Post-cyclosporine-mediated hypertension and nephropathy: amelioration by vascular endothelial growth factor

Duk-Hee Kang, Yoon-Goo Kim, Takeshi F. Andoh, Katherine L. Gordon, Shin-Ichi Suga, Marilda Mazzali, J. Ashley Jefferson, Jeremy Hughes, William Bennett, George F. Schreiner, and Richard J. Johnson. Post-cyclosporine-mediated hypertension and nephropathy: amelioration by vascular endothelial growth factor. Am J Physiol Renal Physiol 280: F727–F736, 2001.—Recent studies have demonstrated a role for microvascular and tubulointerstitial injury in some models of salt-sensitive hypertension. We utilized a model of post-cyclosporin A (CsA) nephropathy and hypertension to test the hypothesis that treatment with an angiogenic factor aimed at ameliorating the microvascular and renal injury would prevent the development of hypertension. CsA was administered with a low-salt diet for 45 days, resulting in a renal lesion characterized by afferent arteriolopathy, focal peritubular capillary loss, and tubulointerstitial fibrosis. We utilized a model of post-cyclosporin A nephropathy on a high-salt diet for 45 days. Placement of rats with established CsA nephropathy on a high-salt diet resulted in the rapid development of salt-sensitive hypertension. VEGF121 treatment resulted in lower blood pressure, and this persisted on discontinuation of the VEGF. VEGF121 treatment was also associated with a decrease in osteopontin expression, macrophage infiltration, and collagen III deposition and markedly stimulated resolution of the arteriolopathy (20.9 ± 7.8 vs. 36.9 ± 6.1%, VEGF vs. vehicle, P < 0.05). In conclusion, CsA-associated renal microvascular and tubulointerstitial injury results in the development of salt-sensitive hypertension. Treatment of animals with established CsA nephropathy with VEGF reduces the hypertensive response and accelerates histological recovery. The vascular protective effect of VEGF may be due to the improvement of arteriolopathy. Angiogenic growth factors may represent a novel strategy for treating CsA-associated hypertension and renal disease.

THE IMPORTANCE OF THE KIDNEY in the pathogenesis of most forms of salt-sensitive hypertension is undisputed, but the specific genetic and acquired alterations in renal structure and function that result in the hypertensive state remain complex and poorly defined. We have hypothesized that some forms of salt-sensitive hypertension result from acquired microvascular and tubulointerstitial injury induced by episodic or transient vasoconstriction (21). In support of this hypothesis, we have recently reported that transient infusion of angiotensin II results in acute hypertension in association with tubulointerstitial injury, arteriolar lesions, and peritubular capillary loss (24). On stopping of angiotensin II, blood pressure returns to normal, but hypertension can subsequently be unmasked by placement of rats on a high-sodium diet. Similar findings could also be shown in rats infused with the catecholamine phenylephrine (20).

Another condition in which salt-sensitive hypertension is commonly observed occurs in patients treated with cyclosporin A (CsA) (8). CsA is an immunosuppressive drug commonly used in organ transplantation, but its use is complicated by the development of hypertension in 40–100% of patients (33, 37). CsA is also associated with the development of microvascular and tubulointerstitial disease in the kidney, consisting of hyalinosis of the afferent arteriole in association with tubular atrophy and “striped” interstitial fibrosis (4, 25, 26). The pathogenesis of the renal injury is thought to be mediated by renal vasoconstriction and ischemia (26, 30), and this appears to result from direct effects of CsA as well as by the ability of CsA to alter intrarenal vasoactive mediators such as renin, endothelin-1, and nitric oxide (NO) (7, 16, 28, 32). CsA also may promote endothelial injury, dysfunction, and cytotoxicity (7, 16, 34, 43) and can inhibit endothelial cell proliferation (14, 18, 36) as well as various functions...
critical for angiogenesis, such as matrix metalloproteinase secretion, chemotaxis, and morphogenesis (18). The endothelial dysfunction induced by CsA may also have a pathogenic role in the development of hemolytic uremic syndrome, which can also complicate CsA therapy (41).

Recently, a rat model of CsA nephrotoxicity has been developed with most of the histological features of CsA nephropathy in humans, including arteriolar hyalinosis and striped fibrosis (29). The model can be induced relatively rapidly if rats are placed on a low-salt diet, which may potentiate renal vasoconstriction by further augmenting the renin-angiotensin system. Because the disease is induced by a low-salt diet, blood pressure is typically normal during the time CsA is administered (29). However, once sufficient tubulointerstitial disease is induced, exposure of the rats to a high-salt diet will result in the rapid development of hypertension even if CsA is discontinued (19). Furthermore, rats administered the same amount of CsA, but who are initially placed on a normal-salt diet so that the tubulointerstitial injury is minimal, fail to develop hypertension with the same sodium challenge (Andoh TF, Bennett W, and Johnson RJ, unpublished observations). These studies suggest that hypertension induced by CsA may result from renal structural changes independently of the direct effect of CsA.

The observation that CsA nephropathy and hypertension are associated with renal vasoconstriction, endothelial dysfunction, and arteriolopathy suggested to us that the disease may be amenable to therapy with vascular endothelial growth factor (VEGF). VEGF mediates both endothelial cell proliferation and survival and has important roles in angiogenesis, vascular remodeling, and repair (15, 22). VEGF also stimulates local NO production, resulting in increased vascular permeability and vasodilatation (27, 42).

We therefore examined whether VEGF could reverse the nephropathy and prevent the development of hypertension induced by CsA. Because we were more interested in therapy as opposed to prevention, we administered the VEGF once the microvascular disease and tubulointerstitial injury induced by CsA were established. Furthermore, the VEGF was administered to rats in which the CsA was stopped, allowing us to determine the effect of VEGF on renal structure without the confounding possibility that VEGF could be directly interacting with CsA. We report that VEGF accelerates recovery of the tubulointerstitial lesion and microvascular disease induced by CsA and reduces blood pressure and that this appears to be mediated by a specific improvement in the arteriolopathy.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats, weighing 200–220 g, (Simonsen Laboratory, Gilroy, CA) were housed in individual cages in a temperature- and light-controlled environment. All rats received a low-salt diet (0.125% NaCl, Zeigler Bros., Gardners, PA) with water ad libitum, which is about threefold less than in a normal-salt diet, and has been previously shown to accelerate CsA nephropathy (29).

Experimental protocol. Chronic CsA nephropathy was established with daily subcutaneous injection of CsA (Neoral, Norvatis, East Hanover, NJ) (Fig. 1). CsA was diluted in olive oil to a final concentration of 15 mg/ml and administered at a dose of 15 mg·kg⁻¹·day⁻¹ for 45 days while animals were on a low-salt diet. After the end of 45 days, body weight, blood pressure, blood urea nitrogen (BUN), and 24-h urinary protein excretion were evaluated, and whole blood CsA levels were measured by HPLC. At this time point, six rats were killed under anesthesia with xylazine and ketamine, and both kidneys were obtained for baseline histological evaluation of chronic CsA nephropathy. Thereafter, body weight and blood pressure–matched rats with chronic CsA nephropathy were randomly assigned to a VEGF (n = 6) or vehicle (n = 6) treatment group. Table 1 shows the baseline body weight and functional data in both groups at day 45, indicating that there were no significant differences in any of the parameters before VEGF or vehicle treatment.

After a 5-day washout period, the diet was switched to high salt (4% NaCl, Zeigler Bros.), and VEGF₁₂₁ (50 µg/kg sc; Scios, Sunnyvale, CA) or vehicle (PBS, pH 7.2) was administered twice a day for 14 days. The VEGF₁₂₁ isoform was chosen because it is the only isoform of VEGF that has no heparin-binding ability (15) and therefore results in a therapeutically effective plasma level when administered subcutaneously. The dose of VEGF₁₂₁ used (100 µg·kg⁻¹·day⁻¹) was selected as it has been found to be effective in inducing renal angiogenesis in a model of thrombotic microangiopathy (23). At this dose, VEGF attains a peak plasma level of 50 ng/ml at 100 min after injection and is still detectable in the

Table 1. Baseline data after 45 days of CsA before VEGF/vehicle administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VEGF (n = 6)</th>
<th>Vehicle (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>388 ± 7</td>
<td>391 ± 8</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>105.3 ± 3.6</td>
<td>106.8 ± 3.7</td>
</tr>
<tr>
<td>BUN, mg/dl</td>
<td>52.8 ± 5.4</td>
<td>53.1 ± 6.8</td>
</tr>
<tr>
<td>CsA level, ng/ml</td>
<td>3,700 ± 280</td>
<td>3,688 ± 160</td>
</tr>
</tbody>
</table>

Values are means ± SD. n, No. of rats/group; CsA, cyclosporin A; VEGF, vascular endothelial growth factor; BP, blood pressure; BUN, blood urea nitrogen.
plasma at 5 ng/ml after 6 h. It is important to note that these plasma levels of VEGF do not affect systemic blood pressure in normal rats (Terjung R, Abraham J, and Schreiner G, personal communication). Seven days after VEGF, or vehicle was discontinued, animals were placed in metabolic cages for collection of 24-h urine. The next day, the rats were anesthetized with xylazine and ketamine, a blood sample was obtained, and both kidneys were collected for histological evaluation.

Systolic arterial blood pressure was measured at each time point by tail-cuff sphygmomanometer using an automated system with a photoelectric sensor (IITC, Life Science, Woodland Hills, CA) that has been shown to be closely correlated with intra-arterial measurement of blood pressure (6). The blood pressure of rats was measured without anesthesia. Rats were conditioned carefully at least three times on separate days before the first measurement of blood pressure. Blood pressure was recorded as the mean value of three separate measurements obtained at each session.

Renal morphology and immunohistochemistry. Tissue for light microscopy and immunoperoxidase staining was fixed in methyl Carnoy’s solution and embedded in paraffin. Four-micrometer sections were stained with periodic acid-Schiff (PAS) reagent, and counterstained with hematoxylin. Indirect immunoperoxidase staining of tissue sections was performed with specific monoclonal and polyclonal antibodies directed to the following antigens: endothelial cells with the mouse monoclonal antibody JG-12 to a 70-kDa cell membrane antigen present on rat endothelial cells (gift from Dr. Dortscho Kerjaschki, Univ. of Vienna, Austria) (23) and monoclonal anti-endothelial cell antibody RECA-1 (gift of A. Duijvestijn, Univ. of Limberg, The Netherlands) (12); VEGF with rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA); osteopontin with goat anti-osteopontin antibody 199 (gift from C. Giachelli, Univ. of Washington, Seattle, WA); endothelial nitric oxide synthase (eNOS) with mouse anti-NOS III (Transduction Laboratory, Lexington, NY); and monocyte/macrophages with mouse monoclonal antibody ED-1 (Serotec, Indianapolis, IN). Controls included omission of the primary antibody and substitution of the primary antibody with preimmune rabbit or mouse serum.

To detect endothelial cell proliferation, double immunostaining was performed with an endothelial cell antibody (JG-12) and an antibody to the proliferating cell nuclear antigen (PCNA) (19A2, Coulter, Hialeah, FL). PCNA antibody and JG-12 were incubated simultaneously overnight at 4°C, followed sequentially by biotinylated rabbit anti-mouse IgM serum, peroxidase-conjugated avidin D, color development with diaminobenzidine with nickel chloride, followed by incubation in 3% H2O2/methanol for 20 min to eliminate any remaining peroxidase activity. Subsequently, sections were incubated with biotinylated horse anti-mouse IgG for 30 min at room temperature, followed by peroxidase-conjugated avidin D and diaminobenzidine.

Quantification of morphological data. The peritubular capillary rarefaction index was obtained by averaging the percentage of squares in 10 × 10 grids showing no capillaries (by JG-12 staining) by using at least 10 sequential fields at ×100 magnification. The minimum possible capillary rarefaction index is 0, and the maximum score is 100, whereby the latter would indicate a complete absence of JG-12-positive cells (17).

The mean number of proliferating endothelial cells (JG-12- and PCNA-positive cells) of peritubular capillaries in each biopsy was calculated in a blinded manner as the mean number of positive cells in 30 sequentially selected 0.25-mm² grids at ×200 magnification.

The degree of VEGF and eNOS expression in the cortex and outer medulla was measured by computer image analysis (Optimas 6.2, Media Cybernetics, Silver Spring, MD). In each biopsy, the background negative staining was calibrated to zero, and the area in each field of positive staining above the background level was measured. The measurement was assessed by computer analysis of the integrated logarithm of the inverse gray value, which is proportional to the total amount of absorbing material in the light path. This system allowed one to measure quantitatively the percent area of positive staining in each biopsy. The degree of osteopontin expression in the renal cortex was assessed with the same method.

The mean number of macrophages (ED-1-positive cells) in each biopsy was calculated in a blinded manner by averaging the total number of positive cells in 30 sequentially selected 0.25-mm² grids at ×200 magnification.

Interstitial fibrosis was scored semiquantitatively by a blinded observer on biopsies stained with PAS stain (Fig. 2), using the following scoring system as previously described (29): 0 = normal interstitium and tubules; 1 = mild fibrosis with mild interstitial thickening between the tubules; 2 = moderate fibrosis with moderate interstitial thickening between the tubules; and 3 = severe fibrosis with severe interstitial thickening between the tubules.

Tubular injury was scored semiquantitatively by a blinded observer who examined at least 40 cortical fields (×100 magnification) of PAS-stained biopsies. Tubular injury was defined as tubular dilatation, tubular atrophy, tubular cast formation, sloughing of tubular epithelial cells, or thickening of the tubular basement membrane. Only cortical tubules were included in the following scoring system: 0 = no tubular injury; 1 = <10% of tubules injured; 2 = 10–25% of tubules injured; 3 = 26–50% of tubules injured; 4 = 51–75% of tubules injured; 5 = >75% of tubules injured.

CsA-induced arteriolaropathy of the afferent arteriole was quantitatively scored by counting the percentage of juxtaglomerular afferent arterioles with hyalinosis per total number of juxtaglomerular afferent arterioles available for examination at ×200 magnification, with a minimum of 50 glomeruli/assessed biopsy (Fig. 2). Afferent arterioles were identified by their locations’ being adjacent to the vascular pole of glomeruli, by the presence of an elastic lamina, and by their having fewer endothelial cells than efferent arterioles. All quantification of afferent arteriolaropathy was performed blinded.
Urinary nitrite and nitrate. Urinary nitrate and nitrite were measured by a two-step process: step 1 was the conversion of nitrate to nitrite by using nitrate reductase, and step 2 was the addition of the Griess reagent (Cayman Chemical, Ann Arbor, MI), which is a mixture of sulfonic acid and \(N\)-(1-naphthyl) ethylenediamine. Optical density was measured by spectrophotometry.

Statistical analysis. All data are presented as means \(\pm\) SD. Differences in the various parameters between VEGF- and vehicle-treated rats were evaluated by unpaired comparisons for nonparametric data. The relationship among variables were assessed by Pearson correlation analysis. Significance was defined as \(P < 0.05\).

RESULTS

Endothelial and vascular injury occurs in chronic CsA nephropathy. Rats treated with CsA and a low-salt diet for 45 days developed classic findings of chronic CsA nephropathy (Fig. 3) with striped tubulointerstitial fibrosis and afferent arteriolopathy (Fig. 2). Many tubules were either atrophied or dilated with the expression of the macrophage adhesive protein osteopontin (Fig. 3B). The interstitium was infiltrated by macrophages (ED-1-positive cells) and expanded with type III collagen in a typical striped pattern of interstitial fibrosis (Fig. 3C).

Microvascular injury was also apparent in chronic CsA nephropathy. Arterioles showed an accumulation of eosinophilic material in the vascular wall (Fig. 2). This characteristic finding in chronic CsA nephropathy (arteriolar hyalinosis) was identified in 40.1 \(\pm\) 8.3% of juxtaglomerular afferent arterioles available for examination. A focal loss of peritubular capillaries, as noted by staining with two different antibodies to endothelial cells (RECA-1 and JG-12), was also present (Fig. 3D).
whereas glomerular capillaries were preserved. The BUN level was significantly increased compared with the pre-CsA level (53.0 ± 5.9 vs. 15.4 ± 5.7 mg/dl, P < 0.001). Blood pressure was unchanged during the period the rats were on CsA with a low-salt diet (Table 1).

The capillary loss, arteriolopathy, and evidence of vasoconstrictive injury in CsA-treated rats raised the possibility that CsA could be acting by altering the local expression of the angiogenic and vasodilatory growth factor VEGF. In normal rats and in rats on a low-salt diet, VEGF was constitutively expressed in tubules (proximal, medullary thick ascending limb, and collecting duct epithelial cells) in the outer medulla and medullary rays (Fig. 3F; %positive area of tubular VEGF staining in normal kidney 14.3 ± 5.2%, means ± SD). In contrast, CsA-treated rats had a diffuse loss of VEGF expression that was most pronounced in the outer medulla (Fig. 3E).

Effect of VEGF on the salt-sensitive hypertension induced by CsA. The administration of CsA to rats for 45 days resulted in significant tubulointerstitial and microvascular injury, but blood pressure remained normal because rats were administered a low-salt diet to facilitate the development of the nephropathy (Table 2). To study the salt-sensitive hypertension associated with CsA, rats were switched to a 4% NaCl diet. The CsA was also stopped so that any effects of VEGF observed would more likely be a consequence of modulation of the renal injury rather than a specific interaction with CsA. Furthermore, previous studies have reported that experimental CsA nephropathy results in permanent, irreversible renal lesions (10, 13).

The placement of rats with established CsA nephropathy on a high-salt diet resulted in the rapid development of hypertension even though the CsA had been discontinued (Fig. 4) and the renal function improved (Table 2). Blood pressure became significantly elevated by day 4 of the high-salt diet (Fig. 4).

Subcutaneous administration of VEGF significantly lowered the blood pressure in response to the high-salt

**Table 2. Changes in renal function during VEGF/vehicle administration**

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN, mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>52.8 ± 5.4</td>
<td>23.8 ± 6.3*</td>
<td>33.3 ± 7.2*</td>
<td>28.6 ± 14.2*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>53.1 ± 6.8</td>
<td>23.2 ± 6.7*</td>
<td>31.3 ± 10.6*</td>
<td>24.7 ± 4.8*</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>0.92 ± 0.21</td>
<td>0.61 ± 0.15*</td>
<td>0.55 ± 0.11*</td>
<td>0.52 ± 0.11*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.79 ± 0.19</td>
<td>0.64 ± 0.10*</td>
<td>0.60 ± 0.07*</td>
<td>0.58 ± 0.15*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.01 vs. day 0.

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**Table 3. Changes in histological parameters before and after VEGF/vehicle administration**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>At End of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 Days of CsA</td>
</tr>
<tr>
<td>Interstitial fibrosis score (range 0–3)</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Arteriolopathy, %</td>
<td>40.1 ± 8.3</td>
</tr>
<tr>
<td></td>
<td>(24)</td>
</tr>
<tr>
<td>PTC rarefaction, %</td>
<td>4.8 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>(24)</td>
</tr>
<tr>
<td>Cortical osteopontin, %</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>Collagen type III, %</td>
<td>6.5 ± 1.0</td>
</tr>
<tr>
<td>Macrophages in tubulointerstitial area, cells/mm²</td>
<td>194.4 ± 35.2</td>
</tr>
<tr>
<td>Tubular VEGF, %</td>
<td>7.8 ± 2.3</td>
</tr>
</tbody>
</table>

Values are means ± SD, with nos. in parenthesis representing afferent arterioles available/kidney section. PTC, peritubular capillary. *P < 0.05 vs. 45 days of CsA. †P < 0.05 vs. 45 days of CsA and vehicle.
diet during the post-CsA injection period, and this effect persisted 1 wk after VEGF was stopped (Fig. 4). VEGF administration did not induce changes in body weight.

Effect of VEGF replacement on chronic CsA nephropathy. The discontinuation of CsA with a switch to a high-salt diet resulted in mild but significant improvement of tubulointerstitial fibrosis both in the VEGF and vehicle-treated groups (Table 3). However, no difference in either the interstitial fibrosis score (1.1 ± 0.4 vs. 1.2 ± 0.5, VEGF vs. vehicle group, *P* = not significant (NS)) or the tubular injury score (1.9 ± 0.5 vs. 2.1 ± 0.9, VEGF vs. vehicle group, *P* = NS) was noted between VEGF- and vehicle-treated rats at the end of the study.

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**Fig. 5.** Effect of VEGF on CsA nephropathy. Compared with control rats (A, C, E, G), VEGF treatment (B, D, F, H) resulted in minimal differences by light microscopy (A and B; PAS staining, ×50) but significant decreases in tubular osteopontin expression (C and D; ×100), macrophage infiltration (E and F; ×200), and collagen type III deposition (G and H; ×200).
Renal function, as assessed by BUN and creatinine measurements, also improved after cessation of CsA. Again, there were no differences in the levels of BUN and creatinine between VEGF and vehicle groups at the end of the study (Table 2).

Effect of VEGF on osteopontin expression, macrophage infiltration, and collagen type III deposition. Despite the comparable tubulointerstitial changes by routine light microscopy (PAS), a significant decrease in %positive cortical area of osteopontin expression (1.4 ± 0.5 vs. 2.3 ± 0.2%, VEGF vs. vehicle group, P < 0.001), macrophage infiltration (96.1 ± 25.3 vs. 159.9 ± 29.2 cells/mm², VEGF vs. vehicle group, P < 0.01), and collagen type III deposition (2.3 ± 0.7 vs. 4.1 ± 0.9%, VEGF vs. vehicle group, P < 0.001) was present in VEGF-treated rats compared with controls (Fig. 5). Cortical osteopontin expression correlated positively with final blood pressure at the time of death (r² = 0.75, P < 0.01) (Fig. 6A). There was also a significant relationship between blood pressure and interstitial macrophage infiltration (r²=0.64, P < 0.01) and collagen type III deposition (r² = 0.47, P < 0.05).

Effect of VEGF on arteriolopathy and capillary rarefaction. VEGF administration was associated with a 45% reduction in the percentage of glomeruli with afferent arteriolar hyalinosis compared with the vehicle-treated group (20.9 ± 7.8 vs. 36.9 ± 6.1%, VEGF vs. vehicle group, P < 0.05). The frequency of arteriolopathy in the vehicle-treated rats was not different from the value at the end of the CsA treatment (40.1 ± 8.0%), documenting that, unlike the tubulointerstitial lesions, there was no spontaneous recovery from the arteriolopathy. Interestingly, there was a significant positive correlation between blood pressure and the frequency of arteriolopathy in VEGF- and vehicle-treated individual rats (Fig. 6B, r² = 0.74, P < 0.01).

There was no statistically significant difference in peritubular capillary density between VEGF- and vehicle-treated rats with chronic CsA nephropathy despite the tendency for a lower capillary rarefaction index in the VEGF group (1.6 ± 0.9 vs. 2.1 ± 2.2%, VEGF vs. vehicle group, P = NS). Double staining with JG-12 and PCNA also showed no significant difference in proliferating endothelial cells of peritubular capillary between the two groups.

The degree of VEGF immunostaining, expressed as the %positive area of the whole kidney cross section, was also comparable in VEGF and vehicle group (9.9 ± 2.2 vs. 9.7 ± 3.3%, VEGF vs. vehicle group, P = NS).

Effect of VEGF on NO. There was no difference in %positive area for eNOS immunostaining (7.2 ± 3.6 vs. 6.3 ± 2.9%, P = NS). Twenty-four-hour urinary nitrite and nitrate concentrations at the end of the study were also comparable (2,932 ± 738 vs. 2,516 ± 564 pmol/day, P = NS), suggesting that VEGF administration for 14 days did not significantly alter systemic and/or renal NO production when evaluated at the final time point.

DISCUSSION

We have been interested in the renal mechanisms responsible for salt-sensitive hypertension (21). We have found that temporary renal vasoconstriction, whether it be by angiotensin II (24) or catecholamines (20), results in tubulointerstitial and microvascular injury and the development of salt-sensitive hypertension that persists despite removal of the original stimulus. The mechanism of CsA-associated hypertension appears to involve a similar pathway. CsA is a potent renal vasoconstrictive agent that also results in microvascular and tubulointerstitial injury. Once the renal injury is induced by CsA, placement of rats on a high-salt diet results in the development of hypertension.
despite discontinuation of the CsA (19). In contrast, rats administered the same dose of CsA and placed on a normal-salt diet but with minimal renal injury do not develop hypertension on exposure to high salt (Andoh TF, Bennett W, and Johnson RJ, unpublished observations).

In this study, we have further dissected the pathogenesis of post-CsA-induced salt-sensitive hypertension. Specifically, we have attempted to clarify the role of tubular and microvascular injury in its pathogenesis. Rats were treated with CsA for 45 days and during this time received a low-salt diet (0.125% NaCl, 3-fold lower compared with a normal-salt diet) that has been used to accelerate the development of CsA nephropathy (29, 39, 40). Tissue obtained at the end of this period showed classic histological changes consistent with CsA nephropathy, including arteriolar hyalinosis and chronic tubulointerstitial disease. A focal loss of peritubular capillary endothelial cell staining was also documented by using two different endothelial cell-specific antibodies (RECA-1 and JG-12). Coincident with the microvascular injury was the loss of VEGF immunostaining, particularly in the tubules in the outer medulla. We have observed a similar finding in rats with microvascular and tubulointerstitial injury induced by angiotensin II (31). Because VEGF is an endothelial cell trophic and survival factor (3, 15), its loss in the kidney could cause a predisposition to a loss of peritubular capillaries and augment ischemia-driven tubulointerstitial injury.

Once the renal injury was established, the CsA was discontinued and the rats were placed on a high-salt diet to induce salt-sensitive hypertension. As previously observed, rats became hypertensive, and the increase in blood pressure correlated directly with the degree of osteopontin expression (a marker of tubular injury), collagen III deposition (a marker of interstitial fibrosis), and macrophage infiltration at the time of death. Interestingly, however, the tubulointerstitial injury generally improved in the rats after discontinuation of CsA despite the development of hypertension (see Table 3). Most histological parameters improved, including osteopontin expression, macrophage infiltration, peritubular capillary density, and interstitial fibrosis scores. However, one important feature of CsA nephropathy, which is afferent arteriolopathy, remained unchanged. The observation that CsA nephropathy is partially reversible contrasts with previous studies that reported that the structural changes in chronic CsA nephropathy remain unchanged after CsA withdrawal despite an improvement in the renal hemodynamic and functional measurements (10, 13). The reason for this discrepancy may relate to the diet, as maintenance of a low-salt diet (which was done in earlier studies) stimulates the renin-angiotensin system and might therefore maintain medullary ischemia via angiotensin II-mediated vasoconstriction (5) or stimulate fibrogenesis through direct effects on tubular cells and fibroblasts (38). A switch to a high-salt diet may ameliorate these angiotensin II-mediated effects.

An important new finding in this study was that VEGF administration blunted the development of salt-sensitive hypertension after CsA exposure. The observation that the blood pressure-lowering effect persisted after VEGF was stopped suggests that this benefit was not a direct systemic vasodilatory effect of VEGF. Actually, the dose used does not lower blood pressure in normal animals. VEGF is known to stimulate the expression of eNOS (27), and the L-arginine-N0 pathway is altered in CsA nephropathy (1, 9). However, in our study we could not demonstrate that this was the mechanism of protection, as urinary nitrates and nitrates (a reflection of systemic and renal NO production and/or metabolism) were not different between VEGF and control groups, and there were no apparent differences in renal eNOS expression.

The most probable mechanism by which VEGF lowered blood pressure is by acceleration of the recovery from tubulointerstitial and microvascular injury. Indeed, VEGF-treated rats had less osteopontin expression, macrophage accumulation, and collagen type III deposition compared with vehicle-treated controls. The most impressive finding was that VEGF dramatically improved afferent arteriolopathy. The afferent arteriole is recognized for its critical role in hypertension, and increased afferent arteriolar resistance is considered to be a major determinant for salt sensitivity and blood pressure (2). We also observed a relationship between the frequency of afferent arteriolopathy and blood pressure in our model. A reduction in arteriolopathy might be expected to reduce afferent arteriolar resistance and increase renal blood flow.

The mechanism by which VEGF improves the arteriolopathy may relate to the known trophic and survival properties of VEGF for endothelial cells (15). Indeed, VEGF is important in vascular remodeling. Several recent studies have shown that blocking VEGF may accelerate arterial wall thickening of precapillary arterioles in the lung (35). VEGF may also stimulate NO and prostaglandins in endothelial cells that block smooth muscle cell growth. A reduction in surrounding tissue ischemia secondary to VEGF-mediated vasodilation may also inhibit local production of growth factors and cytokines that stimulate smooth muscle growth (35). A previous study has also documented that the arteriolopathy induced by CsA is mediated by angiotensin II and that angiotensin II is also known to cause endothelial cell apoptosis in vitro (11). This suggests that VEGF may be counteracting the effects of angiotensin II on this lesion.

In conclusion, these studies suggest that post-CsA hypertension is mediated by renal microvascular and tubulointerstitial injury. Treatment with VEGF ameliorates the hypertensive response, an effect that may relate to the ability of VEGF to improve arteriolopathy and reduce tubulointerstitial injury. Thus these studies suggest that VEGF may be useful in the treatment of post-CsA-associated hypertension and nephropathy.

D.-H. Kang is a recipient of the International Fellowship Award of the International Society of Nephrology and the Postdoctoral Train-
REFERENCES


