Infiltrating T lymphocytes in the kidney increase oxidative stress and participate in the development of hypertension and renal disease

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De Miguel C, Guo C, Lund H, Feng D, Mattson DL. Infiltrating T lymphocytes in the kidney increase oxidative stress and participate in the development of hypertension and renal disease. Am J Physiol Renal Physiol 300: F734–F742, 2011. First published December 15, 2010; doi:10.1152/ajprenal.00454.2010.—The present studies examined the role and mechanism of action of infiltrating T lymphocytes in the kidney during salt-sensitive hypertension. Infiltrating T lymphocytes in the Dahl salt-sensitive (SS) kidney significantly increased from 7.2 ± 1.8 × 10³ cells/2 kidneys to 18.2 ± 3.9 × 10³ cells/2 kidneys (n = 6/group) when dietary NaCl was increased from 0.4 to 4.0%. Furthermore, the expression of immunoreactive p67phox, gp91phox, and p47phox subunits of NADPH oxidase was increased in kidneys (of oxidative stress) also increased from 367 to 49 to 688 nmol/day (n = 8/group) when NaCl intake was increased in Dahl SS rats. Studies were then performed on rats treated with a daily injection of vehicle (5% dextrose) or tacrolimus (0.25 mg·kg⁻¹·day⁻¹ ip), a calcineurin inhibitor that suppresses immune function, during the period of high-NaCl intake (n = 5/group). In contrast to the immune cell infiltration, increased NADPH oxidase expression, and elevated urine TBARS excretion in vehicle-treated Dahl SS fed high salt, these parameters were unaltered as NaCl intake was increased in Dahl SS rats administered tacrolimus. Moreover, tacrolimus treatment blunted high-salt mean arterial blood pressure and albumin excretion rate (152 ± 3 mmHg and 20 ± 9 mg/day, respectively) compared with values in dextrose-treated Dahl SS rats (171 ± 8 mmHg and 74 ± 28 mg/day). These experiments indicate that blockade of infiltrating immune cells is associated with decreased oxidative stress, an attenuation of hypertension, and a reduction of renal damage in Dahl SS rats fed high salt.

reactive oxygen species generation; chronic renal insufficiency

OXIDATIVE STRESS, defined as a persistent imbalance between the production of highly reactive molecular species (mainly oxygen and nitrogen) and antioxidant defenses, has been implicated in pathophysiological conditions that affect the cardiovascular system (12, 28, 35). Increased levels of oxidative stress have been described in experimental models of hypertension (2, 6, 20) and hypertensive patients (22, 35).

A number of studies in animal models of hypertension demonstrated elevations of blood pressure by stimulation of reactive oxygen species (ROS) generation (24, 44, 47). Moreover, treatment with a variety of antioxidants reduces blood pressure in several genetic and experimental models of hypertension (4, 7, 30, 40, 48). One of the most important biological mechanisms for the production of ROS results from the generation of superoxide (O₂⁻) from O₂ by the enzyme NADPH oxidase (14). Stimulation of NADPH oxidase appears to be the primary source of oxidants in systemic arterial vessels in renovascular hypertension (16), ANG II-induced hypertension (8, 34), DOCA-salt hypertension (42, 49), chronic renal insufficiency (47), and the spontaneously hypertensive rat (49). In humans, NADPH oxidase is the principal source of O₂⁻ in vascular smooth muscle cells (1), and levels of NADPH oxidase are increased in patients with essential hypertension (45).

Infiltration of immune cells into the kidney has been documented in many animal models of nonimmune hypertension (15, 27, 29, 33); however, the exact mechanism by which this infiltration may lead to an elevation of mean arterial pressure (MAP) and/or renal damage has not yet been elucidated. Chronic inhibition of the immune system has been shown to attenuate hypertension and renal damage in different animal models of hypertension (10, 13, 25, 27, 31, 33) and human patients (17, 41), further suggesting a relationship between the immune system and the disease. Interestingly, elevated intrarenal oxidative state is often correlated to immune cell infiltration, and it has been proposed that both processes participate in a vicious positive feedback cycle (13), where ROS activate the synthesis of several proinflammatory chemokines and cytokines (11, 32, 39), that in turn stimulate the renal infiltration of immune cells. Moreover, different immune cell types contain the necessary machinery for the production of ROS (5, 19, 23), making them capable of further increasing the intrarenal levels of free radicals.

Although some studies demonstrated a relationship between infiltrating immune cells and oxidative stress in other models of salt-sensitive hypertension (3, 8, 38), little is known about this interconnection in the Dahl salt-sensitive rat. Our laboratory previously demonstrated that immunosuppressive treatment with mycophenolate mofetil ameliorates the sodium-sensitive hypertension and renal damage that occur in this rat strain (10, 25). Studies described in this manuscript were designed to test the hypothesis that renal infiltration of immune cells following an increase in the sodium intake mediates further development of hypertension and renal damage by increasing the intrarenal levels of free radicals.

METHODS

Experimental Animals

Experiments were performed on male Dahl salt-sensitive (SS) rats (SS/JHS/HSDMcwi) obtained from a colony maintained at the Medical College of Wisconsin. Some experiments were performed on outbred Sprague-Dawley (SD) rats obtained from Harlan Laboratories (Madison, WI). All experiments were performed with age-matched (littermates when possible) animals serving as control and experimental subjects. This experimental design minimized the impact of group-to-group variation on the experimental results. The Dahl SS breeders and weanlings were maintained on purified AIN-76A rodent diet (Dyets, Bethlehem, PA) containing 0.4% NaCl. At 9 wk of age, the salt

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content of the diet was increased to 4.0% NaCl and maintained for the
remaining 3 wk of the study; all experimental measurements were
made during the final week of the study (i.e., during the third week of
the 4.0% NaCl diet). Other Dahl SS rats, as described below, were
maintained on the 0.4% NaCl diet for the final 3 wk of the study;
experimental measurements in these rats were also made at 12 wk of
age. The SD rats, which served as a normotensive control strain in this
experiment, were fed the 0.4% NaCl diet from the time they were
received at Medical College of Wisconsin; the rats were then fed 4.0%
NaCl for the 3-wk experimental period and experimental variables
were measured after 3 wk of the high-salt diet. All the animals were
provided water and food ad libitum, and the protocols were approved
by the Medical College of Wisconsin Institutional Animal Care and
Use Committee.

Experimental Design

Group 1. The initial studies were performed in two subgroups of
Dahl SS rats. When the animals were 9 wk old, half of the animals
were switched from 0.4% NaCl to 4.0% NaCl for the final 3 wk of the
study. The remaining animals were maintained on 0.4% NaCl. Over-
night urine samples were collected on the last night of the study for
measurement of creatinine, protein, and excretion of thiobarbituric
acid-reactive substances (TBARS). On the last day of the study, the
animals were deeply anesthetized with pentobarbital sodium (50
mg/kg ip) and blood and kidneys were collected for T lymphocyte
isolation and TBARS measurement in renal tissue.

Group 2. Dahl SS rats were maintained on 0.4% NaCl until 9 wk
of age when the dietary NaCl content was increased to 4.0%; at the
time of the increase in salt intake, the animals were randomly assigned
to receive daily injections of the immunosuppressant agent tacrolimus
(0.25 mg·kg⁻¹·day⁻¹ ip) or vehicle (dextrose). The daily injections
started on the same day as the diet was changed and lasted for the final
3 wk of the protocol. When the rats were 10 wk old, polyvinyl
catheters were placed in the femoral artery, the animals were permit-
ted to recover, and daily blood pressure recordings were made at 12
wk of age. Daily blood pressure measurements were made during a 3-
to 4-h period with solid state pressure transducers and a general
purpose amplifier. The output of the amplifiers was directed into an
A/D convertor and a personal computer; the data were collected at 100
Hz and minute averages of MAP were calculated on line. The mean
of three daily MAP averages was used as the blood pressure value for
each rat.

Overnight urine samples were collected on the final night of the
study for measurement of creatinine, protein, and TBARS excretion.
On the last day of the study, the animals were deeply anesthetized
with pentobarbital sodium (50 mg/kg ip) and blood and kidneys were
collected for T lymphocyte isolation and TBARS measurement in
renal tissue.

Group 3. To test for the effects of the immunosuppressive agent in
normal rats, a group of SD rats were fed 4.0% NaCl chow and randomly
assigned to receive daily injections of tacrolimus (0.25
mg·kg⁻¹·day⁻¹ ip) or vehicle (dextrose). The daily injections started
on the same day as the diet was changed and lasted for 3 wk. As
described above, the rats were instrumented and daily blood pressure
recordings were made at 12 wk of age. Overnight urine samples were
collected on the final night of the study.

Group 4. Dahl SS rats were maintained on 0.4% NaCl until 9 wk
of age when the dietary NaCl content was increased to 4.0%; at the
time of the increase in salt intake, the animals were randomly assigned
to receive normal water or water containing the antioxidant Tempol
(10 mM; Sigma, St. Louis, MO). The treatment started on the same
day as the sodium content of the diet was increased and lasted for 3
wk. MAP in these rats was determined after 3 wk on high-salt diet
with implanted telemetry transmitters. Daily blood pressure measure-
ments were made during a 3- to 4-h period; the mean of three daily
MAP averages was used as the blood pressure value for each rat. In
addition, an overnight urine sample was obtained to quantify urinary
albumin excretion rate.

Surgical Preparation

Rats were deeply anesthetized with a mixture of ketamine (75
mg/kg ip), xylazine (10 mg/kg ip), and acepromazine (2.5 mg/kg ip),
with supplemental anesthesia administered as needed. With the use of
aseptic techniques, polyvinyl catheters were placed in the femoral
artery for measurement of arterial pressure. Catheters were tunneled
subcutaneously and exteriorized at the back of the neck. In some rats,
a telemetry transmitter (Data Sciences International) for measuring
arterial blood pressure was aseptically implanted in the carotid artery,
with the body of the transmitter implanted subcutaneously in the back
of the animal. Animals were maintained on warming trays during and
following surgery. Analgesics and antibiotics were administered after
surgery to control pain and infection. Rats were allowed to recover for
5–7 days before the experimental protocol started.

T Lymphocyte Isolation

Infiltrating immune cells were isolated from the kidney using
methods we previously described (10). Briefly, the rats were anesthe-
itized with pentobarbital sodium (50 mg/kg ip), the kidneys were
flushed with heparinized saline, and the kidneys were minced and
incubated in a phosphate-buffered solution containing collagenase.
The digested renal tissue was centrifuged on Histopaque to isolate
mononuclear cells; the mononuclear cells were counted by hemacy-
tometry and incubated with rat Pan T cell antibody coupled to
magnetic microbeads (MACS Rat Pan T Cells Microbeads, Miltenyi
Biotec); the T lymphocytes were then isolated with a magnetic
column (MACS Separation Columns, Miltenyi Biotec) and counted.
Circulating T cells were isolated from the blood using a protocol
similar to the one described for the kidneys.

Histological and Immunohistochemical Analysis

Kidneys were obtained for histological and immunohistochemical
analysis using methods we previously described (10, 25). The kidneys

![Fig. 1. Thiobarbituric acid-reactive substances (TBARS) in urine (A) and in
renal tissue (B) of Dahl salt-sensitive (SS) rats maintained on 0.4 or 4.0% NaCl
diet for 3 wk. *P < 0.05 vs. 0.4% NaCl-fed animals.](http://ajprenal.physiology.org/)}
were fixed in a 10% formaldehyde solution; and the tissue was paraffin embedded (Microm HMP 300), cut in 3-μm sections (Microm HM355S), mounted, and stained with Gomori’s One-Step Trichrome. For immunohistochemistry, slides were deparaffinized and incubated with Proteinase K for antigen retrieval. The primary monoclonal antibody used to detect T cells was anti-CD43 (Abcam). A biotinylated horse anti-mouse secondary antibody was used for development with avidin-biotinylated horseradish peroxidase complex (Vectastain ABC kits; Vector Laboratory, Burlingame, CA). The slides were lightly counterstained with aniline blue dye and photographed.

Urine and Renal Tissue TBARS Analysis

Overnight urine samples and renal protein homogenates were used to assess TBARS. At the end of the studies, the animals were deeply anesthetized with pentobarbital sodium (50 mg/kg ip) and their kidneys were collected, snap-frozen, and stored at −80°C. Before analysis, the kidneys were thawed, homogenized, and sonicated over ice in RIPA buffer. TBARS were measured using a TBARS assay kit (Cayman Chemical, Ann Arbor, MI). Decomposition of the unstable peroxides derived from polyunsaturated fatty acids results in the formation of malondialdehyde, which is quantified fluorometrically (excitation wavelength: 530 nm; emission wavelength: 550 nm) following its controlled reaction with thiobarbituric acid. Measurement of TBARS is a well-established method for screening and monitoring lipid peroxidation (50), a sign of oxidative stress.

Western Blot Analysis

Blotting experiments were performed with 100 μg of protein obtained from a renal tissue homogenate or protein from 2.5 × 10⁶ T lymphocytes. Proteins were separated on an 8–16% SDS-PAGE gel, transferred to a nitrocellulose membrane (Bio-Rad), blocked for 1 h at room temperature, and incubated overnight with primary antibody at 4°C. The primary antibodies used against the different subunits of NADPH oxidase were gp91phox (BD), p67phox (Upstate), p47phox (Santa Cruz Biotechnology), and p22phox (Santa Cruz Biotechnology). Bound primary antibodies were detected with a horseradish peroxidase-labeled secondary antibody and visualized by chemiluminescence. Band intensities were quantified using densitometry (UNSCAN-IT Gel Automated Digitizing System, Silk Scientific) and normalized to β-actin expression.

Fig. 2. A: total number of T lymphocytes isolated from both kidneys of Dahl SS rats maintained on 0.4 or 4.0% NaCl diet for 3 wk. Immunohistochemical localization of T cells in the renal cortex (B and C) and medulla (D and E) of Dahl SS rats fed 0.4% (B and D) or 4.0% NaCl chow (C and E). T cells were localized in the renal tissue with antibodies directed against the cell surface marker CD43 (original magnification ×20). *P < 0.05 vs. 0.4% NaCl-fed animals.
Statistical Analysis

Data are presented as means ± SE. For comparisons within a group, data were analyzed using a one-way ANOVA for repeated measures with a Tukey’s post hoc test. For comparisons between groups, the data were analyzed using a two-way ANOVA for repeated measures with a Holm-Sidak post hoc test. A probability value of P < 0.05 was considered significant.

RESULTS

Effects of Elevated Sodium Intake on Oxidative State, Expression of NADPH Oxidase, and Immune Cell Infiltration in SS Rats

Elevated sodium consumption in Dahl SS rats was associated with an increased oxidative state in the kidneys. After 3 wk of high-salt diet, the urinary excretion of TBARS was significantly greater than the levels presented by the same animals during the low-salt period (688 ± 92 vs. 367 ± 49 nmol/day, respectively; n = 7/group), indicating that the systemic oxidative stress of these animals had increased with the elevated sodium intake (Fig. 1A). Renal tissue TBARS levels were also significantly increased in the animals that were fed the high-salt diet for 3 wk, with values that were ~1.3 times greater than those in the low salt-fed animals (3.2 ± 0.1 vs. 2.3 ± 0.1 nmol/mg of tissue protein; n = 7/group; Fig. 1B). The elevated sodium intake was associated with a significantly increased infiltration of T lymphocytes (18.2 ± 3.9 vs. 7.2 ± 1.8 × 10^5 cells/2 kidneys; n = 6/group), as indicated in Fig. 2A. An immunohistochemical analysis localized the increased number of T cells to areas surrounding glomeruli in the renal cortex (Fig. 2, B and C) and damaged tubules in the renal outer medulla (Fig. 2, D and E) of the Dahl SS rats fed the high-salt diet.

Effects of Chronic Administration of Tacrolimus on MAP, Albumin Excretion Rate, and Renal Damage

Figure 3 illustrates the effects of chronic administration of the immunosuppressant agent tacrolimus on MAP (Fig. 3A) and albumin excretion rate (Fig. 3B). Dahl SS rats were administered daily intraperitoneal injections of vehicle (dextrose) or tacrolimus (0.25 mg·kg⁻¹·day⁻¹) during the 3-wk period that they were fed 4.0% NaCl. Chronic treatment with tacrolimus significantly attenuated the development of hypertension and albumin excretion rate. MAP values for vehicle-treated animals averaged 171 ± 7 mmHg, while tacrolimus-treated animals presented average values of 152 ± 2 mmHg (n = 5/group). Treatment with tacrolimus also decreased the albumin excretion rate from 64 ± 11 to 20 ± 8 mg/day (n = 5/group).

Daily administration of tacrolimus ameliorated the development of renal damage (Fig. 4, A–D). The vehicle-treated Dahl SS rats fed 4.0% NaCl for 3 wk had both glomerular (blue-stained fibrotic tissue and collapsed capillary structure) and tubular damage (red protein deposition casts indicating blocked tubules in the outer medulla). Visibly less glomerular and tubular injury was observed in the kidneys of the tacrolimus-treated rats. Renal damage, evaluated by the glomerular injury index (Fig. 4E) and the percentage of renal medullary area consisting of protein casts (Fig. 4F), was significantly decreased in rats treated with tacrolimus (n = 5/group). The renal damage in tacrolimus-treated rats was similar to that we previously described in Dahl SS rats maintained on a 0.4% NaCl diet (10).

To test for nonspecific effects of the immunosuppressive agent, additional studies were performed in SD rats fed the 4.0% NaCl chow and administered vehicle or tacrolimus for 3 wk (n = 4–5/group). At the end of the 3-wk period, MAP was significantly elevated (133 ± 4 mmHg) in the tacrolimus-treated rats compared with the vehicle-treated group (120 ± 3 mmHg). Albumin excretion rate was unaltered between the vehicle-treated (5.1 ± 2.3 mg/day) and tacrolimus-treated rats (9.1 ± 7.5 mg/day).

Influence of Tacrolimus on Oxidative State and Renal Immune Infiltration

In contrast to the increase in oxidative stress observed in untreated Dahl SS rats fed 4.0% NaCl chow (n = 12/group), there was no increase in TBARS excretion in tacrolimus-treated SS rats fed 4.0% NaCl (510 ± 208 nmol/day) compared with TBARS excretion rate (551 ± 80 nmol/day) during the low-salt period. Chronic treatment with tacrolimus during the high-salt period significantly decreased the absolute numbers of infiltrating T lymphocytes in the kidneys (18.8 ± 2 × 10^5 cells/2 kidneys in vehicle-treated animals vs. 10.4 ± 2.2 × 10^5 cells/2 kidneys in tacrolimus-treated animals; n = 6/group; Fig. 5A). Although tacrolimus administration tended to decrease blood T lymphocytes in the rats, this decrease was not significant (4.7 ± 1.1 × 10^6 vs. 8.3 ± 1.9 × 10^6 cells/ml; n = 5/group).

Immunoblotting protocols were performed to study differences in expression of NADPH oxidase in renal tissue when the Dahl SS rats were fed 0.4 or 4.0% NaCl or fed 4.0% NaCl...
and receiving tacrolimus treatment. As indicated in Fig. 5B, the elevation of the sodium content of the diet was accompanied by a significant increase of the renal expression of p67phox, one of the subunits that form NADPH oxidase. When the expression of p67phox was normalized to \( \beta \)-actin, Dahl SS rats fed 4.0% NaCl presented 1.6 times greater p67phox than the animals that were fed 0.4% NaCl (1.19 ± 0.1 vs. 0.73 ± 0.1 arbitrary units; \( n = 4–6 \) /group). Expression of p67phox in renal tissue was significantly decreased in the animals treated with tacrolimus during the high-salt period (0.56 ± 0.1 arbitrary units). Tacrolimus-treated animals presented levels of p67phox expression in the renal tissue that were not different than the low salt-fed animal levels, indicating that the enhanced oxidative state in the kidney of Dahl SS rats fed 4.0% NaCl was attenuated by this immunosuppressive treatment.

**NADPH Oxidase Expression in Isolated T Lymphocytes**

Additional experiments were then performed to determine whether T lymphocytes isolated from kidneys of Dahl SS rats are capable of participating in free radical formation. As illustrated in Fig. 6, both renal tissue and isolated T cells express the NADPH oxidase subunit p67phox. Moreover, when normalized to \( \beta \)-actin, the expression was significantly greater in the T cells compared with renal tissue. In addition, gp91phox, p22phox, and p47phox subunits of NADPH oxidase were also identified in T cells, indicating that the infiltrating cells have the molecular machinery needed for the production of ROS. Of the different subunits, significantly greater expression of p67phox and gp91phox was observed in T cells compared with tissue. Figure 7 illustrates the calculated NADPH oxidase expression contributed by infiltrating T lymphocytes in kidneys of Dahl SS rats fed low- or high-salt diet for 3 wk (\( n = 6 /\)group). The significant increase in infiltrating T cells contributes to a significantly greater expression of p67phox, gp91phox, and p47phox in the kidneys of Dahl SS rats.

**Effects of Tempol on MAP and Albumin Excretion Rate**

A final set of studies was performed to assess the effects of the antioxidant tempol on MAP (Fig. 8A) and albumin excretion rate (Fig. 8B) in Dahl SS rats fed high salt. The salt-sensitive increase in blood pressure was significantly ameliorated in rats that were treated with tempol (10 mmol/l; Sigma)
during the high-salt period (126 ± 1 vs. 146 ± 2 mmHg in the control animals; n = 6/group). In addition, chronic administration of tempol to Dahl SS rats fed the high-salt diet significantly reduced the urinary albumin excretion rate by 46% (121 ± 19 vs. 223 ± 36 mg/day; n = 6/group), indicating that the renal damage in these animals was diminished by treatment with this SOD mimetic.

**DISCUSSION**

The present studies demonstrate that T lymphocytes that infiltrate the kidney can participate in the development of SS hypertension and renal disease in Dahl SS rats by increasing the intrarenal level of free radicals. These experiments demonstrate an association between increased renal infiltration of T cells, increased oxidative stress, and increased expression of NADPH oxidase in the renal tissue of Dahl SS rats fed a high-salt diet. Moreover, chronic administration of the immunosuppressant agent tacrolimus during the period of high-salt intake decreased T lymphocyte infiltration, reduced the oxidative state and renal expression of the NADPH oxidase subunit p67phox, and ameliorated hypertension and renal disease. The attenuation of SS hypertension and albuminuria observed with the administration of the antioxidant tempol also highlights the importance of ROS production by the infiltrating T lymphocytes. Taken together, these data indicate that infiltrating T lymphocytes are capable of participating in the progression of hypertension and renal damage by elevating intrarenal oxidative stress in the Dahl SS rat.

Oxidative stress has been implicated in pathophysiological conditions that affect the cardiovascular system. Increased levels of oxidative stress have been described in experimental models of hypertension (2, 6, 20) and hypertensive patients (22, 35). Treatment with a variety of antioxidants has been shown to decrease arterial blood pressure in several experimental and genetic models of hypertension (4, 7, 30, 40, 48). Several groups also demonstrated the association between elevated levels of intrarenal ROS and renal damage (44), and the deleterious effects that deficiencies in catalase (21, 24) or superoxide dismutase (26, 37) have on renal function.

Renal infiltration of immune cells has been reported in many experimental models of nonimmune hypertension (15, 27, 29, 31, 33); however, the exact mechanisms by which these infiltr-
Although our data describe a significant inhibition of renal T cell infiltration with administration of the immunosuppressant agent tacrolimus, we cannot ensure that these effects are limited to this immune cell type. Tacrolimus is an inhibitor of calcineurin, a widely distributed enzyme; therefore, it is possible that the effects of administration of this drug are not limited to T lymphocytes and could affect other immune cell types or even resident cells within the kidney. Recent studies by Guzik et al. (15), however, demonstrated the importance of T lymphocytes in hypertension. They demonstrated that RAG1−/− mice, which lack both T and B lymphocytes, fail to develop ANG II-induced hypertension, and only become hypertensive after an adoptive transfer of T cells. In addition, the importance of NADPH oxidase within the T cells to maintain the hypertensive phenotype was also highlighted by those studies, by showing that transfer of T cells lacking p47phox subunit only partially recovered the hypertensive phenotype in mice (15). These studies, together with our present data, implicate T lymphocytes in the elevation of the oxidative state during hypertensive conditions.

Treatment with the antioxidant tempol has been proven to decrease hypertension and renal damage in Dahl SS rats (18, 26). In the present set of experiments, tempol treatment during the high-salt period ameliorated hypertension and albuminuria without affecting the infiltration of T cells into the kidney, which remained elevated. Tempol (4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl) is a small cell-permeable cyclic nitroxide with SOD activity (46); hence, it is capable of catalyzing the conversion of superoxide to hydrogen peroxide, scavenging in this way the $O_2^-$ being produced intracellularly. Therefore, it seems probable that tempol ameliorates hypertension and albuminuria by scavenging the $O_2^-$ that the infiltrating T lymphocytes produce within the kidney. It is possible, however, that the effects of free radical scavenging in the heart, aorta, brain, or other organs and tissues important in blood pressure regulation could also be involved in the antihypertensive responses observed in the present study.

An interesting observation in the present study is the antihypertensive effect of tacrolimus in the Dahl SS rats fed the high-salt diet but the prohypertensive effects of tacrolimus in normal SD rats fed the same diet. The observation in the SD rats is consistent with previous reports (43) and is likely a result of effects of this agent to decrease nitric oxide production by calcineurin-independent effects (9). In contrast to the prohypertensive effects in normal rats, however, administration of tacrolimus to the Dahl SS rat decreased infiltration of T cells in the kidney, decreased renal oxidative stress, and attenuated sodium-dependent hypertension and renal disease in these rats. A ready explanation is not apparent for this observation; it may be, however, that the reduction in infiltrating cells has a far greater impact on the progression of disease in this model than the results of any change in NO production, particularly in the Dahl SS rat which is known to have a predisposition to endothelial dysfunction.

In conclusion, the data presented in this manuscript indicate that high-salt intake is associated with increased infiltration of T lymphocytes, oxidative stress, and expression of NADPH oxidase in the renal tissue of Dahl SS rats. Chronic immunosuppression with tacrolimus ameliorates hypertension and renal damage by decreasing renal infiltration of T cells and their production of free radicals. All these data together indicate that...
renal infiltrating T lymphocytes are capable of participating in the development or progression of hypertension and renal damage by increasing the intrarenal oxidative stress.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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