Immune suppression prevents renal damage and dysfunction and reduces arterial pressure in salt-sensitive hypertension


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Tian N, Gu JW, Jordan S, Rose RA, Hughson MD, Manning RD Jr. Immune suppression prevents renal damage and dysfunction and reduces arterial pressure in salt-sensitive hypertension. Am J Physiol Heart Circ Physiol 292: H1018–H1025, 2007.—The goal of this study was to test the hypothesis that renal infiltration of immune cells in Dahl S rats on increased dietary sodium intake contributes to the progression of renal damage, decreases in renal hemodynamics, and development of hypertension. We specifically studied whether anti-immune therapy, using mycophenolate mofetil (MMF), could help prevent increases in renal NF-kB activation, renal infiltration of monocytes/macrophages, renal damage, decreases in glomerular filtration rate (GFR) and renal plasma flow, and increases in arterial pressure. Seventy-four 7–8-wk-old Dahl S, Rapp strain rats were maintained on an 8% Na, 8% Na + MMF (20 mg·kg⁻¹·day⁻¹), 0.3% Na, or 0.3% Na + MMF diet for 5 wk. Arterial and venous catheters were implanted at day 21. By day 35, renal NF-kB in 8% Na rats was 47% higher than in 0.3% Na rats and renal NF-kB was 41% lower in 8% Na + MMF rats compared with the 8% Na group. MMF treatment significantly decreased renal monocyte/macrophage infiltration and renal damage and increased GFR and renal plasma flow. In high-NA Dahl S rats mean arterial pressure increased to 182 ± 5 mmHg, and MMF reduced this arterial pressure to 124 ± 3 mmHg. In summary, in Dahl S rats on high sodium intake, treatment with MMF decreases renal NF-kB and renal monocyte/macrophage infiltration and improves renal function, lessens renal injury, and decreases arterial pressure. This suggests that renal infiltration of immune cells is associated with increased arterial pressure and renal damage and decreasing GFR and renal plasma flow in Dahl salt-sensitive hypertension.

SEVERAL MECHANISMS have been found to contribute to the etiology of salt-sensitive hypertension, including reduced levels of NO (7) and elevated oxidative stress (12, 29). In addition to these factors, there is emerging evidence indicating that the immune system may play an important role in salt-sensitive hypertension.

In several models of hypertension, renal tubulointerstitial infiltration of macrophages and lymphocytes occurs. Renal immunocompetent cells have been found in DOCA hyperten-
sion (27), post-angiotensin II (ANG) salt-sensitive hypertension (21), hypertension following NO inhibition (20), protein overload nephropathy (1), the spontaneously hypertensive rate (SHR) (22) and the double-transgenic rat (dTGR) that has human renin and angiotensinogen genes (18). Anti-immune therapy administered to each of the above models of hypertension successfully decreased arterial pressure (24).

Dahl salt-sensitive hypertension is characterized by increases in oxidative stress, severe renal damage, and decreases in renal hemodynamics (29); however, the role of renal immune cell infiltration in Dahl salt-sensitive hypertension is not clear. The transcription factor NF-kB stimulates the generation of proinflammatory cytokines and adhesion molecules. The present study was designed to determine whether Dahl S rats on a high-Na diet experience activation of renal NF-kB and renal infiltration of monocytes/macrophages. We especially studied the ability of the anti-immune agent mycophenolate mofetil (MMF) to decrease renal monocytes/macrophages and renal NF-kB activation and to prevent the hypertension, renal dysfunction, and renal damage normally associated with Dahl S rats on a high-Na diet for 5 wk.

METHODS

Animal protocol and experimental measurements. Studies were conducted in 74 conscious 7–8-wk-old male Dahl S rats, Rapp strain (Harlan Sprague Dawley, Indianapolis, IN) with the authorization of the Institutional Animal Committee. Rats arrived at our laboratory when they were 6–7 wk old, and a 1-wk recovery was afforded before one of three diets was started.

Studies were conducted in Dahl S rats that were randomly divided into four groups: 8% Na (n = 7), 8% Na + MMF (n = 8), 0.3% Na (n = 7), and 0.3% Na + MMF (n = 7). Chronic arterial and venous catheters were implanted through the femoral artery and vein with aseptic techniques and isoflurane anesthesia (1%) after 3 wk on the different Na diets. Catheters exited the scapular region through a swivel apparatus. Pulsatile arterial pressure signals were sent to a digital computer at 500 Hz for 4 s of each minute throughout the entire 24-h period to determine arterial pressure and heart rate. Rats recovered for 3–4 days before a 10-day (days 26–35) collection of arterial pressure and renal functional data.

The 8% Na + MMF and 0.3% Na + MMF treatment groups received MMF (CellCept, Roche Pharmaceutical) by gastric gavage in daily doses of 20 mg/kg body wt for 3 wk before the surgery day. Because oral MMF is insoluble in water, it was suspended in 0.5 ml of water by vigorous agitation immediately before administration. Then, during the final 1 wk, injectable MMF was infused continuously at 20 mg·kg⁻¹·day⁻¹ through the venous catheter.

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined at the end of the 5-wk period in conscious rats as we have done previously (12, 28, 29) by measuring the iothalamate and aminohippurate concentrations of a 4-h fasted plasma sample after tritiated iothalamate and aminohippurate sodium were infused intravenously for 24 h (2, 5). Renal vascular resistance was calculated from arterial pressure and renal blood flow data assuming a hematocrit of 40, based on previous results. At the end of the 5-wk period rats were anesthetized with isoflurane, and the kidneys were removed to provide tissue for various assays. Renal cortical and medullary tissues were homogenized, and chemiluminescence was determined with 5 μM lucigenin as we have done previ-

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ously (13, 29). The amounts of protein in renal tissues and urine were quantified with the Lowry and Bradford assays, respectively. An additional 45 Dahl S rats were kept on one of the three diets for 5 wk to provide additional renal tissue for the different assays, and their arterial pressures were not different from those of the catheterized groups.

Measurement of renal monocytes/macrophages and NF-κB activation. Monocytes/macrophages in the kidney sections collected at 5 wk of the various diets were measured by indirect immunoperoxidase methodology (21) using ED-1, a monoclonal antibody to monocytes/macrophages (Chemicon). NF-κB activation was measured with two techniques. In the first method, whole kidney nuclear protein extracts and electrophoretic mobility shift assay (EMSA) for NF-κB were performed by a previous method (11) with some modification. EMSA detection was performed with a Gelshift NF-κB p65 Kit (Active Motif, Carlsbad, CA) according to the manufacturer’s instructions. In the second method, 10 μg of the nuclear extract from each kidney was used in the TransAM NF-κB p65 kit (Active Motif), which can measure the binding of activated NF-κB to its consensus sequence attached to a microwell plate, according to the manufacturer’s instructions.

Analysis of glomerular necrosis, glomerular sclerosis, and tubulointerstitial injury. A periodic acid Schiff-hematoxylin renal section from each rat was examined at ×200 magnification, and necrotic and sclerotic glomeruli were counted as a proportion of all consecutively examined glomeruli as we have done previously (29). In a Masson trichrome-stained section from each rat, tubulointerstitial injury was measured as the proportion of the number of points overlying increased amounts of blue-stained interstitial tissue, dilated cast-containing tubules, or tubules showing acute injury divided by the number of points on the grid overlying nonglomerular and nonvascular cortex, as we have done previously (29).

Measurement of renal glutathiones, glutathione peroxidase, and superoxide dismutase activity. GSH and oxidized glutathione (GSSG) in the renal cortex and medulla of 8% Na and 8% Na + MMF rats were measured with the fluorescent detection of dansyl derivatives by HPLC according to the method of Jones (8). Renal cortical glutathione peroxidase (GPx) and cortical and medullary superoxide dismutase (SOD) activities were measured with kits from Oxis International according to the manufacturer’s instructions.

Statistical analysis. Statistical comparisons of data from animals on 8% Na, 8% Na + MMF, 0.3% Na, and 0.3% Na + MMF diets were performed by analysis of variance followed by a Fisher least significant difference test for post hoc analysis or a Mann-Whitney U-test. Differences were considered to be statistically significant if \( P < 0.05 \). All data are expressed as means ± SE.

RESULTS

Renal monocyte/macrophage infiltration responses to MMF. Figure 1 indicates that tubulointerstitial infiltration of monocytes/macrophages was markedly higher in Dahl S rats on an
8% Na diet compared with Dahl S rats on a 0.3% Na diet. Infiltration of renal monocytes/macrophages was significantly lower in high-Na rats treated with MMF compared with high-Na rats.

Renal NF-κB responses to MMF. Renal NF-κB was measured by two techniques: an EMSA and an ELISA method (TransAM NF-κB p65 binding assay). EMSA NF-κB data are shown in Fig. 2, A–C. Figure 2A shows a representative EMSA gel blot, and Fig. 2B shows average gel blot data. Renal NF-κB p65 binding activity in Dahl S rats after 5 wk of an 8% Na diet was 47% higher than that in the 0.3% Na group (n = 5; P < 0.01), as shown in Fig. 2B. MMF treatment in high-Na rats resulted in a 41% lower value in renal NF-κB p65 binding activity compared with the high-Na group (n = 5; P < 0.01). Figure 2C illustrates the competition experiments in which the signal of renal NF-κB p65 binding activity was completely blocked by addition of excess unlabeled wild-type, but not mutant, NF-κB p65 oligonucleotide. Results from the ELISA-TransAM NF-κB p65 binding assay shown in Fig. 2D indicate that there was a 43% higher binding of activated renal NF-κB p65 to its consensus sequence in the 8% Na group compared with the 0.3% Na group (P < 0.05). MMF treatment in high-Na rats resulted in a 38% lower value in renal NF-κB p65 binding activity of Dahl S rats compared with the high-Na group (P < 0.05).

GFR, renal plasma flow, and renal vascular resistance responses to MMF. Figure 3 illustrates that GFR was significantly lower in the 8% Na group (2.3 ± 0.2 ml/min) compared with the 8% Na + MMF (3.9 ± 0.2 ml/min), 0.3% Na (3.2 ± 0.2 ml/min), and 0.3% Na + MMF (3.5 ± 0.2 ml/min) groups. ERPF was also significantly lower in the 8% Na group (7.7 ± 0.8 ml/min) compared with the 8% Na + MMF (14.4 ± 0.7 ml/min), 0.3% Na (11.7 ± 1.7 ml/min), and 0.3% Na + MMF (12.6 ± 1.0 ml/min) groups. The data show that treatment with MMF prevented a decrease in GFR and ERPF in high-Na rats. As shown in Fig. 3, renal vascular resistance (in mmHg·ml⁻¹·min) was significantly higher in the 8% Na group (49.5 ± 5.4) compared with the 8% Na + MMF (17.6 ± 1.4), 0.3% Na (19.3 ± 3.3), and 0.3% Na + MMF (15.0 ± 1.1) groups. There were no significant differences in renal vascular resistance when comparing the 0.3% Na and 0.3% Na + MMF groups.

Mean arterial pressure, heart rate, and urinary protein excretion responses to MMF. Figure 4 indicates that arterial pressure was noticeably lower in the 8% Na Dahl S rats treated with MMF compared with the 8% Na rats. Arterial pressures are presented for the last 10 days of the 5-wk experiment. On
day 35 mean arterial pressure was 182 ± 5 mmHg in the 8% Na group and 124 ± 3 mmHg in the 8% Na MMF group (P < 0.05). Arterial pressure in the high-Na MMF group remained significantly higher than in the 0.3% Na group. MMF treatment had little effect on the heart rate of 8% or 0.3% Na Dahl S rats.

Figure 4 also shows that from day 25 through day 35 urinary protein excretion, an index of renal damage, was significantly lower in the 8% Na + MMF group compared with the 8% Na group. MMF treatment had little effect on the heart rate of 8% or 0.3% Na Dahl S rats.

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Histological analyses of kidneys. Figure 5 shows representative kidney sections for each group of rats. In the 8% Na rats (Fig. 5, top left), the kidneys displayed fibrinoid arterial and arteriolar necrosis and fibrinoid glomerular necrosis, especially in rats with the highest pressures. A small fraction of glomeruli at all levels of the cortex showed global or segmental glomerular sclerosis. Some interlobular arteries were thrombosed; some tubules contained red blood cells, degenerate cells, and cell debris; and hemorrhage occurred in the interstitium close to necrotic arterioles and glomeruli. Tubulointerstitial accumulation of immune cells was evident in the high-Na group, and this was confirmed by the ED-1 results presented in Fig. 1.

In the 8% Na + MMF rats (Fig. 5, top right), the kidneys had only occasional glomeruli that showed fibrinoid glomerular and arteriolar necrosis. Glomeruli were found at all levels of the cortex that demonstrated segmental and global glomerular sclerosis. The intima of some arterioles and small arteries demonstrated hyperplasia. Tubulointerstitial injury, which was much less common than in the high-Na rats, exhibited some areas with dilated tubules surrounded by increased amounts of interstitial connective tissue. In the 0.3% Na and 0.3% Na + MMF groups (Fig. 5, bottom), the kidneys had a normal appearance, with infrequent sclerotic glomeruli in the subcapsular cortex.

Average glomerular and tubulointerstitial damage responses to MMF. Figure 6 indicates that the tubulointerstitial damage averaged 0.27 ± 0.07 (fractional area) in the high Na group, and the 8% Na + MMF rats had a fractional area of 0.08 ± 0.02. Figure 6 also shows that the glomerular necrosis was markedly higher in the 8% Na Dahl S rats compared with either the 8% Na + MMF or the 0.3% Na group. The amount of glomerular necrosis in the Dahl S rats in the high-Na + MMF group was noticeably lower than in the high-Na group.

Renal SOD activity. Renal SOD activity decreased in the high-Na group compared with the low-Na group but did not significantly increase during MMF administration. Cortical

Fig. 3. Glomerular filtration rate (GFR; top), effective renal plasma flow (ERPF; middle), and renal vascular resistance (bottom) responses in Dahl S rats in 8% Na (n = 7), 8% Na + MMF (n = 8), 0.3% Na (n = 7), and 0.3% Na + MMF (n = 7) groups. †P < 0.05, high Na alone group vs. high Na + MMF group. *P < 0.05 vs. low Na alone group.

Fig. 4. Mean arterial pressure (top), heart rate (middle), and urinary protein excretion (bottom) responses in Dahl S rats in 8% Na (n = 7), 8% Na + MMF (n = 8), 0.3% Na (n = 7), and 0.3% Na + MMF (n = 7) groups. †P < 0.05, high Na alone group vs. high Na + MMF group. *P < 0.05 vs. low Na alone group.
SOD activity (in U/mg) averaged 498 ± 62 (P < 0.05 compared with 0.3% Na group), 572 ± 62, and 1,008 ± 136 in the 8% Na, 8% Na + MMF, and 0.3% Na groups, respectively. Medullary SOD activity (in U/mg) averaged 400 ± 74 (P < 0.05 compared with 0.3% Na group), 490 ± 39, and 1,081 ± 121 in 8% Na, 8% Na + MMF, and 0.3% Na groups, respectively.

Renal cortical and medullary glutathione and cortical O$_2^-$ release responses to MMF. The GSH-to-GSSG ratio in both renal cortical and medullary tissue was significantly higher in high-Na + MMF rats compared with high-Na rats. In the renal cortex the GSH-to-GSSG ratio was 0.25 ± 0.03 in the high-Na group (n = 7) and 0.94 ± 0.05 (P < 0.05) in the high-Na + MMF group (n = 7). In the renal medulla the GSH-to-GSSG ratio was 0.37 ± 0.11 in the high-Na group (n = 7) and 1.82 ± 0.75 (P < 0.05) in the high-Na + MMF group (n = 7). The cortical levels of GPx in the 8% Na group averaged 54 ± 6 mU/mg in the cortex and did not significantly change in the 8% Na + MMF, 0.3% Na, or 0.3% Na + MMF groups. O$_2^-$ release from the renal cortex was 63 ± 3 cpm/mg in the 8% Na group (n = 8), which was significantly greater than the values of 44 ± 6 cpm/mg in the 8% Na + MMF group (n = 4). Renal cortical O$_2^-$ release was not significantly different in the 0.3% Na group and the 0.3% Na + MMF group.

DISCUSSION

The major findings of this study are that placing Dahl S salt-sensitive rats on a high-Na diet activated renal NF-κB, markedly increased renal macrophage infiltration, and caused hypertension and renal dysfunction and damage. Treatment of high-Na Dahl S rats with MMF resulted in lower renal NF-κB, renal macrophage infiltration, renal damage, renal vascular resistance, and arterial pressure and increased GFR and renal plasma flow. These data suggest that renal infiltration of immune cells is one of the major contributors to elevated blood pressure, renal damage, and decreases in GFR and renal plasma flow in Dahl salt-sensitive hypertension.

Previous studies have shown that several models of experimental hypertension are characterized by renal infiltration of immunocompetent cells. Treatment of these animal models with MMF has been shown to be an effective way to reduce renal immune cells and to reduce arterial pressure. In double-transgenic rats harboring both human renin and angiotensinogen genes, renal infiltration of immunocompetent cells is activated, and MMF attenuated this infiltration and decreased oxidative stress (18). ANG II administration in rats causes a secondary salt-sensitive hypertension after the ANG II is stopped; this is accompanied by increased renal monocytic cells, increased oxidative stress, and elevations in systolic blood pressure and renal vasoconstriction, and these changes were blunted with MMF (6, 21). Other forms of hypertension that experience an increased presence of renal immune cells are nitro-L-arginine methyl ester (L-NAME) hypertension (20), high-protein-induced proteinuria (1), aging Zucker rats (23), and SHR (22), and MMF ameliorated these changes. In the above types of hypertension, the common thread is the renal
control of arterial pressure in rats with ANG II-induced renal injury prevented 70–75% of the renal injury, but this left 25–30% of the renal injury unexplained (17). Salt-sensitive hypertensive models have shown considerable renal injury and renal immune cell infiltrate including aging Zucker rats (23), high-protein-induced proteinuria (1), DOCA-salt rats (3), post-ANG II hypertension (6), and l-NAME-hypertensive rats (20). Inhibition of the inflammatory response in each of the above models with MMF improved the high blood pressure and reduced renal damage (24). However, whether the improvement in renal damage was caused by a reduction in blood pressure or a reduction in inflammation is not clear. Studies in dTGRs in some cases have shown blood pressure independence from renal damage. Lowering blood pressure to control values with hydralazine, reserpine, and hydrochlorothiazide in the dTGR decreased renal monocytes/macrophages but only delayed renal damage by 1 wk, but a human renin inhibitor normalized blood pressure and reduced renal immune cells and renal damage (15). Dexamethasone treatment of dTGRs did not change blood pressure but reduced renal immune cells and renal damage, whereas MMF treatment of these rats decreased blood pressure, renal immune cells, and renal damage (18). Antioxidant treatment of dTGRs reduced blood pressure, renal immune cells, and renal damage (14). There seems to be evidence supporting both blood pressure independence and dependence of renal damage in different hypertensive models.

The inhibition of the renal immune system with MMF may have protected the kidney from damage by several mechanisms. First, a possible mechanism of renal protection is the lowering of arterial pressure, which has been shown to be highly important in preventing kidney damage (4). This would clearly reduce mechanical stress on the kidney. Second, MMF reduced renal oxidative stress in the high-Na group, which may have directly reduced renal damage. Third, MMF may have reduced renal damage by decreasing renal levels of cytokines, which could have been significantly impacted by the decrease in renal NF-κB.

A high-Na diet in the present study caused a 47% increase in renal NF-κB, and the administration of MMF to Dahl S rats on high Na caused a marked decrease in NF-κB. Stimulation of the transcription factor NF-κB causes generation of proinflammatory cytokines and adhesion molecules (16). Since MMF treatment decreased NF-κB, resulting in a decrease in renal interstitial monocytes/macrophages in the present study, it is likely that NF-κB activation is associated with renal infiltration of the macrophages (24). Therefore, NF-κB activation seems to play an important role in Dahl salt-sensitive hypertension.

Oxygen free radicals are known stimulators of NF-κB (25) and thus can indirectly cause inflammation. In the present study, oxygen free radicals were elevated in the kidneys of Dahl S rats on high Na, as evidenced by elevated renal O$_2^\cdot$ and marked decreases in the renal GSH-to-GSSG ratio. It is likely that the result of the excess oxidative stress in the present study was an increase in NF-κB activation, which in turn caused a nonspecific inflammation in Dahl S rats that contributed to the hypertension and the related renal damage.

Elevations in NF-κB can have prohypertensive effects by increasing renal immune cells, which release ANG II (24). In experimental hypertensive models such as overload proteinuria (1), post-NO synthesis inhibition (20), and the SHR (22), lymphocytes and macrophages infiltrating the kidney express infiltration of immunocompetent cells, which when inhibited by MMF decreases any elevations in blood pressure and renal damage and increases renal blood flow. The data in the present study indicate that renal infiltration of immune cells plays an important role in Dahl salt-sensitive hypertension and that MMF was an effective way to reverse any immune cell-mediated changes.

Several other approaches to immunosuppression have prevented hypertension or renal damage in models of experimental hypertension. Chronic immunosuppressant therapy with cyclophosphamide in 3-wk-old SHRs or the New Zealand Black mouse caused a significant attenuation of hypertension (9, 26). Cyclophosphamide had no effect on 3-wk-old Wistar rats (9). Thymus implants in the SHR increase the functional capability of T cells, possibly by increasing T helper cells. Finally, in 16-wk-old SHRs, the progression of hypertension was prevented with thymus implants (19).

There is a question in this study and in many others as to whether high pressure causes the renal injury or renal injury causes the hypertension. The relationship of renal immune cells in this renal damage is also of great interest. Normally, immune cell infiltration occurs in response to tissue damage; therefore, in the present study it is likely that the high blood pressure in the Dahl S rats may have caused some renal damage and initiated an immune response that exacerbated the renal damage. However, MMF caused parallel decreases in renal monocytes/macrophages, arterial pressure, and renal damage, making it difficult to differentiate cause and effect. Other studies have addressed this issue more directly. Servo
ANG II. The ANG II can have renal vasoconstrictor and renal sodium-retaining effects, thus increasing blood pressure. Aldosterone production could also be increased by ANG II. The Dahl S rat has been shown to have an increase in intrarenal ANG I during a high-Na diet (10), despite the presence of a very low blood ANG II concentration. Intrarenal ANG II can also stimulate NAD(P)H oxidase, which releases O$_2^{-}$, which has been shown to cause a rightward shift in the pressure natriuresis mechanism and thus an increase in arterial pressure (7, 12, 29). Perhaps this link between NF-κB and O$_2^{-}$ through ANG I explains why the anti-immune substance MMF caused a decrease in renal NF-B and thus a decrease in renal oxidative stress.

Since MMF caused a decrease in renal cortical O$_2^{-}$ release and an increase in renal cortical and medullary GSH/GSSG in the present experiment, it is important to determine whether MMF has a direct antioxidant effect or whether MMF causes a decrease in oxidative stress due to a decrease in O$_2^{-}$-producing immune cells. In the present study, MMF caused no significant change in renal cortical or medullary SOD, suggesting that the MMF effects on high-Na Dahl S rats do not involve a change in renal SOD activity. Previous studies have also looked at the potential role of MMF in reducing oxidative stress. In a study in adriamycin-induced renal failure in rats, MMF did not cause any direct antioxidant effect (30). In that study the following indexes of oxidative stress were not changed by MMF: renal cortical thiobarbituric acid-reactive substances, renal malondialdehyde, renal 4-hydroxynonenal, GPs, manganese and copper-zinc SOD, and proximal tubule ferric iron (30). Several studies have shown that the immune cells that invade the kidney during hypertension actively produce O$_2^{-}$ (20, 21, 24). Treatment of these hypertensive models with MMF reduced monocye/macrophage infiltration in the kidney, which proportionally decreased the O$_2^{-}$-positive cells (20, 21, 24). Therefore, the antioxidant effects of MMF are likely not caused by a direct antioxidant effect but are due to the reduction in renal infiltration of superoxide-producing immune cells. Data from the present study support this conclusion in that MMF caused no increase in renal cortical O$_2^{-}$ production in the 0.3% Na group.

The decline in renal hemodynamic function in the Dahl S rats on high Na is evident in Fig. 3, and importantly, MMF treatment markedly attenuated the reductions in GFR, renal plasma flow, and renal vascular resistance. MMF actually caused a slight increase in GFR in the 8% Na group compared with the 0.3% Na group, which could have been caused by the 25 mmHg greater arterial pressure in the high-Na group coupled with a normal renal vascular resistance. The decreases in GFR and renal plasma flow in the high-Na rats could have been caused by several factors. First, the glomerular and vascular necrosis evident in Fig. 5 could decrease the number of viable nephrons, resulting in a decrease in GFR and renal blood flow. Second, plasma and urinary isoprostanes have been shown in our laboratory (13) to increase during high Na intake in Dahl S rats, and isoprostanes act as vasoconstrictors. Third, intrarenal ANG II, another vasoconstrictor, may increase in Dahl S rats on high Na as has been seen before in other laboratories (10). Fourth, excess release of renal O$_2^{-}$ as occurred in the present study could have inactivated NO, thus decreasing renal vasorelaxation and causing more vasoconstriction. Therefore, the anti-immune treatment with MMF in the present study may have attenuated the progression of renal damage and the reduction in GFR and renal plasma flow normally seen in Dahl salt-sensitive hypertension by inhibiting vasoconstriction or enhancing vasodilation.

MMF treatment in Dahl S rats on a high-Na diet caused marked decreases in renal damage as evidenced by decreases in urinary protein excretion and striking decreases in tubulointerstitial and glomerular damage. In fact, the glomeruli, tubules, and interstitium of the high-Na Dahl S rats experienced severe glomerular necrosis and tubulointerstitial damage, as seen in Fig. 5. Kidneys from high-Na Dahl S rats have an appearance similar to that of kidneys from patients with florid malignant hypertension. The MMF treatment in high-Na Dahl S rats prevented the glomerular and arteriolar necrosis and shifted the histopathology toward a more stable glomerulosclerosis and arterial intimal hyperplasia. Similarly, the acute tubulointerstitial injury in the high-Na Dahl S rats was markedly blunted in rats treated with MMF. The improvement in renal damage with MMF treatment suggests that renal immune cell infiltration may lead to renal damage in Dahl salt-sensitive hypertension. However, our data do not exclude the lessening in renal damage because of the reduction in arterial pressure.

In conclusion, MMF treatment significantly decreased renal NF-κB activation, which markedly decreased renal macrophage infiltration. MMF decreased mean arterial pressure, renal tubulointerstitial damage, glomerular necrosis, and urinary protein excretion and increased renal plasma flow and GFR. In Dahl salt-sensitive hypertension, increased renal infiltration of immune cells may play an important role in increasing arterial pressure and renal damage and decreasing GFR and renal plasma flow. Treatment with MMF decreases renal NF-κB and renal immune cell infiltration, blunts renal dysfunction, lessens renal injury, and decreases arterial pressure in Dahl salt-sensitive hypertension.

GRANTS

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