the rat with 1.25% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4) and processed for morphologic studies. The frequency of focal and segmental glomerulosclerosis (FSGS) was determined by examining all glomerular profiles (>100) contained in one or two coronal sections from each rat stained using the periodic acid-Schiff method. The number of glomeruli showing criteria for sclerosis [23] was expressed as a percentage of the total examined per rat by an experienced pathologist (H.G.R.), who was blinded as to the group from which the sections came.

Glomerular hemodynamic studies

In two additional groups of identically prepared F344→LEW-transplanted rats receiving either losartan (50 mg/L, N = 6) in their drinking water or drinking water alone (N = 6), micropuncture and gene expression studies were carried out at 20 weeks. The dose of losartan was lowered in this group in order to maintain SBP > 100 mm Hg and to avoid hypotension under anesthesia. For these studies, anesthesia was induced with methohexitol (10 to 20 mg/kg, IV) and maintained with a slow bolus injection of Inactin (0.1 mL/100 g body wt, IV; Byk Gulden, Konstanz, Germany). After the induction of anesthesia, the rats were placed on a thermostatically regulated table. A standard surgical preparation for micropuncture was employed. A femoral artery catheter was inserted for blood pressure measurement and collection of blood (250 µL) for baseline as well as intermittent sampling of plasma protein concentration (C_{A}), hematocrit (Hct), and plasma inulin concentration. Two catheters were inserted into the femoral vein for administration of Inactin and constant infusion of the following solutions throughout the experiments: bovine salt-poor albumin (40 g/L) at 100 µL/min to replace protein and volume losses during surgery (0.5% body wt), 7.5% inulin, and 0.75% para-aminohippurate (Merck, Rahway, NJ, USA) in 0.9% saline at 27 µL/min. The left kidney was then exposed through a midline incision, and the ureter was catheterized. Following a 60-minute stabilization period, timed urine collections were obtained for determination of the whole-kidney glomerular filtration rate (GFR). During these periods, four exactly timed proximal tubule fluid samples were collected for determination of single nephron GFR (SNGFR). At the midpoint of the periods, arterial blood was sampled for measurement of C_{A} and plasma inulin concentration. Hydraulic pressures were then measured in four to six proximal tubules under free-flow (P_{f}) and stop-flow (P_{SF}) conditions. Stop-flow conditions were created upstream of single nephron obstructions produced by microinjection of bone wax into proximal tubule segments. Pressure measurements were obtained using micropipettes of 1 to 2 µm tip diameter filled with 2 mol/L NaCl connected to a servo-null pressure measuring device (Instrumenta-
tion for Physiology and Medicine, San Diego, CA, USA). 

C\textsubscript{A} was determined using a clinical refractometer; arterial plasma colloid osmotic pressure, P\textsubscript{A}, was derived from C\textsubscript{A} using the Landis-Pappenheimer equation. P\textsubscript{GC} was estimated from P\textsubscript{G} + P\textsubscript{A} and \Delta P from P\textsubscript{GC} − P\textsubscript{TP}. Urine volumes were determined gravimetrically. Concentrations of inulin in blood and plasma were assessed by an anthrone method [24] and in tubule fluid samples by a microimmunofluorescence method [25].

At the end of the study, portions of renal allograft cortex were excised, snap-frozen in liquid nitrogen, and stored at −80°C for later RNA extraction. Coronal sections were frozen in gel (OCT; Sakura Finetek, Torrance, CA, USA) for later examination by immunohistochemistry.

Reverse transcription-polymerase chain reaction

Total RNA was extracted from frozen kidney cortex samples using a guanidinium isothiocyanate/phenol-chloroform isolation method (ULTRA-spec.; Biotex, Houston, TX, USA) [23]. The isolated total RNA was air dried and resuspended in diethyl pyrocarbonate-treated water, and the approximate quantity of RNA was determined by spectrophotometry (O.D. 260). The purity of the RNA was then confirmed with an O.D. 260/280 ratio for all samples more than 1.8. Total RNA (2.5 μg) was used for first-strand cDNA synthesis employing 1.2 μg of oligo (dT)\textsubscript{12-18} and the superscript reverse transcriptase (RT) method according to manufacturer’s recommendations (GIBCO-BRL, Gaithersburg, MD, USA). Non-looping, nonoverlapping, oligonucleotide primer pairs from separate exons were obtained for each gene studied; including β-actin, from Clontech (Palo Alto, CA, USA) or Genosys (The Woodlands, TX, USA). The specific primers for interleukin-2 (IL-2), interferon-γ (IFN-γ), IL-1, IL-6, TGF-β, tumor necrosis factor-α (TNF-α), RANTES, MCP-1, and β-actin followed previously published sequences [22]. The primer sequence for endothelin was as follows: 5'-GCTATGAATCGAT CCGAT-3' and 5'-CATGGATCACTGCCCTAA-3'.

Competitive polymerase chain reaction (PCR) for semiquantitation of mRNA was performed as reported previously [26]. Briefly, for each 25 μL amplification, 2.5 μL of first-strand cDNA product was used. Twofold to tenfold serial dilutions of known quantities of PCR MIMICs were added to the PCR reaction containing constant amounts of sample target cDNA. RT-PCR conditions were optimized for each cytokine and growth factor amplified. PCR products (5 μL) were run on an ethidium bromide-stained 1.5% agarose gel, and gene-specific bands were visualized and photographed under ultraviolet light. The quantities of MIMIC and target cDNA were compared by band-intensity analysis using a PC SCANJET and NIH Image and Adobe Photoshop software (Adobe Inc., San Jose, CA, USA). Tissues were tested for individual cytokine mRNA transcripts as well as for the control β-actin mRNA transcript. Band intensity for each cytokine was adjusted for expression of β-actin. Twofold differences were detectable. This method has been noted to be as accurate as scintillation counting of \textsuperscript{3}P-labeled PCR products [27]. Each PCR reaction was repeated twice in all samples and did not differ appreciably from one another.

Immunohistology

Mouse monoclonal antibodies to rat monocytes/macrophages (ED-1) and to the rat intercellular adhesion molecule-1 (ICAM-1; CD54) and to T cells (directed against the T-cell receptor α/β chains) were obtained from Bioproducts for Science (Indianapolis, IN, USA); TNF-α, TGF-β, RANTES, and MCP-1 were from Genzyme (Boston, MA, USA). Biotinylated donkey anti-mouse IgG and streptavidin peroxidase-conjugated complexes were obtained from Dako (Carpenteria, CA, USA). Cryostat sections (4 μm) of cortical samples were fixed in acetone and then incubated (room temperature, 1 h) with a blocking solution [Tris-buffered saline (TBS) with 1% nonfat milk, 0.05% Tween 20, 0.02% sodium azide and 105°C heat-inactivated donkey serum]. Optimally diluted primary monoclonal antibodies (mAbs; 0.1 mol/L TBS containing 1% nonfat milk, 0.05% Tween 20, and 0.02% sodium azide) were then used (room temperature, 1 to 24 h), followed by incubation with bridging antibodies and peroxidase-conjugated complexes. Control sections were prepared by replacing the primary mAb with either dilution buffer or normal mouse serum. The peroxidase reaction was developed with 3,3-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA). Stained sections were evaluated in duplicate or triplicate by counting the labeled cells within 10 high-power fields (HPFs)/section or by analysis in semiquantitative fashion in which the relative abundance of each one was judged from (+) minimal to (+++) very abundant.

Statistical analysis

Statistical comparisons between groups were made using factorial or repeated-measures analysis of variance (ANOVA), with post hoc testing where appropriate [26]. FSGS scores were compared using a Wilcoxon test and RT-PCR data by the Mann–Whitney method. Data are presented as mean ± SEM unless stated otherwise. The null hypothesis was rejected at \( P < 0.05 \).

RESULTS

Graft function and morphology

As shown in Table 1, all rats grew at similar rates without significant differences in body weights between the LOS and CON groups. Rats receiving losartan aver-
Table 1. Body weight in the two groups of rats over 24 weeks

<table>
<thead>
<tr>
<th>Week</th>
<th>Body weight g</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>291 ± 11</td>
</tr>
<tr>
<td>9</td>
<td>324 ± 6.5</td>
</tr>
<tr>
<td>12</td>
<td>340 ± 6.8</td>
</tr>
<tr>
<td>15</td>
<td>346 ± 8.8</td>
</tr>
<tr>
<td>18</td>
<td>347 ± 7.0</td>
</tr>
<tr>
<td>21</td>
<td>368 ± 5.6</td>
</tr>
<tr>
<td>24</td>
<td>367 ± 5.9</td>
</tr>
</tbody>
</table>

Losartan (N = 9) 292 ± 9.9 309 ± 7.3 330 ± 5.5 348 ± 12 358 ± 10 368 ± 13 367 ± 10
Control (N = 9) 292 ± 6.9 309 ± 7.3 330 ± 5.5 348 ± 12 358 ± 10 368 ± 13 367 ± 10

Data are mean ± SEM. No statistically significant differences between groups were found by repeated measures ANOVA.

Table 2. Systolic blood pressure (SBP) in the study groups over 24 weeks

<table>
<thead>
<tr>
<th>Week</th>
<th>SBP mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>86 ± 4.8a</td>
</tr>
<tr>
<td>9</td>
<td>96 ± 4.1a</td>
</tr>
<tr>
<td>12</td>
<td>102 ± 4.6</td>
</tr>
<tr>
<td>15</td>
<td>96 ± 4.4a</td>
</tr>
<tr>
<td>18</td>
<td>111 ± 5.1</td>
</tr>
<tr>
<td>21</td>
<td>110 ± 3.4</td>
</tr>
<tr>
<td>24</td>
<td>113 ± 3.6</td>
</tr>
</tbody>
</table>

Losartan (N = 9) 86 ± 4.8a 96 ± 4.1a 102 ± 4.6 96 ± 4.4a 111 ± 5.1 110 ± 3.4 113 ± 3.6
Control (N = 9) 122 ± 5.7 124 ± 8.3 120 ± 4.0 121 ± 4.0 121 ± 7.0 124 ± 7.0 119 ± 6.0

Data are mean ± SEM.

*P < 0.05 vs. control by repeated measures ANOVA and by Scheffé’s test.

Fig. 1. Proteinuria. Symbols are: (■) losartan group; (■) control group. Progressive rises in urinary protein excretion rates seen in the control rats are abolished in rats receiving losartan. *P < 0.05 by ANOVA and Scheffé’s test.

Fig. 2. Glomerulosclerosis. The percentage of glomeruli involved with focal and segmental glomerulosclerosis (FSGS) is depicted in a box and whiskers plot. The horizontal margins of the box represent the 75th percentile, and the error bars represent the 90th percentile. The circles denote the range. FSGS in vehicle-treated rats (Control) was characterized by a high variance, whereas the losartan rats showed little variance in sclerosis with four rats scoring zero. Because of the unequal variances between the groups, nonparametric testing was used for statistical comparison (Wilcoxon test, *P < 0.05).

Aged SBPs that were initially lower when compared with controls, although at weeks 12 and 18 through 24, levels of SBP in LOS rats rose to approximate those of CON rats, which remained normotensive throughout the study (Table 2). As depicted in Figure 1, progressive increases in UprotV were observed over 24 weeks in CON rats, whereas in LOS rats, UprotV remained at levels that were essentially normal and significantly lower than those observed in CON (F = 6.6, P < 0.05 by repeated-measures ANOVA). In keeping with the prevention of proteinuria in LOS rats, structural injury was also less at 24 weeks (Fig. 2), with the percentage of glomeruli affected by FSGS averaging 0.3 ± 0.2% in LOS versus 4 ± 2% in CON rats (P < 0.05 by Wilcoxon test). Four of the nine rats in the LOS group showed no evidence of glomerular injury whatsoever.

Hemodynamic studies

For the hemodynamic studies, the losartan dose was adjusted in an attempt to minimize differences in SBPs
between the two groups (Table 3). Nevertheless, at 20 weeks, mean arterial pressure (MAP), measured under anesthesia, was still significantly lower in LOS rats than CON (Table 4) but was within the autoregulatory range. Accordingly, GFR was undiminished in LOS rats at this lower MAP. Average values for renal plasma flow rates and filtration fraction were also similar between LOS and CON rats. SNGFR showed a nonsignificant tendency to be lower in LOS versus CON rats, and both values were elevated when compared with values of SNGFR obtained in this laboratory from age-matched, ungrafted normal rats (40 nL/min). Average values for PGC were elevated in CON rats to levels consistent with glomerular capillary hypertension (Table 4), despite MAP in these rats remaining within the normotensive range. Treatment with losartan was associated with significantly lowered values for PGC and ΔP, the glomerular transcapillary hydraulic pressure difference, when compared with CON. Values for LOS rats approximated values typically obtained from normal unoperated, two-kidney rats in our laboratory (55 mm Hg). 

**Intragraft expression studies**

Reverse transcription-polymerase chain reaction studies on allograft cortex revealed an unambiguous effect of losartan treatment on the expression of a variety of cytokines implicated in the development of chronic renal allograft injury in this model. Expression of RANTES, inducible nitric oxide synthase (iNOS), IL-1, IL-6, TGF-β, ET, and MCP-1, associated with macrophage activity, were all significantly reduced (Fig. 3). Interestingly, levels of expression of IL-2, IFN-γ, and TNF-α were not significantly affected by treatment and did not differ significantly between CON and LOS rats. Immunohistochemical analysis revealed characteristic features of chronic rejection in kidney allografts in CON rats, including mononuclear cell infiltration in perivascular and interstitial areas and early arteriosclerosis, as well as early glomerular injury. Sections of allografts from losartan-treated rats, on the other hand, showed markedly fewer mononuclear cells, with ED-1+ cell numbers averaging 52 ± 9 cells per 10 HPFs in LOS versus 112 ± 10 in CON (P < 0.05) and T-cell numbers averaging 47 ± 7 in LOS versus 86 ± 10 in CON rats (P < 0.05; Table 5). Whereas RANTES, MCP-1, and ICAM-1 staining in interstitial areas was clearly diminished in LOS rats (averaging 2+ vs. 3+ in controls), staining patterns for TNF-α, TGF-β, and platelet-derived growth factor (PDGF), while abundant in tubulointerstitial regions and present in glomeruli, were highly variable, and no clear differences could be discerned between the LOS and CON rats.

**DISCUSSION**

Chronic renal allograft failure remains a major obstacle to transplantation fulfilling its promise as the treatment of choice for end-stage renal disease [28]. Thus, there is an urgent need to identify mechanisms of chronic renal allograft injury to provide the basis for developing therapeutic strategies to enhance graft longevity and preserve adequate renal allograft function. Several lines of evidence suggest that antigen-independent mechanisms play a major role in the progression of chronic renal allograft “rejection” [1–6]. Since the involvement of RAS-dependent mechanisms is a consistent finding in a diversity of models of experimental CRD, pharmacological interruption of this system is an obvious candidate approach for preservation of renal allograft function. Moreover, our previous work has demonstrated the importance of inadequate nephron mass to the development of chronic rejection in kidney allografts in CON rats, including by a reduction of glomerular and tubulointerstitial injury initiated by elevated PGC. An alternative but not mutually exclusive pathway to injury could involve angiotensin-dependent fibrogenesis or potentiation of antigen-specific injury processes. Indeed, Benediktsson et al observed protective effects from pharmacological inhibi-

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**Table 3. Systolic blood pressure (SBP) over 20 weeks**

<table>
<thead>
<tr>
<th>Week</th>
<th>SBP mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>87 ± 4.5</td>
</tr>
<tr>
<td>12</td>
<td>106 ± 5.4</td>
</tr>
<tr>
<td>20</td>
<td>107 ± 6.3</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

*P < 0.05 versus control by repeated measures ANOVA and by Scheffe’s test.*

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**Ziai et al: Renoprotective effects of losartan**
Angiotensin II stimulates the synthesis and elaboration of extracellular matrix components by glomerular mesangial cells in culture [16]. Interestingly, recent evidence also suggests that Ang II may promote the synthesis of a variety of cell-adhesion molecules and cytokines, possibly via reactive oxygen intermediates [29, 30] and activation of the transcription factor nuclear factor-κB (NF-κB) [17, 18]. Molecules with NF-κB-responsive elements include ICAM-1 and MCP-1. Coordinated expression of these latter factors might be expected to promote local macrophage recruitment in the allograft. Macrophages also express angiotensin receptors [31], and angiotensin has been shown to stimulate macrophage recruitment directly [32]. From our data, however, it is not possible to determine whether losartan has had a direct effect on macrophage activation in the F344→LEW renal allograft. However, our findings may be considered consistent with Ang II-stimulated transcriptional activation of ICAM-1, RANTES, and MCP-1, with resulting enhanced recruitment, activation, and intragraft retention of macrophages. In turn, the expanded macrophage population could account for the increased transcription of iNOS, IL-1, IL-6, and TGF-β that was detected. Macrophages have been implicated as important contributors to graft injury in this model. Inhibition of macrophage activation ameliorated graft injury [33], whereas interventions designed to increase macrophage activation exacerbated chronic graft injury [34].

While the previously mentioned observations are consistent with the hypothesis that Ang II contributes directly to the up-regulation of renal cortical cytokine expression characteristic of this model, it is possible that Ang II exerts these effects via altered hemodynamics. Recent in vitro evidence raises the possibility that the transduction of mechanical forces by mesangial and other cell types present in the kidney may lead to increased cytokine expression [35]; this may be analogous to the recent in vivo observation that systemic hypertension up-regulates the expression of MCP-1 in the vascular smooth muscle cells of rats [36]. Thus, altered glomerular hemodynamics, for example, increased \( P_{GC} \), could have a direct effect on gene expression in the kidney. It has also been suggested that the increased uptake of filtered protein by the renal tubule epithelial cells in proteinuric

### Table 4. Renal physiological parameters at 20 weeks of study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Losartan Mean ((N = 6))</th>
<th>Control Mean ((N = 6))</th>
<th>(P)</th>
<th>(\Delta P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm\ Hg)</td>
<td>107±1.84</td>
<td>132±1.91</td>
<td>0.05</td>
<td>2.5</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>60</td>
<td>83</td>
<td>0.05</td>
<td>1.3</td>
</tr>
<tr>
<td>SNGFR (mL/min)</td>
<td>7.1</td>
<td>7.3</td>
<td>0.26</td>
<td>0.4</td>
</tr>
<tr>
<td>RPF (mL/min)</td>
<td>0.26</td>
<td>0.25</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Ff</td>
<td>38±1.3</td>
<td>53±1.4</td>
<td>0.26</td>
<td>1.8</td>
</tr>
<tr>
<td>C(_A) (mL/min)</td>
<td>5.2</td>
<td>5.6</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>C(_G) (g/dL)</td>
<td>16</td>
<td>18</td>
<td>54±1.4</td>
<td>71±5.9</td>
</tr>
<tr>
<td>(\Delta P_{GC})</td>
<td>41±2.3</td>
<td>1.2</td>
<td>2.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Abbreviations are: MAP, mean arterial pressure; GFR, whole kidney glomerular filtration rate; SNGFR, single nephron glomerular filtration rate; RPF, renal plasma flow; Ff, filtration fraction; \( P_{SF} \), tubular stop flow pressure; \( P_{TF} \), tubular free flow pressure; \( C_{A} \), afferent arteriolar plasma protein concentration; \( C_{G} \), effenter arteriolar oncotic pressure; \( C_{G} \), glomerular capillary pressure; \( D_{P} \), glomerular transcapillary pressure difference.

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**Table 5.** T-cell and macrophage numbers

<table>
<thead>
<tr>
<th>SBP (mm\ Hg)</th>
<th>T cells</th>
<th>CD-1 MO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losartan ((N = 6))</td>
<td>47±7(^*)</td>
<td>52±9(^*)</td>
</tr>
<tr>
<td>Control ((N = 6))</td>
<td>86±10</td>
<td>112±10</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. \(^*\) \(P < 0.05\) vs. control by Mann–Whitney test.
states may stimulate local cytokine expression [37, 38]. Accordingly, the favorable hemodynamic effects of losartan could also alter the expression of proinflammatory cytokines in renal cortex by reducing the passage of proteins through the glomerular filtration barrier and reducing their uptake by the renal tubules. Finally, it is possible that losartan blocks direct actions of Ang II on tubule cells to promote cytokine expression and elaborate extracellular matrix components [39, 40].

While it is apparent that the RAS, either directly or indirectly, is involved prominently in injury processes and macrophage recruitment and activation in this model, we do not see any evidence to support an interaction of the RAS with T-cell activation at this late stage, insofar as the levels of expression of IFN-γ and IL-2, classic products of activated T cells, are similar in LOS rats when compared with controls at 20 weeks. Previous studies suggest that the influence of T cells occurs earlier in this model; therefore, it is possible that an angiotensin-independent effect on T-cell activation would be evident at earlier time points or that the sensitivity of the RT-PCR for IFN-γ and IL-2 is compromised because of low levels of expression of these factors. The finding of significantly fewer T cells in the LOS allografts versus CON rats could be consistent with this latter interpretation.

In summary, these data show that the AT1 RA losartan is effective in reducing \( P_{GC} \) and proteinuria in long-surviving F344–LEW renal allografts. These effects are associated with a reduction in allograft macrophage infiltration and attenuated expression of molecules involved in macrophage recruitment. Together with the finding of down-regulated expression of several classic cytokine products of activated macrophages in losartan-treated rats, these findings suggest a central role for Ang II in the cellular and molecular events leading to chronic renal allograft injury in this model.

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Reprint requests to: Harald S. Mackenzie, M.D., Renal Division, Department of Medicine, MRB4, Brigham and Women’s Hospital, 75 Francis Street, Boston, Massachusetts 02115, USA.

E-mail: hmackenzie@rics.bwh.harvard.edu

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