Renal Denervation Prevents Immune Cell Activation and Renal Inflammation in Angiotensin II–Induced Hypertension

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Rationale: Inflammation and adaptive immunity play a crucial role in the development of hypertension. Angiotensin II and probably other hypertensive stimuli activate the central nervous system and promote T-cell activation and end-organ damage in peripheral tissues.

Objective: To determine if renal sympathetic nerves mediate renal inflammation and T-cell activation in hypertension.

Methods and Results: Bilateral renal denervation using phenol application to the renal arteries reduced renal norepinephrine levels and blunted angiotensin II–induced hypertension. Bilateral renal denervation also reduced inflammation, as reflected by decreased accumulation of total leukocytes, T cells, and both CD4+ and CD8+ T cells in the kidney. This was associated with a marked reduction in renal fibrosis, albuminuria, and nephrinuria. Unilateral renal denervation, which partly attenuated blood pressure, only reduced inflammation in the denervated kidney, suggesting that this effect is pressure independent. Angiotensin II also increased immunogenic isoketal-protein adducts in renal dendritic cells (DCs) and increased surface expression of costimulation markers and production of interleukin (IL)-1α, IL-1β, and IL-6 from splenic DCs. Norepinephrine also dose dependently stimulated isoketal formation in cultured DCs. Adoptive transfer of splenic DCs from angiotensin II–treated mice primed T-cell activation and hypertension in recipient mice. Renal denervation prevented these effects of hypertension on DCs. In contrast to these beneficial effects of ablating all renal nerves, renal afferent disruption with capsaicin had no effect on blood pressure or renal inflammation.

Conclusions: Renal sympathetic nerves contribute to DC activation, subsequent T-cell infiltration and end-organ damage in the kidney in the development of hypertension. (Circ Res. 2015;117:547-557. DOI: 10.1161/CIRCRESAHA.115.306010.)

Key Words: dendritic cells ■ inflammation ■ lymphocyte ■ norepinephrine ■ sympathetic nerve block

Inflammation, and in particular adaptive immunity, contributes to the development of hypertension. Recombination activating gene 1–deficient (RAG-1−/-) mice lacking lymphocytes are relatively resistant to hypertension caused by angiotensin II, norepinephrine, or deoxycorticosterone acetate–salt challenge.1,2 Similar protective effects have also been observed in mice with severe combined immune deficiency and in Dahl salt–sensitive rats with deletion of the RAG-1 gene.3,4 Adoptive transfer of T cells to RAG-1−/- mice restores hypertension and end-organ dysfunction in response to hypertensive stimuli.1,2 T lymphocytes and other inflammatory cells accumulate in the kidneys and vasculature during hypertension and produce cytokines, such as interleukin (IL)-17A, interferon (IFN)-γ, and tumor necrosis factor α, which in turn lead to vascular remodeling and renal sodium retention, augmenting blood pressure elevation.1,4 The mechanisms underlying T-cell activation are not yet fully understood.

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T-cell activation requires antigen presenting cells, in particular dendritic cells (DCs) and the phenomenon of costimulation.5 We have previously shown that DCs from hypertensive mice have increased surface expression of the costimulatory B7 ligands (CD80 and CD86), suggestive of DC maturation and activation.6 Pharmacological blockade or genetic deletion of CD80/CD86 prevents T-cell activation in response to angiotensin II and reverses both angiotensin II and DOCA-salt

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implicated in both pro- and anti-inflammatory responses.\textsuperscript{10–12} immune cells possess adrenergic receptors, which have been shown to modulate inflammation, whereas activation of sympathetic outflow by deletion of the NADPH oxidase subunit p22\textsuperscript{phox} in the subfornical organ enhances T-cell activation.\textsuperscript{2,8,9} The mechanisms underlying a potential anti-inflammatory role of RDN. We determined the efficacy of RDN in both preventing and reversing hypertension and sought to understand mechanisms underlying a potential anti-inflammatory role of RDN. We determined the efficacy of RDN in both preventing and reversing hypertension and examined the effect of both renal efferent and afferent nerves in modulating renal inflammation.

The central nervous system orchestrates much of the inflammation caused by angiotensin II and probably other hypertensive stimuli. Inhibition of sympathetic outflow by AV3V lesioning or by deletion of the NADPH oxidase subunit p22\textsuperscript{phox} in the subfornical organ reduces hypertension and T-cell activation, whereas activation of sympathetic outflow by deletion of the extracellular superoxide dismutase in the subfornical organ enhances T-cell activation.\textsuperscript{2,8,9} The mechanisms underlying this link between the central nervous system and T-cell activation are, however, not well understood. In keeping with this, a recent study by Mathis et al\textsuperscript{16} showed that RDN reduces albuminuria and renal cortical monocyte chemoattractant protein expression in mice with experimental systemic lupus erythematosus. In this study, we tested the hypothesis that renal sympathetic nerves modulate renal inflammation and T-cell activation in hypertension and sought to understand mechanisms underlying a potential anti-inflammatory role of RDN. We determined the efficacy of RDN in both preventing and reversing hypertension and examined the effect of both renal efferent and afferent nerves in modulating renal inflammation.

Renal denervation (RDN) has been used as a potential treatment for hypertension for several decades.\textsuperscript{13} Some, but not all, animal studies and clinical trials indicate that renal sympatheticectomy effectively reduces blood pressure and is expected to slow the progression of chronic renal disease.\textsuperscript{14,15} Independent of its therapeutic efficacy in humans, RDN provides a useful platform on which to study the relationship between sympathetic nerves modulate renal inflammation and T-cell activation.

RDN reduces renal inflammation, perhaps by reducing DC activation and ultimately formation of effector T cells that secrete proinflammatory cytokines. In keeping with this, a recent study by Mathis et al\textsuperscript{16} showed that RDN reduces albuminuria and renal cortical monocyte chemoattractant protein expression in mice with experimental systemic lupus erythematosus. In this study, we tested the hypothesis that renal sympathetic nerves modulate renal inflammation and T-cell activation in hypertension and sought to understand mechanisms underlying a potential anti-inflammatory role of RDN. We determined the efficacy of RDN in both preventing and reversing hypertension and examined the effect of both renal efferent and afferent nerves in modulating renal inflammation.

### Methods

The Institutional Animal Care and Use Committee of Vanderbilt University approved all animal protocols. A detailed description of the materials and methods can be found in the Online Data Supplement.

### Results

**Effects of RDN on Catecholamine Content and Hypertension**

In initial studies, we examined the efficacy of renal artery phenol application in producing RDN. Norepinephrine content was markedly decreased in denervated kidneys compared with the sham-treated kidneys (Figure 1A). In contrast, renal epinephrine, which is largely derived from the adrenal glands, was not altered by phenol application. Western blots for tyrosine hydroxylase, the rate-limiting enzyme for catecholamine biosynthesis, confirmed successful denervation (Figure 1B). We also confirmed that this technique does not interrupt innervation of adjacent lymph nodes and the adrenal gland, as verified by the neuronal marker β3 tubulin expression and catecholamine content in lymph nodes and adrenal glands, respectively (Online figure I).

The hypertensive response to angiotensin II, assessed by radiotelemetry, was markedly blunted in mice that had previously undergone bilateral RDN. In sham-operated mice, systolic pressure increased to 163±4 mm Hg in response to angiotensin II infusion (Figure 1C). This was reduced to and 127±4 mm Hg in mice that had undergone bilateral RDN (Figure 1C). Likewise,

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**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CCR7</td>
<td>C–C chemokine receptor type 7</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
</tr>
<tr>
<td>eNOS</td>
<td>endothelial nitric oxide synthase</td>
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<tr>
<td>IFN-γ</td>
<td>interferon-γ</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>Klf4</td>
<td>Kruppel-like factor 4</td>
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<tr>
<td>RAG-1</td>
<td>recombination activating gene 1</td>
</tr>
<tr>
<td>RDN</td>
<td>renal denervation</td>
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<td>VCAM-1</td>
<td>vascular cell adhesion molecule 1</td>
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hypertension.\textsuperscript{6} We recently demonstrated that proteins oxidatively modified by highly reactive γ-ketoaldehydes (isoketals) are formed in hypertension and accumulate in DCs, leading to T-cell activation and proliferation.\textsuperscript{7}

Figure 1. Renal denervation (RDN) reduces sympathetic drive in the kidney and attenuates angiotensin II–induced hypertension. A, Mice underwent phenol application to 1 renal artery. Three weeks later, catecholamines were extracted from the innervated and denervated kidney homogenates and analyzed by high-performance liquid chromatography (n=4 in both the groups). B, Western blot showing tyrosine hydroxylase (TH) in innervated and denervated kidneys. C and D, Effect of renal denervation on the hypertensive response to 2 weeks angiotensin II infusion (490 ng/kg per minute). Data in (A) and (B) were analyzed by paired t tests and data in (C) and (D) with 2-way ANOVA with repeated measurements, n=10 and 8 in each group. BP indicates blood pressure. *P<0.05, **P<0.01, ***P<0.001.
Diastolic pressure was reduced from 131±5 mm Hg to 111±5 mm Hg (P<0.001) by RDN (Figure 1D).

**Effects of RDN on Renal Inflammation and T-Cell Activation in Angiotensin II–Induced Hypertension**

To examine the effect of denervation on renal inflammation, we prepared single-cell suspensions of renal homogenates and performed flow cytometry. As apparent in Figure 2A and in the mean data, angiotensin infusion increased the presence of all leukocyte subsets, including total leukocytes, identified by CD45, total T cells, and both CD4+ and CD8+ T cells. RDN prevented the increased renal accumulation of leukocytes in response to angiotensin II infusion (Figure 2B–2G). Of note, the number of memory T cells, as defined as the CD44high, was also increased by angiotensin II, and this was normalized by RDN. RDN has recently been reported to have beneficial effects on vascular inflammation in apolipoprotein−/− mice.17 In keeping with this, we also found that RDN reduced aortic infiltration of total leukocytes, total T cells, CD4+ T cells, and CD8+ T cells (Online figure II).

To confirm our flow cytometry results and to localize inflammatory cells in the kidney, CD3+ and F4/80+ cells in the kidney were visualized using immunohistochemistry. T cells were increased in both the renal cortex and the medulla by angiotensin II infusion (Figure 3A and 3B) and this was prevented by RDN. Monocyte/macrophages, as identified by

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**Figure 2. Effects of bilateral renal denervation on renal leukocyte and T-cell infiltration.** Mice underwent bilateral renal denervation and 1 week later had osmotic minipumps for angiotensin II infusion implanted. Two weeks later, kidneys were harvested for flow cytometry. Representative flow cytometry of kidneys from mice with sham surgery or bilateral renal denervation are shown in (A). Live singlet cells were gated for total leukocytes (CD45+), total T cells (CD3+), CD4+ and CD8+ T cells. CD44 expression was examined in CD4+ and CD8+ T cells. Fluorescence-minus-one controls for CD44hi cells are shown as dotted lines. Mean data are shown for total leukocytes (B), T cells (C–E), and memory T-cell accumulation (F and G) in response to angiotensin II was examined in either sham-treated or denervated kidneys. Data were analyzed using 2-way ANOVA, n=5 to 10 in each group. RDN indicates renal denervation. *P<0.05, **P<0.01.
F4/80 staining, were also increased by angiotensin II infusion, and were reduced in the renal medulla by denervation. Further flow cytometry studies using a gating strategy recently described by Jakubzick et al\(^1\) showed that the majority of these F4/80+ cells were monocytes and not macrophages (Online figure III). Taken together, these data indicate that sympathetic innervation plays a major role in mediating renal inflammation in the angiotensin II–induced hypertension.

We further examined the effect of RDN on renal injury in hypertension. Angiotensin II infusion caused collagen deposition in both the renal cortex and the medulla (Figure 3A and 3B), as evidenced by Masson Trichrome staining. This was almost completely prevented by RDN. Increased superoxide production was observed in numerous cell types of the kidney in hypertensive mice, but predominantly in vascular endothelial cells. This was attenuated by RDN (Figure 3C). Angiotensin II infusion induced both albuminuria and nephritinuria in sham-operated mice, and these were also reduced by denervation (Figure 3D and 3E).

Local Effects of Sympathetic Nerves on Renal Inflammation in a Unilateral RDN Model

To determine if renal nerves directly contribute to inflammation or if renal inflammation is predominantly because of high blood pressure, we performed unilateral RDN. Using this approach, both kidneys are exposed to a similar pressure, but only 1 is denervated. Unilateral RDN also reduced the hypertensive response to a 2-week infusion of angiotensin II, albeit not as effectively as bilateral denervation (146±5 versus 164±3 mm Hg; \(P<0.05\); Figure 4A). Interestingly, significantly fewer total leukocytes (Figure 4B), lymphocytes (Figure 4C), and both CD4+ and CD8+ T cells (Figure 4E and 4F) accumulated in the denervated kidney as compared with the innervated kidney. Combining with the results from bilateral denervation, this suggests that there is a significant proinflammatory effect of sympathetic innervation, independent of pressure elevation (Online figure IV).

**Effect of RDN on Chemoattractant and Adhesion Molecules**

The homing of inflammatory cells to an affected tissue depends on local levels of adhesion and chemoattractant molecules. We, therefore, used polymerase chain reaction to detect mRNA levels of selected proteins known to attract immune cells. Hypertension caused a striking increase in the vascular cell adhesion molecule 1 (VCAM-1), intercellular cell adhesion molecule 1 monocyte chemotactic protein 1, and regulated on activation, normal T cell expressed and secreted. RDN significantly attenuated, and in the case of VCAM-1 and regulated on activation, normal T cell expressed and secreted, completely prevented this response (Figure 5A–5D). Nitric oxide has anti-inflammatory properties, and we found that mRNA for the endothelial nitric oxide synthase (eNOS) was markedly reduced by angiotensin II–induced hypertension.
and this was partly corrected by RDN (Figure 5E). An important transcription factor that modulates eNOS transcription is Kruppel-like factor 4 (Klf4). Hypertension was associated with a striking reduction of Klf4, and this was completely prevented by RDN (Figure 5F).

We considered the hypothesis that RDN increases renal blood flow, as eNOS and Klf4 are known to be shear responsive. We, therefore, measured renal blood flow using magnetic resonance imaging in angiotensin II–infused mice subjected to unilateral RDN surgery and found no significant change in renal perfusion caused by denervation (Online figure V).

**Effect of RDN on DC Activation in Angiotensin II–Induced Hypertension**

T-cell activation is dependent on antigen-presenting cells, and in particular DCs. These cells present antigen and provide costimulation to initiate T-cell proliferation and activation. On maturation, DCs increase expression of CD80 and CD86. We, therefore, performed additional studies to determine if RDN affects DC activation and their ability to promote hypertension. Subtypes of splenic DCs were characterized according to the surface markers I-Ab, CD11c, CD11b, and B220 (Online figure VI). Angiotensin selectively increased CD80 and CD86 expression by ≈26% and 15% in CD11b+/CD11c+ DCs, and this was prevented by RDN (Figure 6A and 6B).

We have recently found that isoketal-protein adducts are formed in hypertension. These accumulate in DCs and promote DC activation, causing DCs to express costimulatory molecules, produce cytokines, and stimulate T-cell proliferation. We performed additional studies using flow cytometry to detect intracellular isoketal-protein adducts in sham-operated and RDN mice. As we previously reported, splenic CD11c+CD11b+ DCs from hypertensive mice robustly accumulated isoketal adducts, and RDN prevented this (Figure 6C). One explanation for these findings is that renal sympathetic nerves stimulate formation of isoketal adducts in the kidney, perhaps directly in DCs. To address this, we examined isoketal adducts in the DCs within the kidney shortly after the onset of angiotensin II infusion. In sham mice not receiving angiotensin II, only 25% of DCs stained positively for isoketal-protein adducts. In contrast, nearly 80% of the DCs isolated from kidneys 5 days after the onset of angiotensin II contained isoketal-adducted proteins. RDN markedly reduced these (Figure 6D).

Activated DCs produce cytokines that influence the inflammatory milieu, in large part by guiding T-cell polarization. To examine if RDN alters DC cytokine production, splenic DCs were cultured in RPMI for 24 hours and cytokines released into the media were measured using Luminex. Angiotensin II infusion increased splenic DC IL-1α, IL-1β, and IL-6 production 2- to 6-fold, and these increases were completely

**Figure 4. Local anti-inflammatory effect of unilateral renal denervation.** Mice underwent unilateral renal denervation by application of phenol to the left renal artery. Systolic blood pressure in mice after 2 weeks of angiotensin II infusion measured by tail cuff (A, n=8 for Sham and 7 in both unilateral and bilateral RDN). Infiltration of total leukocytes (CD45+), T cells (CD3+), and both CD4+ and CD8+ T cells, in the innervated and denervated kidneys was determined by flow cytometry (B–E, n=6 per group). Data in (A) was analyzed using 1-way ANOVA. Data in (B)–(E) were analyzed using paired t test, n=6. Ang indicates angiotensin; and BP, blood pressure. *P<0.05, **P<0.01, ***P<0.001.
prevented by bilateral RDN (Figure 6E–6G). Other cytokines, including IL-12, IL-23, GM-CSF, and TGF-β were not altered by RDN (Online Table I).

**Role of Activated DCs in Priming Hypertension and Effect of RDN**

To further determine if renal innervation affects the ability of DCs to promote hypertension and renal inflammation, we performed adoptive transfer experiments. Mice underwent RDN or sham surgery as described above, and 10 days later underwent infusion of angiotensin II (490 ng/kg per minute) for 2 weeks. 1×10⁶ splenic DCs were isolated from these animals and injected by tail vein into recipients. Ten days later, the recipients were treated with low-dose angiotensin (140 ng/kg per minute) for 2 weeks. This experimental paradigm is illustrated in Figure 7A. DCs from mice with intact renal nerves markedly augmented the hypertensive response to this generally subpressor dose of angiotensin II (Figure 7B and 7C). In contrast, DCs from denervated animals did not alter the hypertensive response to low-dose angiotensin II. Flow cytometry revealed that adoptive transfer of DCs from angiotensin II–infused mice with intact renal nerves increased renal accumulation of total leukocytes, CD3⁺ cells, and both CD8⁺ T cells in response to low-dose angiotensin II. These values were reduced by 30% to 50% in mice that had received DCs from mice with RDN (Figure 7D–7H). These data illustrate an important contribution of DCs to hypertension, and show that this is modulated by the sympathetic nervous system.

**Role of Afferent Renal Nerves in Angiotensin II–Induced Hypertension**

Activation of renal afferent nerves has been reported to produce hypertension in response to calcineurin,¹⁹ renal failure,²⁰ and renal injury.²¹ To examine a role of afferent renal nerves in modulating inflammation, we applied capsaicin to the renal arteries. This marked reduction of the afferent nerve marker calcitonin gene-related peptide in the renal pelvis, but had no effect on renal norepinephrine levels (Online Figure VIIA and VIIB). Disruption of the afferent nerves using this approach did not alter either the hypertension (Online Figure VII) or renal inflammation (Online Figure VIID–VIIH) caused by angiotensin II.

**Effect of RDN After the Onset of Hypertension**

In clinical trials, RDN is usually performed after the onset of hypertension. To mimic this clinical scenario, we infused...
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angiotensin II for 4 weeks in mice, and performed RDN after 2 weeks. Other mice underwent a sham denervation procedure at 2-week angiotensin II infusion. As shown in Figure 8A and 8B, RDN after the onset of hypertension lowered blood pressure by ≈15 mm Hg, compared with that observed in mice undergoing sham denervation, during the ensuing 2 weeks. This value was significantly less that the blood pressure reduction observed when denervation was performed at the onset of angiotensin II infusion (149±4 mm Hg versus 129±3 mm Hg; *P<0.001). Of note, this 4-week protocol resulted in substantially more leukocytes in the kidney than observed after 2 weeks of angiotensin II, but RDN substantially reduced total leukocytes and all T-cell subsets as demonstrated by flow cytometry (Figure 8C–8F).

Direct Effect of Sympathetic Stimuli in Isoketal Adduct Formation in DCs

To examine if sympathetic stimuli directly affect DC function in hypertension, adrenergic receptor expression in splenic DCs was quantified using real-time polymerase chain reaction (Online figure VIIIA), and further confirmed by Western blot (Online figure VIIIB). We found that DCs contain mRNA for \( \alpha_1D, \alpha_2A, \alpha_2B, \alpha_2C, \beta_1, \) and \( \beta_2 \) adrenergic receptors. In most cases, adrenergic receptors were downregulated by angiotensin II infusion, with the exception of \( \beta_2 \) adrenergic receptors, which were increased. To further determine if adrenergic signaling contributes to DC activation, bone marrow–derived DCs were cultured with norepinephrine, and isoketals were measured by flow cytometry 1 week later. Norepinephrine dose...
dependently increased isoketal-protein adducts in those cells. A similar response was observed with DCs obtained from β2 receptor–deficient mice (Figure 8C and 8D). In contrast to the effects of norepinephrine, neuropeptide Y, another sympathetic nerve transmitter, had no effect on DC isoketal-protein content (Online figure VIIIE).

Potential Role of C–C Chemokine Receptor Type 7 in Homing of DCs From the Kidney to Secondary Lymphoid Organs

Our data about the effect of RDN on splenic DC function and isoketal content are compatible with the hypothesis that these cells are initially activated in the kidney and migrate to secondary lymphoid organs such as the spleen, where they interact with T cells to promote T-cell activation (Figure 8G). To gain further insight into this hypothesis, we performed additional studies on C–C chemokine receptor type 7 (CCR7)–deficient mice. CCR7 is used by DCs to allow homing to secondary lymphoid organs. The hypertensive response to angiotensin II was identical to that observed in wild-type mice (Online figure IXA). Likewise, DCs in the kidney demonstrated an increase in CD80 and CD86 (Online figure IXB), however, there was no effect of angiotensin II infusion on these DC surface markers in the spleen (Online Figure IXC). Angiotensin infusion failed to induce inflammatory cell infiltration in the kidneys of CCR7−/− mice (Online Figure IXC–IXF). However, memory CD8+ T cells were increased (Online Figure IXH).

Discussion

In this study, we show that the renal sympathetic nerves play an important role in activation of adaptive immunity in hypertension. RDN attenuates the hypertension caused by chronic angiotensin infusion and prevents T-cell infiltration and subsequent renal injury. Renal sympathetic outflow also promotes DC maturation as defined by upregulation of costimulatory molecules and augmented proinflammatory cytokine production during angiotensin II infusion, and all these changes are reversed by RDN. In accordance with these, DC adoptive transfer experiments further indicate that renal nerves indeed contribute to DC activation, which in turn promote T-cell activation and hypertension. Our data also indicate that efferent but not afferent renal nerves mediate these effects.

Before our present study, the interplay between renal sympathetic nerves and inflammation was poorly defined. As in previous studies, we found that hypertension is associated accumulation of leukocytes, T cells, and both CD4+ and CD8+ T cells in the kidney. In recent studies, we have shown that T cells that infiltrate the kidney in hypertension have an effector memory phenotype. Importantly, RDN not only reduces the total number of immune cells in the kidney but also memory T cells. We have found that activated T cells in hypertension produce IL-17A, TNF-α, and IFN-γ, and we and others have shown that these affect vascular and renal function. For example, prolonged exposure of renal proximal tubular cells to IFN-γ enhances production of angiotensinogen by renal
epithelial cells, which modulates tubular sodium transport. In keeping with this concept, we have recently shown that angiotensin II infusion increases phosphorylation of the Na–K–2Cl cotransporter the NaCl cotransporter and Ste20/SPS-1-related proline–alanine-rich kinase in wild-type mice but not in IFN-γ−/− mice. In this study, we also found evidence that IL-17A and IFN-γ modulate abundance of Na/H-exchanger isoform 3 and the motor myosin VI during angiotensin II infusion. It is therefore likely that the reduction of effector memory T cells in the kidney contributes to the antihypertensive effect of RDN.

The homing of immune cells to sites of inflammation is mediated by local increases in adhesion and chemotactic molecules. In keeping with this, we found that angiotensin II infusion increases renal expression of VCAM-1, intercellular cell adhesion molecule 1, monocyte chemotactic protein 1, and regulated on activation, normal T cell expressed and secreted, and that these are all attenuated by RDN. In contrast, angiotensin II–induced hypertension is associated with a marked decrease in eNOS mRNA expression, and this is completely prevented by denervation. Nitric oxide has myriad anti-inflammatory effects on the endothelium, reducing expression of VCAM-1, monocyte chemotactic protein 1, regulated on activation, normal T cell expressed and secreted, intercellular cell adhesion molecule 1, and monocyte adhesion. In many cases, this has been attributed to modulation of transcription factors, such as nuclear factor-κB, activator protein 1, and cAMP response element-binding protein. In this regard, the Klf4 modulates endothelial function by promoting eNOS expression, reducing VCAM-1 expression and exerting antithrombotic effects. Thus, the enhancement of eNOS and Klf4 expression could explain at least a part of the anti-inflammatory effects of RDN.

A critical finding in this study is that RDN reduces DC activation and the ability of these cells to convey hypertensive response renal wrapping.33 In contrast, activation of sensory afferents enhances hypertension in a feed-forward fashion.30 It is likely that sympathetic innervation plays an important intermediate role in such interplay.

A striking finding in this study is that RDN affected the function of DCs in the spleen. One explanation for this is that DCs arising from the kidney migrate to the spleen, where they can activate T cells that promote systemic inflammation (Figure 8G). Our data in CCR7−/− mice are compatible with this concept. CCR7 is used by DCs as a homing marker that allows their transmigration to secondary lymphoid organs. In CCR7−/− mice, we continued to observe DC activation in the kidney, but not in the spleen, suggesting that their movement from the kidney to the spleen might be defective. These studies must be interpreted with caution, as CCR7 is also critical for T-cell homing to secondary lymphoid organs. Nevertheless, findings in these animals suggest an interplay between the kidney and spleen, and perhaps other sites of immune activation.

There has been substantial debate as to the role of renal afferent nerves in hypertension. Intrarenal injection of phenol in rats leads to sustained hypertension and increased hypothalamic norepinephrine production, thought secondary to afferent nerve activity.31 Circulating calcitonin-related peptide level, reflecting afferent nerve activity, is elevated in this model.32 Likewise, activation of renal afferent nerves enhances hypertension in response renal wrapping.33 In contrast, activation of sensory nerves has been proposed to buffer the increase in blood pressure caused by angiotensin II. Sensory afferents could conceivably lead to reflex activation of cells within the spleen, as observed in this study. Our data using capsaicin renal sensory denervation
suggest that afferent nerves have little role in blood pressure, regulation of blood pressure, or immune cell activation in response to chronic angiotensin II infusion, but do not discount a role of these in the hypertension caused by other stimuli.

An important issue related to this study is the clinical benefit of RDN in humans. The SYMPLICITY HTN-1 and 2 trials reported a striking lowering of blood pressure by catheter-based radiofrequency renal nerve ablation, with sustained reductions near 30 mm Hg. The Global SYMPLICITY registry confirmed a similar effect on office blood pressure. These studies, however, were not sham controlled or blinded. Questions were raised about study design and the patients included in these trials.

In contrast, the SYMPLICITY HTN-3 trial, which included sham controls and was blinded, showed essentially the same lowering of blood pressure in the sham-treated and denervation-treated patients. It has been suggested that the benefit observed in the earlier trials were because of the Hawthorne effect, regression of blood pressure values to a mean and placebo effects. Questions were raised about study design and the patients included in these trials.

Another issue is that long-standing hypertension in humans, with attendant changes in renal and vascular function, might not be responsive to renal nerve ablation. Indeed, we found that the antihypertensive effect of denervation was less pronounced when applied after the onset of hypertension than when denervation was performed before the onset of angiotensin II infusion. Despite this, we observed a substantial anti-inflammatory effect of RDN performed after the onset of hypertension. It is, therefore, possible that the anti-inflammatory effects of RDN might not be reflected by simple measures of blood pressure, but might have long-term benefit on renal function or nonrenal cardiovascular events.

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Disclosures
None.

References


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Novelty and Significance

What Is Known?

- Renal sympathetic nerves and norepinephrine cause dendritic cell activation and isoketal accumulation.
- RDN prevents dendritic cell and T-cell activation in response to chronic angiotensin II infusion.

This study shows that the kidney is a major site of immune activation in hypertension and that this is mediated by effenter sympathetic nerves and their release of norepinephrine. RDN reduces activation of dendritic cells and ultimately T cells and thus prevents both renal and vascular inflammation. Our findings may help explain why RDN has pleiotropic systemic effects.

What New Information Does This Article Contribute?

- Sympathetic outflow contributes to T-cell activation in hypertension.
- In hypertension, proteins oxidatively modified by highly reactive γ-ke-toaldehydes accumulate in dendritic cells. These are immunogenic and lead to activation of adaptive immunity and end-organ damage.
- Renal denervation (RDN) lowers blood pressure in animals with experimental hypertension and in some studies of hypertensive humans.

What New Information Does This Article Contribute?

- RDN attenuates renal inflammation in angiotensin II–induced hypertension.