Mycophenolate mofetil prevents arteriolopathy and renal injury in subtotal ablation despite persistent hypertension

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Mycophenolate mofetil prevents arteriolopathy and renal injury in subtotal ablation despite persistent hypertension.

Background. Although renal protective effect of interrupting the inflammatory process is well established, it is still controversial if it also prevents the glomerular hemodynamic disturbances that initiate renal injury. We investigated the effects of suppressing inflammation with mycophenolate mofetil (MMF) on glomerular hemodynamics, arteriolar structural changes, and renal histologic injury in rats with subtotal renal ablation.

Methods. Micropuncture studies were performed 30 days after 5/6 nephrectomy in rats untreated and treated with MMF (30 mg/kg/day). Renal histology, immunohistochemistry for lymphocytes, macrophages and inducible nitric oxide synthase (iNOS) expression, as well as afferent arteriolar (AA) morphology was evaluated.

Results. Renal ablation significantly increased proteinuria (6.8 to 82.7 mg/day), mean arterial pressure (MAP) (120 to 166 mm Hg), single-nephron glomerular filtration rate (SNGFR) (34.8 to 56.3 mL/min), glomerular plasma flow (QG) (117.7 to 246.9 mL/min), and glomerular capillary pressure (PGC) (48.9 to 61.0 mm Hg). Afferent resistance (AR), efferent resistance, and ultrafiltration coefficient remained unchanged. Despite persisting arterial hypertension (152 mm Hg), MMF prevented proteinuria (13.3 mg/day), and significantly reduced SNGFR (44.4 mL/min), PGC (49.1 mm Hg), and QA (163.2 mL/min) due to a rise in AR (3.13 vs. 2.18 10^10 dyn/sec/cm^5). Glomerular sclerosis, tubulointerstitial damage, lymphocyte and macrophage infiltration, and iNOS expression were significantly reduced by MMF, in addition hypertrophy of AA resistance evaluated by the media/lumen ratio was prevented (P < 0.001).

Conclusions. Reductions in proteinuria, SNGFR, QA, and PGC, despite elevated MAP, indicate preservation of AA function. These results suggest that inflammation associated arteriolopathy of AA contributes to glomerular hemodynamic disturbances that participate in the progression of renal disease.

Key words: glomerular hemodynamics, arteriolopathy, mycophenolate mofetil, 5/6 nephrectomy, inflammation.

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The mechanisms involved in the progression of renal disease to end-stage renal failure are incompletely understood. Clinical studies have shown that hypertension and proteinuria are closely associated with progression to chronic renal disease [1, 2]. In clinical and experimental studies, therapies that lower blood pressure and reduce proteinuria effectively retard progression, especially drugs that interfere with the effects of angiotensin II (Ang II) [1–5]. Hemodynamic stress induced by glomerular hypertension and hypertrophy are thought to be initiating factors [6]. In addition, proteinuria resulting from increased glomerular capillary pressure and glomerular barrier dysfunction produces proximal tubule protein overload inducing proliferation and inflammatory cells infiltration [7].

There is growing evidence that inflammation plays a critical role in the progression of renal injury, even when the initiating insult is not mediated by immunologic factors, such as the case in renal lesion associated with Ang II-mediated hypertension in double transgenic rats [8, 9], subtotal renal ablation [10, 11], and other models [12–15]. In double transgenic rats for human renin and angiotensinogen genes, characterized by high levels of Ang II, severe hypertension, intense proteinuria and renal damage, Muller et al [8] demonstrated that renal injury is associated with activation of nuclear factor-kappa B (NF-κB), overexpression of NF-κB-dependent proinflammatory cytokines, chemokines, and adhesion molecules, and inflammatory cells infiltration. Inhibition of NF-κB activation with different maneuvers [8, 9, 16] prevented proteinuria and greatly attenuated renal damage, despite persisting hypertension. These observations suggest that in hypertension-induced renal injury, the inflammatory process plays a predominant role, however, it is not known from these studies if suppression of inflammation also prevented the glomerular hemodynamic changes that initiates injury or if renal injury was attenuated despite persistence of these alterations.
Several recent studies in rats with extensive renal ablation evaluated the role of inflammation by suppressing inflammatory cell infiltration with different drugs [10, 11, 17–20]. Administration of mycophenolate mofetil (MMF), an immunosuppressive drug that exerts a selective antiproliferative activity on activated B and T cells and inhibits glycosylation of adhesion molecules [21], prevented lymphocyte and macrophage proliferation and infiltration and reduced progressive renal damage [10, 11, 17, 20]. In every study, the beneficial effects of MMD treatment occurred despite the persistence of systemic hypertension and, in some studies, despite persistence of proteinuria, suggesting that they were not mediated by amelioration of glomerular hemodynamic disturbances responsible for the initiation of renal injury [10, 11, 17, 20]. Remuzzi et al. [20] found that MMF effect was independent of hypertension and proteinuria, Fujihara et al. [10] measured glomerular pressure and found that the rise in glomerular pressure was not changed after 4 weeks of MMF therapy. However, after 8 weeks when rats had considerably higher values of glomerular pressure, MMF therapy was associated with partial reduction of glomerular capillary pressure (PGC) and proteinuria [17]. On the other hand, we recently treated rats with subtotal renal ablation with polysulphate pentosan (PPS), a heparinoid with anti-inflammatory effects and found that, despite persisting hypertension, PPS prevented proteinuria, reduced inflammatory cells infiltration, and significantly attenuated renal injury. Evaluation of glomerular hemodynamics revealed normalization of glomerular hypertension and hyperfiltration due to a rise in afferent arteriolar (AA) resistance [19]. These results suggested that suppression of inflammation improved arteriolar functional capacity to prevent excessive transmission of arterial pressure to glomerular capillaries due to prevention of vascular lesions associated to renal injury.

Thus, although the renal protective effect of interrupting the inflammatory process by different maneuvers is well established, it is still controversial if it also prevents the glomerular hemodynamic disturbances responsible for the initiation of renal injury.

This possibility should be further investigated because the renoprotective effect suppressing inflammation in nonimmune renal injury could be partially due to improvement in glomerular hypertension and hyperfiltration in remnant nephrons as a consequence of preventing arteriolar lesions.

Therefore, in this study we evaluated the effects of suppressing the inflammatory process with MMF on the glomerular hemodynamic pattern, arteriolar structural changes, and renal histologic injury in rats with subtotal renal ablation.

METHODS

Experimental design

Studies were carried out in male Sprague-Dawley rats weighing 250 to 300 g. Three experimental groups were studied: (1) the group sham included nine sham-operated rats; (2) the group with a 5/6 nephrectomy included 11 rats with 5/6 renal mass reduction, which were studied 30 days after renal ablation; and (3) group 5/6 nephrectomy/MMF consisted of 12 rats with 5/6 renal mass reduction that received MMF (5/6 Nx/MMF; F. Hoffman-La Roche, Basel, Switzerland) at a dose of 30 mg/kg/day by gastric gavage for 30 days and then were studied.

Twenty additional rats had 5/6 nephrectomy and were divided in four groups of five rats each, two groups received vehicle (5/6 nephrectomy) and two groups received MMF (5/6 nephrectomy/MMF). One set of 5/6 nephrectomy and 5/6 nephrectomy/MMF was used for light microscopy studies and for the immunohistologic analysis of the infiltrating lymphocytes and macrophages and the other set was used to evaluate inducible nitric oxide synthase (iNOS) expression. Rats were sacrificed at 30 days.

Renal mass reduction

Under anesthesia with sodium pentobarbital, 30 mg/kg intraperitoneally, renal ablation was performed by removal of the right kidney and selective infarction of approximately two thirds of the left kidney by ligation of two or three branches of the renal artery. Sham operation consisted of ventral laparotomy and manipulation of the kidneys and renal pedicle without destruction of renal tissue. Micropuncture studies were performed 30 days after the surgical procedure.

Systolic blood pressure was measured in conscious, restrained rats by tail-cuff plethysmography (Narco Biosystems, Austin, TX, USA). Rats were conditioned twice before the blood pressure was measured at basal period, every week during the first 2 weeks and every 2 weeks for the rest of the study.

A series of 24-hour urine collections for proteinuria were collected in metabolic cages with ad libitum food and water intake in the basal period and at 29 days of the study before the micropuncture experiments.

Micropuncture studies

Rats were anesthetized with sodium pentobarbital (30 mg/kg body weight intraperitoneally), and supplementary doses were instilled as required. The rats were placed on a temperature-regulated micropuncture table at 37°C. Polyethylene tubing was used to catheterize the trachea (PE-240), both jugular veins, femoral arteries (PE-50), and the left ureter (PE-10). The left kidney was exposed and placed in a Lucite holder, which was sealed, covering the kidney surface with Ringer’s solution. Mean arterial
pressure (MAP) was continuously monitored with a pressure transducer (Model p23 Db, Gould, Hato Rey, Puerto Rico, USA) and recorded on a polygraph (Grass Instruments, Quincy, MA, USA). Blood samples were taken periodically and replaced with blood from a normal donor rat.

Rats were maintained euolemic by infusion of isotonic rat plasma (10 mL/kg body weight) during surgery, followed by an infusion of 5% polyfructosan, 2.2 mL/hour (Inutest, Laeversan-Gesellschaft, Austria), and 0.9% sodium saline solution, as vehicle. After 60 minutes, six timed samples of tubule fluid were collected from surface proximal convolutions for determination of flow rate and polyfructosan concentration; intratubular hydrostatic pressure under free flow and stop flow conditions and peritubular capillary pressure were measured with a servo-null device (Servo Nulling Pressure System, Instrumentation for Physiology and Medicine, Inc., San Diego, CA, USA) as previously described [5]. Polyfructosan was measured in plasma and urine samples. Glomerular colloid osmotic pressure was estimated from protein levels taken from the femoral artery (C_v) and surface efferent arterioles (C_k).

Analytical procedures

Polyfructosan concentrations in plasma and urine were determined by the macro-anthrone method of Davidson and Sackner [22]. The volume of fluid collected from individual proximal tubules was estimated from the length of the fluid column in a constant bore capillary tube of known internal diameter. The concentration of tubular polyfructosan was measured by the microfluorescence method of Vureck and Pegram [23]. Protein concentrations in afferent and efferent samples were determined by the method of Viets et al [24]. Protein concentrations in urine were determined by the method of sulfoSalicylic acid [25].

Glomerular filtration rate (GFR), single-nephron glomerular filtration rate (SNGFR), calculated glomerular capillary hydrostatic pressure (P_C), glomerular capillary hydrostatic pressure gradient (∆P), single-nephron filtration fraction (SNFF), single-nephron plasma flow (Q_v), single-nephron blood flow (SNBF), afferent (AR) and efferent (ER) resistances, ultrafiltration coefficient (Kf), and oncotic pressure (π) were calculated according to equations given elsewhere [5, 26].

Histologic studies

Paraffin-embedded sections were stained with hematoxylin-eosin and periodic acid-Schiff (PAS) reagent. Four micrometer sections were examined by two investigators in a blinded fashion. Glomerulosclerosis was defined as the presence of PAS-stained tissue within the glomerular tuft, with loss of cellular elements, collapse of capillary lumen, and entrapment of hyaline material. The severity of glomerulosclerosis was classified as described initially by Raji, Azar, and Keane [27] and used by us in previous communications [28]: grade 0 = no sclerosis, grade 1 = <25% of the glomerulus, grade 2 = 25% to 50% of the glomerulus, grade 3 = 50% to 75% of the glomerulus, and grade 4 = 75% to 100% of the glomerulus. The score of a biopsy was calculated by the equation: [(1 × number glomeruli with grade 1) + (2 × number glomeruli with grade 2) + (3 × number glomeruli with grade 3) + (4 × number glomeruli with grade 4)] × 100/total number of glomeruli examined.

Tubulointerstitial lesions (dilatation, atrophy, cellular infiltration, and fibrosis) were graded on a scale of 0 to 5+, as described in a previous work [5]: 0 = changes in less than 10% of the section, 1+ = changes in up to 20% of the section, 2+ = changes in up to 40% of the section, 3+ = changes in up to 40% of the section, 4+ = changes in up to 80% of the section, and 5+ = changes in more than 80% of the section. Ten to 15 sections were examined in each biopsy and the average score was used as the score of the biopsy.

Quantification of afferent arterioles morphology: Arteriolar morphology was assessed by indirect peroxidase immunostaining for α-smooth muscle actin (α-SMA) (DAKO Corp., Carpinteria, CA, USA). Quantifications were performed blinded. Only vessels adjacent to glomeruli in the outer cortex were selected. AAAs were distinguished from efferent arterioles by the presence of an internal elastic lamina and by thin, flattened endothelial cells [29]. Using immersion-fixed tissue, AA wall thickness was measured by computer image analysis (Image-Pro-Plus) as previously described [29, 30]. For each arteriole, the outline of the vessel and its internal lumen (excluding the endothelium) was generated using computer analysis to calculate the total medial area (outline/inline), in ten arterioles per biopsy. Vessels that were cross-sectioned or not sectioned transversally, providing an asymmetric wall, were excluded from the present study. The media-lumen ratio was calculated by the outline/inline relationship [29, 30]. Although perfusion-fixed tissue is preferred for morphometric measurements [31], immersion-fixed tissue is acceptable for measuring medial thickness [32], and comparisons are valid because all samples were treated with the same method.

Immunohistologic studies

Lymphocyte and macrophage infiltration was studied with the immunoperoxidase technique as described before [33, 34]. Briefly, tissues were successively washed and incubated with 20 mL ExtrAvidin, 2.5 mg/mL (Sigma Chemical Co., St. Louis, MO, USA) and with 30 mL 0.001% biotin in phosphate-buffered saline (PBS). Afterward, tissues were incubated for 2 hours at 37°C with 50 mL of the corresponding primary monoclonal antibody (see later), diluted 1:30 in Tris-saline buffer (TSB), pH 7.8. After washing in TSB for 15 minutes, tissues
were incubated for 1 hour with 30 mL rat antimouse immunoglobulin (IgG) (ab') biotin-conjugated fragments with minimal cross-reactivity with human, horse, and rat serum proteins (Accurate Chemical Corp., Westbury, NY, USA), and finally, for 30 minutes with 60 mL peroxidase-conjugated ExtrAvidin. After a final wash, tissues were incubated for 15 minutes in diaminobenzidine and H2O2 in TSB.

The primary antibodies used were anti-CD5 (mouse monoclonal antirat thymocytes and lymphocytes) and anti-ED-1 (mouse monoclonal antirat monocytes and macrophages) were purchased from Biosource International (Camarillo, CA, USA). Results in glomeruli were expressed as positive cells/glomerular cross-section and in interstitium as positive cells per millimeter squared.

Inducible NOS expression was evaluated by indirect streptavidin-biotin-peroxidase technique using anti-iNOS antibody (DAKO Corp., Carpinteria, CA, USA). Quantifications were performed blinded. Ten noncrossed glomeruli fields (×100) were analyzed per biopsy in an Olympus BX51 microscope (Olympus America, Inc., Melville, NY, USA). Expression of glomerular iNOS was evaluated by computer image analysis (Image-Pro-Plus). Results were expressed as the relationship between the number of positive cells/number of glomeruli nuclei.

Statistical analysis

Data are expressed as mean ± SEM. Data were analyzed by ANOVA for multiple comparison followed by Bonferroni test as a post hoc procedure. P < 0.05 was considered statistically significant.

RESULTS

Renal ablation resulted in development of severe systolic hypertension (>170 mm Hg) (Fig. 1). Treatment with MMF was associated with a less pronounced increment in blood pressure but the difference between treated and untreated groups were not statistically significant (Fig. 1).

Subtotal renal ablation induced an elevation in urine protein excretion of almost ninefold after 30 days. In contrast, the urinary protein excretion in the MMF-treated rats was not significantly different from sham-operated control rats (Fig. 1).

Micropuncture studies

The results obtained in the micropuncture studies are summarized in Table 1. Body weight was not significantly different among the studied groups.

Although whole kidney GFR was comparably reduced by renal ablation in the MMF-treated and untreated groups (Table 1), measurement of single-nephron function demonstrated marked differences in glomerular adaptation between untreated rats and those receiving MMF. In the untreated group there was a 62% increment in SNGFR versus sham animals (P < 0.05). In contrast, the increment of SNGFR in MMF-treated group was only 27% and did not reach statistical significance (Table 1).

The increase in SNGFR was the result of the elevation in the untreated 5/6 nephrectomy group compared to sham animals. PGC increased to 61.5 ± 2.5 mm Hg, whereas in the MMF-treated rats, PGC did not rise, remaining at values similar to sham group (Table 1). Glomerular overperfusion in rats with renal ablation was the result of defective constriction of preglomerular vessels to higher arterial pressure, as indicated by the unchanged AA resistance in the untreated 5/6 nephrectomy group compared to sham animals. In contrast, glomerular hyperperfusion was prevented in rats treated with MMF due to a 70% higher AA resistance. Thus, in the face of persistent systemic hypertension, MMF treatment preserved the capacity of preglomerular vessels to prevent hyperfiltration and glomerular hypertension in remnant nephrons (Table 1).
Finally, the Kf and ER were not significantly different among the studied groups.

**Histologic examination**

The histologic analysis is shown in Table 2. Glomerular volume was larger in the group of rats with renal mass reduction. There were no significant differences in the glomerular hypertrophy in the 5/6 nephrectomy group and the 5/6 nephrectomy/MMF group. As expected, glomerular sclerosis increased significantly in severity 30 days following renal ablation and MMF treatment resulted in an almost fourfold reduction in sclerosis scores in the 5/6 nephrectomy/MMF group (Table 2).

AAs appeared hypertrophic and there was narrowing of the lumen due to thickening of the arterial wall. Analysis of AA morphology by immunostaining for α-SMA demonstrated significant hypertrophy in the 5/6 nephrectomy group, as indicated by a higher media/lumen ratio than sham (6.6 ± 0.9 vs. 3.4 ± 0.2, P < 0.01), which was prevented in rats treated with MMF (2.8 ± 0.2, P < 0.001, Figs. 2 and 3).

Intraglomerular lymphocytes (CD5-positive cells/glomerular cells) were also reduced with MMF treatment but the results were not statistically significant (5/6 nephrectomy = 0.11 ± 0.12, 5/6 nephrectomy/MMF = 0.03 ± 0.07). Similar findings were obtained with intraglomerular macrophage (ED-1-positive cells/ges) in the experimental groups (5/6 nephrectomy = 0.22 ± 0.25, 5/6 nephrectomy/MMF = 0.04 ± 0.08).

In contrast to the findings in glomeruli, tubulointerstitial infiltration of lymphocytes (CD5-positive cells) and macrophages (ED-1-positive cells) in the remnant kidney of the untreated 5/6 nephrectomy group was increased three to six times. MMF treatment reduced drastically the inflammatory infiltrate (Table 2, Fig. 4).

Finally, the expression of glomerular iNOS, which is a marker of inflammation and macrophage activation, was significantly reduced by MMF, from 1.0 ± 0.2 vs. 0.45 ± 0.1, P < 0.05 (Fig. 4).

### DISCUSSION

The renal protective effect of interrupting the inflammatory process without lowering arterial pressure is well established [10, 11, 17–20]. It is still controversial, however, if suppression of inflammation also prevents the hemodynamic disturbances that initiate renal damage. Vascular injury is a constant finding in inflammatory renal lesions [12, 14, 35, 36], and glomerular hemodynamic changes are determined to a great extent by the functional capacity of preglomerular arterioles. Thus, the renoprotective effect exerted by anti-inflammatory may be partially mediated by an improvement of glomerular hemodynamics resulting from amelioration of AA damage. Accordingly, we decided to investigate the role of inflammation on vascular injury and glomerular hemodynamics in rats with subtotal renal ablation, a model of nonimmune renal damage.

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**Table 1. Glomerular hemodynamics in rats with 5/6 nephrectomy and 5/6 nephrectomy plus mycophenolate mofetil (MMF)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>5/6 Nephrectomy</th>
<th>5/6 Nephrectomy/ mycophenolate mofetil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight g</td>
<td>386.1 ± 9.2</td>
<td>366.3 ± 9.7</td>
<td>342.7 ± 11.7</td>
</tr>
<tr>
<td>Kidney weight g</td>
<td>1.60 ± 0.06</td>
<td>1.78 ± 0.16</td>
<td>1.75 ± 0.15</td>
</tr>
<tr>
<td>Mean arterial pressure mm Hg</td>
<td>119.6 ± 2.9</td>
<td>165.8 ± 5.4</td>
<td>152.5 ± 4.9</td>
</tr>
<tr>
<td>Glomerular filtration rate mL/min</td>
<td>1.17 ± 0.05</td>
<td>0.43 ± 0.09</td>
<td>0.47 ± 0.07</td>
</tr>
<tr>
<td>Single-nephron filtration fracture %</td>
<td>0.30 ± 0.02</td>
<td>0.25 ± 0.02</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>Glomerular plasma flow nL/min</td>
<td>34.8 ± 2.7</td>
<td>56.3 ± 4.2</td>
<td>44.4 ± 2.6</td>
</tr>
<tr>
<td>Glomerular capillary pressure mm Hg</td>
<td>117.7 ± 10.4</td>
<td>246.9 ± 35.0</td>
<td>163.2 ± 13.3</td>
</tr>
<tr>
<td>Glomerular laminar flow nL/min</td>
<td>48.9 ± 1.6</td>
<td>61.0 ± 2.5</td>
<td>49.1 ± 1.0</td>
</tr>
<tr>
<td>Efferent resistance 10³ dyn/cm²</td>
<td>2.45 ± 0.14</td>
<td>2.18 ± 0.30</td>
<td>3.13 ± 0.25</td>
</tr>
<tr>
<td>Afferent resistance 10⁴ dyn/cm²</td>
<td>1.54 ± 0.14</td>
<td>1.24 ± 0.22</td>
<td>1.36 ± 0.10</td>
</tr>
</tbody>
</table>

**Table 2. Glomerular histology and immunohistology 30 days after surgery**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>5/6 Nephrectomy</th>
<th>5/6 Nephrectomy/ mycophenolate mofetil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular volume μm³ × 10⁶</td>
<td>1.61 ± 0.10</td>
<td>2.38 ± 0.7</td>
<td>2.48 ± 0.7</td>
</tr>
<tr>
<td>Glomerular sclerosis score, 0 to 400</td>
<td>1.3 ± 2.0</td>
<td>104.4 ± 30.0</td>
<td>29.2 ± 9.9</td>
</tr>
<tr>
<td>Tubulointerstitial score 0 to 5</td>
<td>0.0 ± 0</td>
<td>2.90 ± 0.8</td>
<td>0.71 ± 0.9</td>
</tr>
<tr>
<td>CD5-positive cells/mm²</td>
<td>0.2 ± 0.3</td>
<td>137 ± 64.2</td>
<td>59 ± 39.3</td>
</tr>
<tr>
<td>ED-1-positive cells/mm²</td>
<td>0.7 ± 0.1</td>
<td>194 ± 61.2</td>
<td>34 ± 9.6</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. sham; †P < 0.01 vs. sham; ‡P < 0.001 vs. sham; §P < 0.01 vs. 5/6 nephrectomy; ‡P < 0.001 vs. 5/6 nephrectomy.
The results of the present study confirmed that extensive renal mass ablation is associated with severe arterial hypertension, massive proteinuria, and significant glomerular and tubulointerstitial lesions with lymphocyte and macrophage infiltration. Micropuncture determinations demonstrated glomerular hypertension (+12.1 mm Hg) and hyperfiltration (+61.8%) in remnant nephrons. Treatment with MMF, suppressed inflammation as indicated by a significant reduction in CD5-positive and ED-1-positive cells and a decrease of glomerular expression of iNOS, an inflammation marker.

An important observation was that, despite persisting arterial hypertension, glomerular hemodynamic disturbances were largely prevented. Indeed, glomerular pressure was normalized and glomerular hyperfiltration was reduced to only 20.7%. Similarly, the rise in glomerular plasma flow was significantly attenuated. These changes were due to a higher afferent resistance and suggest that functional capacity of preglomerular vessels was preserved.

Recently, Fujihara et al [10, 17] studied Munich-Wistar rats and evaluated glomerular hemodynamics by measuring whole kidney function and directly measuring glomerular pressure in superficial cortical glomeruli. In contrast to our results, they found that 4 weeks after renal ablation, glomerular hypertension was not changed by MMF therapy [10]. However, in a second study in which

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Fig. 2. Afferent arteriole morphology in remnant kidney tissue. (A) and (B), sham rat. (C) and (D), 5/6 nephrectomy untreated rat at 30 days. (E) and (F), 5/6 nephrectomy/mycophenolate mofetil (MMF)-treated rat at 30 days. (B), (D), and (F) stained with Ab’ anti-α-smooth muscle actin (a-SMA). Arteriolar hypertrophy in 5/6 nephrectomy rats was prevented in MMF-treated rats. (Reproduction of this figure in color was made possible by Roche Mexico, Mexico City, Mexico.)

Fig. 3. Afferent arteriole media/lumen ratio in sham, 5/6 nephrectomy (5/6 Nx), and 5/6 nephrectomy/mycophenolate mofetil (5/6 Nx/MMF) groups. *P < 0.01 vs. sham; †P < 0.001 vs. 5/6 Nx vs. 5/6 Nx/MMF.
rats were evaluated 8 weeks after surgery, when the values of glomerular pressure were considerably higher [17]. MMF therapy was associated with partial reduction of PGC and proteinuria. Treatment with an Ang II AT1 receptor antagonist normalized glomerular pressure and reduced proteinuria close to normal [17]. The main difference with our studies is that we found a clear effect of MMF on renal function, evidenced by the reduction in PGC, SNGFR, and proteinuria, which are independent measurements that indirectly evaluate glomerular pressure and function. We do not have an obvious explanation for these discrepancies; however, there are several possible facts that may contribute to the different results. The rat strains were different. They used Munich Wistar rats and we used Sprague-Dawley. PGC was measured by different methods. They measured it directly in capillaries of superficial glomeruli and we used stop flow method. Previous studies however, demonstrated that stop flow measurements are valid in rats without superficial glomeruli [37–39]. Indeed, the absolute values of PGC at 4 weeks after 5/6 nephrectomy in our studies were not different than those reported by them (61 ± 2.5 vs. 66 ± 3.0 mm Hg) [10]. In contrast, values after MMF treatment were unchanged in their studies and significantly lower in ours (49.1 ± 1 vs. 66 ± 2 mm Hg). Since their rats tolerated considerable lower doses of MMF (10 vs. 30 mg/kg), it is conceivable that a lesser immunosuppressive effect prevented the reduction in proteinuria and restoration of glomerular hemodynamics. Moreover, when we used lower doses of MMF (15 mg/kg) in our 5/6 nephrectomy rats proteinuria was not prevented (unpublished observations). Moreover, Remuzzi et al [20] found in Sprague-Dawley rats with 5/6 nephrectomy that the drug greatly limited the accumulation of lymphocytes but, in contrast to our findings, it had no effect on proteinuria and its effects on histologic lesions were minimal. They suggest the effect of MMF was not due to interference with hemodynamic mechanisms of injury. There is no apparent explanation for these discrepancies with our results, except that the dose of MMF in their study was 33% lower (20 vs. 30 mg/kg given orally). Thus, it appears that higher doses of MMF...

Fig. 4. Immunohistology showing the remnant kidney tissue stained with Ab' anti-CD5 (A) and (B), ED-1 (C) and (D) and inducible nitric oxide synthase (iNOS) (E) and (F). (A), (C), and (E) are 5/6 nephrectomy-untreated rats. (B), (D), and (F) are 5/6 nephrectomy/mycophenolate mofetil (MMF)-treated rats. MMF treatment reduced interstitial infiltration of lymphocytes and macrophages and suppressed iNOS expression in glomeruli. (Reduction of this figure in color was made possible by Roche Mexico, Mexico City, Mexico.)
are required to prevent proteinuria and glomerular hemodynamic alterations in addition to suppressing inflammatory cells infiltration.

Vascular injury is a constant finding in renal inflammatory lesions, particularly when arterial hypertension is present; however, little attention is usually given to the potential role of vascular lesions in contributing to progression of renal damage. Injury of an AA can result in glomerular hemodynamic disturbances that induce proteinuria, which, in turn, can stimulate the inflammatory process, thereby creating a vicious cycle that may contribute to progression of renal disease. In 5/6 nephrectomy rats, the rise in PGC results from inappropriate constriction of the AA that allows the transmission of the systemic hypertension to the glomerular tuft. In the present study, histologic examination and morphometry with image computer analysis showed arteriolopathy of AAs as disclosed by a significant increase in media/lumen ratio indicating hypertrophy of the vessel wall. Treatment with MMF attenuated arteriolopathy and prevented the rise in glomerular pressure despite persisting systemic hypertension, suggesting that preservation of vascular structure also preserved vascular function as indicated by a higher afferent resistance. Vascular resistance is inversely determined by lumen diameter; however, we cannot evaluate AA luminal changes in our studies because we fixed the kidneys by immersion instead of perfusion fixation. Dissociation of vascular hypertrophy from arterial hypertension has been reported in several experimental models. Mervaala et al [40] reported that in double transgenic rats, which develop intense proliferation and infiltration of monocyte/macrophage in vascular wall, triple drug therapy (hydralazine, reserpine, and hydrochlorothiazide) normalized blood pressure, but had almost no effect on vascular cells proliferation. In contrast, suppression of Ang II synthesis with a renin inhibitor lowered blood pressure and prevented vascular proliferation [40]. These findings provide evidence that Ang II promotes an inflammatory response and cellular growth independent of high blood pressure.

Moreover, the effects of MMF on vascular lesion in a nonimmunologic model of renal injury in Munich-Wistar rats were recently studied by Fujihara et al [12]. Chronic inhibition of nitric oxide synthase and high salt diet induced systemic and glomerular hypertension, massive albuminuria, severe renal injury, and inflammation, as well as myointimal proliferation and necrosis of small arteries and arterioles. MMF treatment at doses of 10 mg/kg limited the extent of renal injury and partially decreased albuminuria without lowering blood pressure. Myointimal proliferation of microvessels was not significantly reduced and elevated glomerular pressure persisted, suggesting that arteriolopathy facilitated increased transmission of pressure to glomerular capillaries. In our studies higher doses of MMF prevented both arteriolopathy and glomerular hypertension.

The mechanism by which vascular hypertrophy impairs the ability of preglomerular vessels to limit the transmission of systemic pressure to glomerular capillaries is beyond the scope of this study. Nonetheless, it is well established that inflammation of the vascular wall plays an important role. Activation of transcription factors, NF-κB and activator protein-1 (AP-1) in vascular smooth muscle cells (VSMC) induces a switch of VSMC to a proliferative phenotype [41]. This change promotes growth and increased synthesis and rearrangement of extracellular matrix proteins and decreases the synthesis of contractile proteins, which in turn decrease the response of VSMC to contractile stimulus. [42]. Thus, hypertrophy and increase in collagen content of vascular wall may decrease their ability to contract in response to a rise perfusion pressure. Inhibition of expression of selectins, VSMC proliferation and collagen deposition by MMF [43–46] would maintain the functional capacity of preglomerular vessels.

CONCLUSION

In summary, interrupting inflammation with MMF therapy prevented proteinuria, renal injury, and arteriolopathy of AAs. In addition, normal glomerular pressure was maintained, despite systemic hypertension, suggesting that vascular injury, by perpetuating glomerular hemodynamic stress may contribute to progression of renal disease.

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