Enhancing Renal Lymphatic Expansion Prevents Hypertension in Mice

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ABSTRACT

Rationale: Hypertension is associated with renal infiltration of activated immune cells; however, the role of renal lymphatics and immune cell exfiltration is unknown.

Objective: We tested the hypotheses that increased renal lymphatic density is associated with 2 different forms of hypertension in mice and that further augmenting renal lymphatic vessel expansion prevents hypertension by reducing renal immune cell accumulation.

Methods and Results: Mice with salt-sensitive hypertension or nitric oxide synthase inhibition-induced hypertension exhibited significant increases in renal lymphatic vessel density and immune cell infiltration associated with inflammation. Genetic induction of enhanced lymphangiogenesis only in the kidney, however, reduced renal immune cell accumulation and prevented hypertension.

Conclusions: These data demonstrate that renal lymphatics play a key role in immune cell trafficking in the kidney and blood pressure regulation in hypertension.

Keywords:
Lymphatic capillary, kidney, hypertension, immunity, immune system.

Nonstandard Abbreviations and Acronyms:

B	blood pressure
HT	hypertension
KidVD	kidney-specific VEGF-D overexpression mice
LHTN	L-NAME-induced hypertension
L-NAME	nitro-L-arginine methyl ester hydrochloride
LYVE-1	lymphatic vessel endothelial hyaluronan receptor 1
NFAT5	nuclear factor of activated T-cells 5
SBP	systolic blood pressure
SSHT	salt-sensitive hypertension
VEGF-C	vascular-endothelial growth factor C
VEGF-D	vascular-endothelial growth factor D
VEGFR-3	vascular-endothelial growth factor receptor 3

INTRODUCTION

Hypertension (HTN) affects almost 1 in 2 adults in the US and is the #1 contributor to cardiovascular-renal disease.[1, 2] Of patients with HTN, nearly 50% have salt-sensitive hypertension (SSHTN). Patients with SSHTN are at even greater risk for cardiac events and developing chronic kidney disease than patients with HTN alone. African-Americans are especially at risk since 75% of those with HTN have SSHTN.[3] Current medications used for HTN and SSHTN have many negative side effects and diuretic therapy, the 1st line therapy for SSHTN, can lead to altered potassium and uric acid levels. Therefore, alternative therapies for HTN and especially SSHTN are needed.

Renal inflammation has been identified as a partial contributor to the development of HTN and SSHTN. Humans and animals with HTN and SSHTN exhibit increased numbers of activated immune cells in the kidney.[4-9] Immunosuppression reduces the number of macrophages and T cells in the body,

ameliorates renal immune cell infiltration and inflammation, and prevents the development of experimental SSHTN.[4, 10-12] Since suppressing the immune systems of the ~148 million people with HTN in the U.S. is not feasible, alternative strategies to reduce renal inflammation and blood pressure are needed.

Lymphatic vessels provide a route for immune cell migration out of the interstitium to the draining lymph node. Acute inflammation initially causes lymphatic vessel dilation; however, persistent inflammation and immune system activation induces lymphangiogenesis to potentially enhance leukocyte trafficking from the tissue. Renal lymphangiogenesis is evident in chronic kidney pathologies such as diabetic nephropathy, lupus nephritis, tubulointerstitial nephritis, and IgA nephropathy.[13-15] We recently reported that renal lymphatics are increased in Spontaneously Hypertensive Rats (SHRs) that develop renal injury but not in SHRs resistant to renal injury.[16] Whether lymphangiogenesis is merely indicative of injury is unknown. Likewise, could modulating renal lymphatics aid in limiting the inflammation associated with HTN and specifically SSHTN?

In the current study we utilized 2 different mouse models that mimic patients with SSHTN and HTN patients with elevated levels of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine.[17-20] We also utilized transgenic mice that conditionally overexpress the lymphangiogenic signal vascular-endothelial growth factor D (VEGF-D) only in the kidney.[21] We hypothesized that renal lymphatics are increased in both models of HTN as a marker of inflammation. Additionally, we hypothesized that enhancing renal lymphatic expansion prior to HTN stimuli would prevent renal immune cell accumulation and prevent the development of SSHTN and HTN.

METHODS

The data, analytic methods, and study materials that support the findings of this study are available from the corresponding authors upon reasonable request.

An expanded Methods section is provided in the online-only Data Supplement.

RESULTS

SSHTN mice exhibit increased renal lymphatic vessel density and renal immune cell infiltration.

To test whether renal lymphatic architecture is altered in SSHTN, mice were administered nitro-L-arginine methyl ester hydrochloride (L-NAME) in their drinking water for 2 weeks, followed by a washout period of 2 weeks, and then provided a 4% high salt diet for 3 weeks as described previously.[17] As expected, these mice were hypertensive [systolic blood pressure (SBP): 136±2 vs.103±3 mm Hg, $p<0.05$; Figure 1A]. There were no differences in body weight, kidney weight/body weight, or spleen weight/body weight (Online Table III). We then examined renal lymphatic vessel density by performing immunofluorescence on kidney sections for the lymphatic vessel markers lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1) and podoplanin (data not shown). SSHTN mice had increased lymphatic labeling around the interlobular arteries (Figure 1B). Every interlobular artery per kidney section was counted and the mean number did not differ between groups (Figure 1C). Next, every lumen-containing, LYVE-1+ lymphatic vessel per kidney section was counted and the mean number of lymphatic vessels per kidney section (Figure 1C) and per artery (Figure 1D) were increased significantly in SSHTN mice. Lymphatics in the cortical region are primarily localized to the interlobular arteries. Arteries in control

mice typically had 0 to 2 lymphatic vessels around them, while SSHTN mice had 2-4 lymphatic vessels around most arteries (Figure 1E). In support of these data, gene expression of *Prox1*, a lymphatic endothelial cell transcription factor, and *Vegfr3*, the receptor for the lymphangiogenic signal VEGF-C and VEGF-D, were increased significantly in SSHTN kidneys (Figure 1F). To determine if the increased renal lymphatic vessel density was associated with increased immune cell infiltration, we measured gene expression of various immune cell markers in the kidney. Expression levels of the macrophage marker, *Adgre1* (F4/80), and the Th1 cell marker, *Tbx21*, were increased significantly in SSHTN mice (Figure 1G). Mice given a 4% high salt diet alone for 3 weeks (HS) did not develop HTN (Online Figure IA) and had no change in renal lymphatic vessel density (Online Figures IB and IC). Changes in renal gene expression of lymphatic vessel and immune cell markers associated with SSHTN were also unchanged in HS mice (Online Figures ID and IE). These results demonstrate that renal lymphatic vessel density is increased in SSHTN and is associated with renal infiltration of macrophages and Th1 cells.

Kidney-specific lymphatic augmentation prevents SSHTN due to reduced macrophage and T cell accumulation.

To determine whether enhancing renal lymphatic vessel density could prevent SSHTN, we used mice with tet-responsive kidney-specific overexpression of murine VEGF-D (Kidney-specific VEGF-D overexpression, KidVD+). Doxycycline treatment, and thereby VEGF-D overexpression, was initiated 1 week prior to the 4% salt diet of the SSHTN protocol. The augmentation of renal lymphangiogenesis in KidVD+ mice prevented the development of SSHTN while KidVD- cagemate controls developed SSHTN (SBP: 117±4 vs. 139±5 mm Hg, $p<0.05$; Figure 2A). There were no differences in body weight, kidney weight/body weight, spleen weight/body weight, or heart weight/body weight (Online Table IV). Immunofluorescence of kidney sections for LYVE-1 revealed renal lymphatic expansion in KidVD+ SSHTN mice, most notably throughout the cortex (Figure 2B). Lymphatic structures were confirmed to also be positive for podoplanin and Prox1 (Online Figure II). Quantification of LYVE-1+ pixels per field confirmed this significant increase in lymphatics compared to KidVD- SSHTN mice (Figure 2C). Increased lymphatic density was specific to the kidney with no changes in LYVE-1 staining in the heart, lung, ear skin, liver, thymus, or intestine of KidVD+ mice (Online Figure III and not shown). Supporting the massive increase in renal lymphatic vessel density, KidVD+ SSHTN mice had expected renal mRNA increases in the lymphatic vessel markers *Lyve1* and *Prox1, Vegfd*, and the lymphatic-expressed immune cell trafficking chemokine *Ccl21* (Figure 2D). The immune cell-expressed lymphatic trafficking chemokine receptor to CCL21, *Ccr7*, mRNA was also highly upregulated in KidVD+ SSHTN kidneys compared to controls (Figure 2E). Renal gene expression of the macrophage marker *Adgre1*, which was increased significantly in SSHTN mice, was normalized in KidVD+ SSHTN mice (Figure 2E). There was also a significant decrease in renal mRNA levels of the T cell marker *Cd3e* and the Th1 cell marker *Tbx21* suggesting fewer of these cells in KidVD+ SSHTN kidneys (Figure 2E). To test whether the prevention of SSHTN in KidVD+ mice was associated with decreased renal accumulation of immune cells, we performed flow cytometry analysis and found that KidVD+ SSHTN kidneys had significantly decreased F4/80+ macrophages and CD3e+ T cells compared to KidVD- SSHTN mice (Figure 2F). These results demonstrate that increasing renal lymphatic density prior to the high salt diet challenge prevents the development of SSHTN in KidVD+ mice, likely due to reduced renal accumulation of macrophages and Th1 cells.

LHTN mice exhibit increased renal lymphatic vessel density and renal immune cell infiltration.

To determine whether these renal lymphatic and immune cell changes occur in another model of HTN independent of sodium, we administered L-NAME alone in the drinking water of mice for 2 weeks as described previously (LHTN).[17] We confirmed the development of LHTN in these mice (Figure 3A). There were no differences in body weight, kidney weight/body weight, or spleen weight/body weight (Online Table V). We labeled the kidneys for LYVE-1 and podoplanin and found that renal lymphatic

vessel density was increased significantly in LHTN mice (Figure 3B). Similar to the SSHTN mice, the mean number of interlobular arteries per kidney section did not differ between groups (Figure 3C). The average number of lumen-containing, LYVE-1+ renal lymphatic vessels per kidney section and per interlobular artery were increased significantly in LHTN mice (Figures 3C and 3D). Most arteries in kidneys from LHTN mice had 1 to 4 lymphatic vessels around them while most arteries in kidneys from control mice had 0 to 2 lymphatic vessels (Figure 3E). Expression of *Vegfc* and *Vegfr3* were increased significantly in LHTN kidneys compared to controls (Figure 3F). Kidneys from LHTN mice also had significantly increased mRNA levels of *Ccr7*, *Adgre1*, the dendritic cell marker *CD11c*, and *Tbx21* (Figure 3G). The increases associated with LHTN regressed following a 2-week washout period (LHTN-Wash) including normalization of SBP (Online Figure IVA), renal lymphatic vessel density (Online Figures IVB and IVC), lymphatic vessel markers (Online Figure IVD), and immune cell markers (Online Figure IVE). These data demonstrate that LHTN mice exhibit renal lymphatic expansion that is associated with increased markers of macrophages, dendritic cells, and Th1 cells.

Kidney-specific lymphatic density augmentation prevents LHTN due to reduced macrophage and dendritic cell accumulation.

To induce kidney-specific VEGF-D expression and initiate lymphangiogenesis, we administered doxycycline to KidVD+ and KidVD- mice 1 week before L-NAME administration and continued L-NAME and doxycycline in the drinking water for 3 weeks. KidVD- LHTN mice developed LHTN within a week that remained elevated (Figure 4A). However, enhanced lymphatic expansion prevented the development of LHTN in KidVD+ mice (Figure 4A). No differences were evident in body weight, kidney weight/body weight, spleen weight/body weight, or heart weight/body weight (Online Table VI). KidVD+ LHTN kidney sections exhibited a marked increase in lymphatic vessel coverage (Figure 4B). Quantification of LYVE-1+ pixels per field confirmed the significant increase in cortical lymphatic vessel density (Figure 4C). Renal gene expression levels of lymphatic endothelial cell-related genes *Lyve1*, *Prox1*, *Vegfd*, *Vegfr3*, *Ccl19*, and *Ccl21*, as well as the immune cell-expressed chemokine receptor *Ccr7*, were increased significantly in KidVD+ LHTN mice (Figure 4D). Gene expression of *Adgre1*, *Cd11c*, and *Tbx21*, all of which were upregulated during LHTN, were either decreased significantly or did not increase significantly in the kidneys of KidVD+ LHTN mice (Figure 4E). KidVD+ LHTN mice had significantly decreased levels of F4/80+ macrophages and CD11c+ dendritic cells as determined by flow cytometry (Figure 4F), though unlike in SSHTN, T cell numbers were unaffected. These results collectively demonstrate that genetic augmentation of renal lymphatic density prior to L-NAME administration prevents the development of LHTN, likely due to reduced accumulation of renal macrophages and dendritic cells.

DISCUSSION

The current study supports the concept that renal immune cell accumulation plays a major role in HTN, and identifies that renal lymphangiogenesis is beneficial in this pathogenesis. We demonstrate increased renal lymphatic density occurs in two different models of HTN that, if analogous to other models of inflammation-induced lymphangiogenesis, likely serves as a compensatory mechanism for removal of increased fluid and immune cells. Importantly, we also demonstrate that genetically inducing increased renal lymphatic density prevents HTN and reduces pro-inflammatory renal immune cells.

Renal immune cell infiltration and inflammation play a major role in SSHTN and HTN. In effect, HTN can be viewed as a disease of chronic, low-grade renal inflammation. Lymphangiogenesis is not only a marker of inflammation, but an important adaptation to chronic inflammation. Multiple forms of kidney injury or disease have demonstrated renal lymphangiogenesis. Conversely, blocking this process

results in failed immune cell trafficking and worsened disease, and ligation of renal lymphatics induces proteinuria, tubulointerstitial fibrosis, and mesangial expansion within weeks.[22] A high salt diet fed to control rats and SHRs induces CD68+ macrophages to produce the VEGF-C transcription factor nuclear factor of activated T-cells 5 (NFAT5) leading to dermal and cardiac lymphangiogenesis, respectively, via VEGFR-3.[23, 24] We reported that Fisher 344 aged rats as well as SHRs that develop renal injury exhibit increased renal VEGF-C, VEGFR-3, and lymphatics and this was associated with macrophage infiltration.[16] In the current study, we detected increased renal levels of macrophages and Th1 cells in SSHTN mice and macrophages and dendritic cells in LHTN mice. Renal T cells and monocytes have been previously implicated in several hypertension models.[4, 6, 11, 25, 26] Whether these specific immune subsets (i.e., CD70 T cells[17]) are the primary inflammatory drivers likely depends on the hypertensive stimuli. We are currently exploring whether infiltrating immune cells, elevated blood pressure, and/or increased renal interstitial concentrations of salt and asymmetric dimethylarginine drive lymphangiogenesis in the kidney.

Renal lymphangiogenesis may be beneficial at certain times, but detrimental at others.[27] In renal transplant studies, rejection is associated with renal lymphangiogenesis and inhibition of lymphangiogenesis prolongs allograft survival.[28] Immune trafficking to the draining lymph node increases antigen presentation and propagates allograft immunity. In contrast, establishing more exit routes for immune cells may be beneficial in HTN and SSHTN for maintaining self-tolerance. Weekly intravenous injections of a VEGF-C retrovirus into hypertensive SHRs during the last 4 weeks of a 12-week high salt diet increased cardiac lymphatics, decreased myocardial macrophages, and reduced SBP from 197 to 189 mm Hg.[23] Additionally, inhibition of dermal lymphangiogenesis worsened SSHTN.[24, 29] In the current study, VEGF-D was overexpressed specifically in the kidney to initiate renal lymphatic expansion prior to the HTN stimuli. It is important to reiterate that all mice received doxycycline, which reduced SBP in some reports, to make effects specific to VEGF-D overexpression. VEGF-D driven renal lymphangiogenesis prevented the development of both SSHTN and LHTN and this was associated with decreased renal levels of immune cells. Studies to determine whether augmenting renal lymphatics after SSHTN/HTN is established may therapeutic and how inhibiting renal lymphangiogenesis impacts HTN are underway.

In conclusion, physiologic levels of renal lymphangiogenesis occurs in SSHTN and HTN but are insufficient to significantly enhance immune cell exfiltration. Kidney-specific augmentation of lymphatic density is able to prevent the development of SSHTN and HTN in mice. The current study suggests that renal lymphatic density may be predictive in identifying individuals at risk of developing SSHTN/HTN and identifies a potential new therapeutic strategy for patients incapable of renal lymphatic expansion in response to HTN stimuli.

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DISCLOSURES
None.

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FIGURE LEGENDS

Figure 1. Salt-sensitive hypertension in mice was associated with increased renal lymphatic density and immune cell infiltration. (A) Systolic blood pressure measures in control mice (CON) and mice administered L-NAME in the drinking water for 2 weeks, then 2 weeks of tap water washout, then 3 weeks of 4% salt diet (SSHTN). (B) LYVE-1 immunofluorescence in kidney sections from CON and SSHTN mice. Scale bars = 50 μm. Renal interlobular (IL) artery and lymphatic density in CON and SSHTN mice as determined by (C) mean number of IL arteries and LYVE-1+, lumen-containing lymphatic vessels per kidney section, (D) mean number of LYVE-1+, lumen-containing lymphatic vessels per IL artery, and (E) distribution of the number of LYVE-1+, lumen-containing lymphatic vessels around each artery. Expression changes in (F) lymphatic genes and (G) immune cell marker genes in kidneys from CON and SSHTN mice. Results are expressed as mean ± SEM where appropriate and statistical analyses consisted of Student's t-test. N=6-10 mice in each group. *p<0.05 vs CON.

Figure 2. Genetic augmentation of renal lymphatics prevented salt-sensitive hypertension in mice and decreased renal immune cells. (A) Systolic blood pressure measures in KidVD- and KidVD+ mice administered L-NAME in the drinking water for 2 weeks, then 1 week of tap water washout, then 4 weeks of doxycycline (DOX) water coupled with a 4% salt diet the last 3 weeks (SSHTN). (B) LYVE-1 immunofluorescence in kidney sections from KidVD- and KidVD+ SSHTN mice. Scale bars = 100 μm. (C) Renal lymphatic density in KidVD- and KidVD+ SSHTN mice as determined by LYVE-1+ pixels per field. Expression changes in (D) lymphatic genes and (E) immune cell marker genes in kidneys from control KidVD- and KidVD+ SSHTN mice. (F) Flow cytometry in kidneys from KidVD- and KidVD+ SSHTN and representative dot plots for CD45+/CD3e+ T cells. Results are expressed as mean ± SEM and statistical analyses consisted of Student's t-test. N=6-10 mice in each group. *p<0.05 vs KidVD- or CON.

Figure 3. L-NAME-induced hypertension in mice was associated with increased renal lymphatic density and immune cell infiltration. (A) Systolic blood pressure measures in control mice (CON) and mice administered L-NAME in the drinking water for 2 weeks (LHTN). (B) LYVE-1 immunofluorescence in kidney sections from CON and LHTN mice. Scale bars = 50 μm. Renal interlobular (IL) artery and lymphatic density in CON and LHTN mice as determined by (C) mean number of IL arteries and LYVE-1+, lumen-containing lymphatic vessels per kidney section, (D) mean number of LYVE-1+, lumen-containing lymphatic vessels per IL artery, and (E) distribution of the number of LYVE-1+, lumen-containing lymphatic vessels around each artery. Expression changes in (F) lymphatic genes and (G) immune cell marker genes in kidneys from CON and LHTN mice. Results are expressed as mean ± SEM where appropriate and statistical analyses consisted of Student's t-test. N=6-10 mice in each group. *p<0.05 vs CON.

Figure 4. Genetic augmentation of renal lymphatics prevented L-NAME-induced hypertension in mice by decreasing renal immune cells. (A) Systolic blood pressure measures in KidVD- and KidVD+ mice administered doxycycline (DOX) in the drinking water for 4 weeks coupled with L-NAME the last 3 weeks (LHTN). (B) LYVE-1 immunofluorescence in kidney sections from KidVD- and KidVD+ LHTN mice. Scale bars = 100 μm. (C) Renal lymphatic density in KidVD- and KidVD+ LHTN mice as determined by LYVE-1+ pixels per field. Expression changes in (D) lymphatic genes and (E) immune cell marker genes in kidneys from KidVD- and KidVD+ LHTN mice. (F) Flow cytometry in kidneys from KidVD- and KidVD+ LHTN and representative dot plots for CD45+/CD11c+ dendritic cells. Results are expressed as mean ± SEM and statistical analyses consisted of Student's t-test. N=6-10 mice in each group. *p<0.05 vs KidVD- or CON.

NOVELTY AND SIGNFICANCE

What Is Known?

- Renal inflammation is part of the etiology of hypertension.
- Lymphangiogenesis is a necessary process in inflammation resolution.

What New Information Does This Article Contribute?

- Endogenous lymphatic expansion occurs in two murine models of hypertension.
- Genetically increasing renal lymphangiogenesis prevents hypertension.

Hypertension can be characterized as a disease of chronic renal inflammation. Inflammation and increased immune cells cause a localized expansion of lymphatic vessels through secretion of pro-lymphangiogenic factors in a host of preclinical and clinical studies. This lymphangiogenesis is essential for clearing fluid, chemokines, and immune cells, and, thereby, resolving inflammation. Here, endogenous renal lymphatic expansion is demonstrated in two murine models of hypertension. These models also demonstrate increased renal inflammation and immune cell accumulation. In an inducible model of augmented renal lymphangiogenesis, hypertension is completely prevented under these conditions. Enhanced lymphatic density reduced macrophage, dendritic cell, and T cell populations in the kidney. This work demonstrates a novel role for renal lymphatic vessels in hypertension and kidney health and provides a significant new target for regulating or assessing blood pressure.

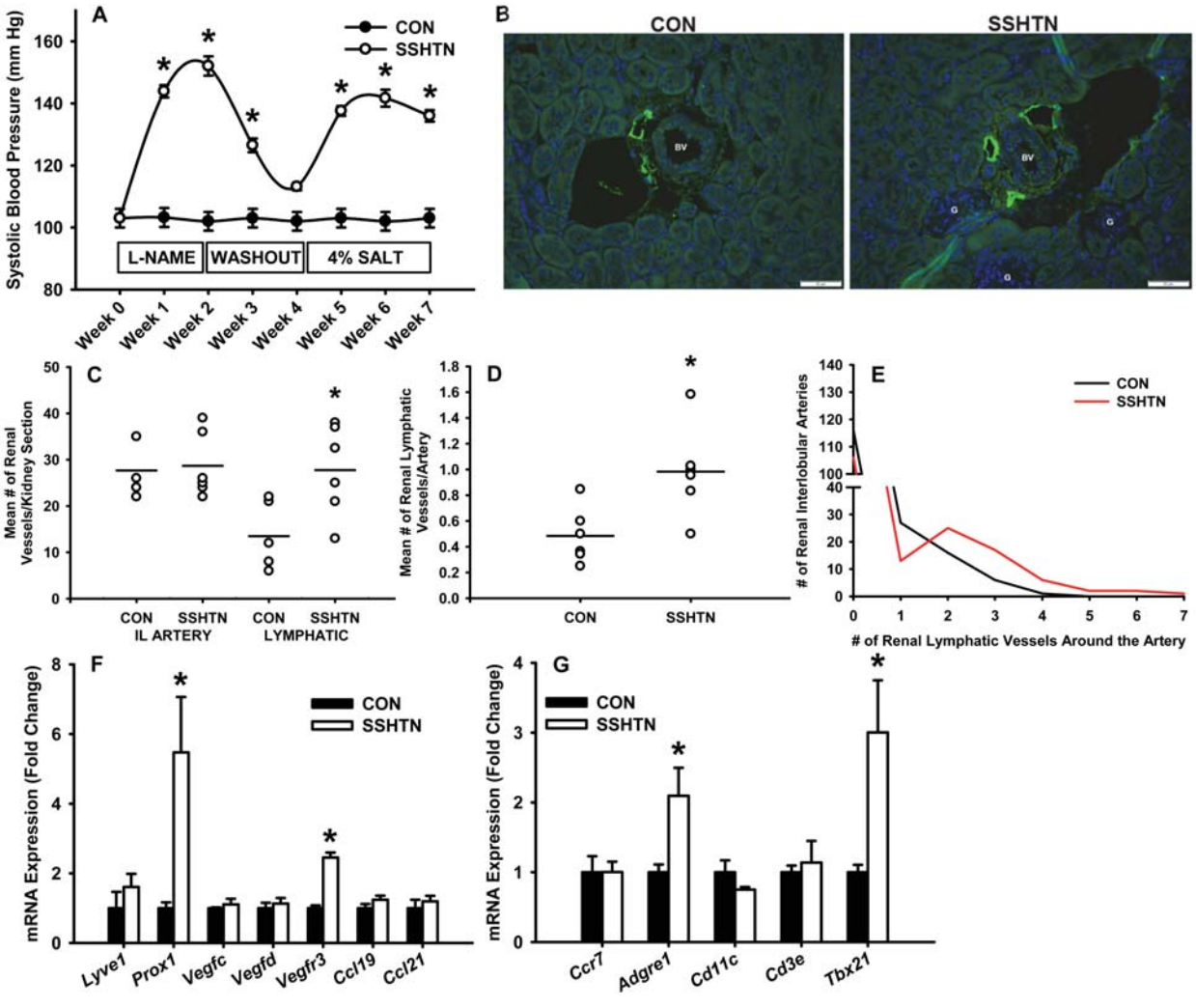
A
Systolic Blood Pressure (mm Hg)
CON
SSHTN
L-NAME
WASHOUT
4% SALT
Week 0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
B
CON
SSHTN
BV
G
C
Mean # of Renal Vessels/Kidney Section
CON
SSHTN
IL ARTERY
LYMPHATIC
D
Mean # of Renal Lymphatic Vessels/Artery
E
# of Renal Interlobular Arteries
# of Renal Lymphatic Vessels Around the Artery
F
mRNA Expression (Fold Change)
Lyve1
Prox1
Vegfc
Vegfd
Vegfr3
Ccl19
Ccl21
G
Ccr7
Adgre1
Cd11c
Cd3e
Tbx21

Figure 1

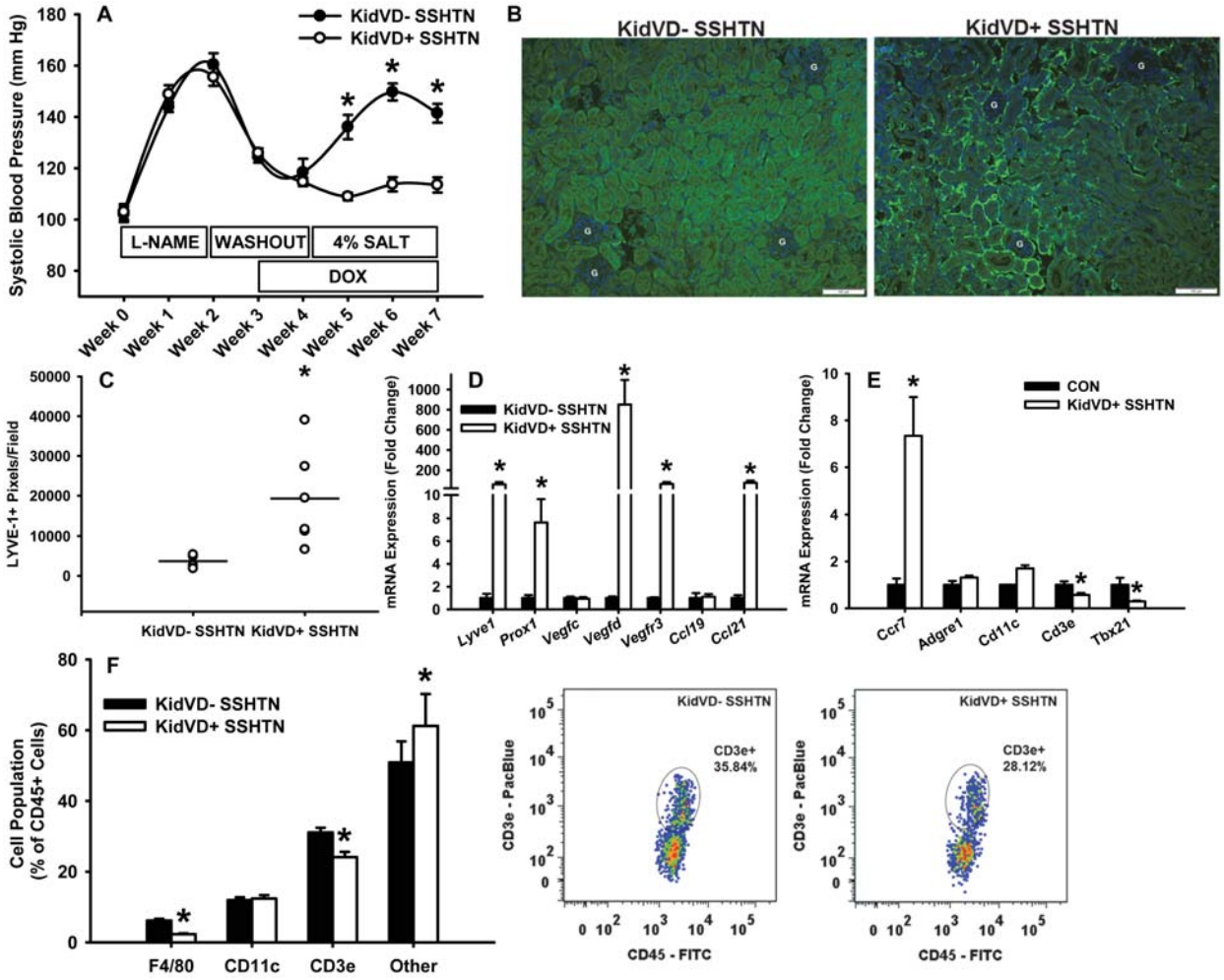
A
KidVD- SSHTN
KidVD+ SSHTN
Systolic Blood Pressure (mm Hg)
180
160
140
120
100
80
L-NAME
WASHOUT
4% SALT
DOX
Week 0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
B
KidVD- SSHTN
KidVD+ SSHTN
C
LYVE-1+ Pixels/Field
50000
40000
30000
20000
10000
0
KidVD- SSHTN
KidVD+ SSHTN
D
KidVD- SSHTN
KidVD+ SSHTN
mRNA Expression (Fold Change)
1000
800
600
400
200
10
8
6
4
2
0
Lyve1
Prox1
Vegfc
Vegfd
Vegfr3
Ccl19
Ccl21
E
CON
KidVD+ SSHTN
mRNA Expression (Fold Change)
10
8
6
4
2
0
Ccr7
Adgre1
Cd11c
Cd3e
Tbx21
F
KidVD- SSHTN
KidVD+ SSHTN
Cell Population
(% of CD45+ Cells)
80
60
40
20
0
F4/80
CD11c
CD3e
Other
KidVD- SSHTN
CD3e+
35.84%
CD3e - PacBlue
CD45 - FITC
KidVD+ SSHTN
CD3e+
28.12%
CD3e - PacBlue
CD45 - FITC

Figure 2

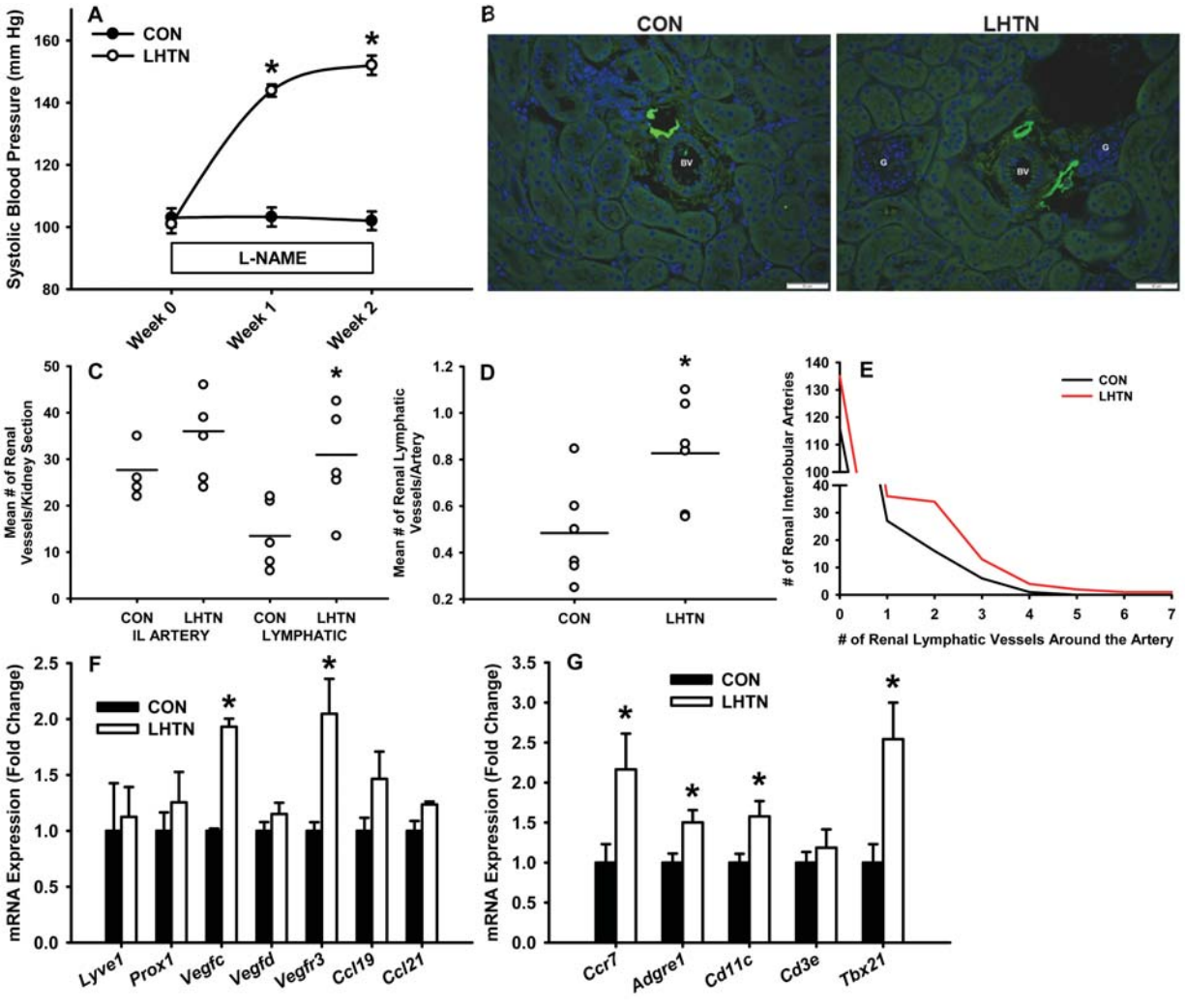
A
CON
LHTN
Systolic Blood Pressure (mm Hg)
160
140
120
100
80
L-NAME
Week 0
Week 1
Week 2
B
CON
LHTN
BV
G
C
Mean # of Renal Vessels/Kidney Section
50
40
30
20
10
0
CON
LHTN
CON
LHTN
IL ARTERY
LYMPHATIC
D
Mean # of Renal Lymphatic Vessels/Artery
1.2
1.0
0.8
0.6
0.4
0.2
CON
LHTN
E
# of Renal Interlobular Arteries
140
130
120
110
100
40
30
20
10
0
CON
LHTN
0
1
2
3
4
5
6
7
# of Renal Lymphatic Vessels Around the Artery
F
mRNA Expression (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
CON
LHTN
Lyve1
Prox1
Vegfc
Vegfd
Vegfr3
Ccl19
Ccl21
G
mRNA Expression (Fold Change)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CON
LHTN
Ccr7
Adgre1
Cd11c
Cd3e
Tbx21

Figure 3

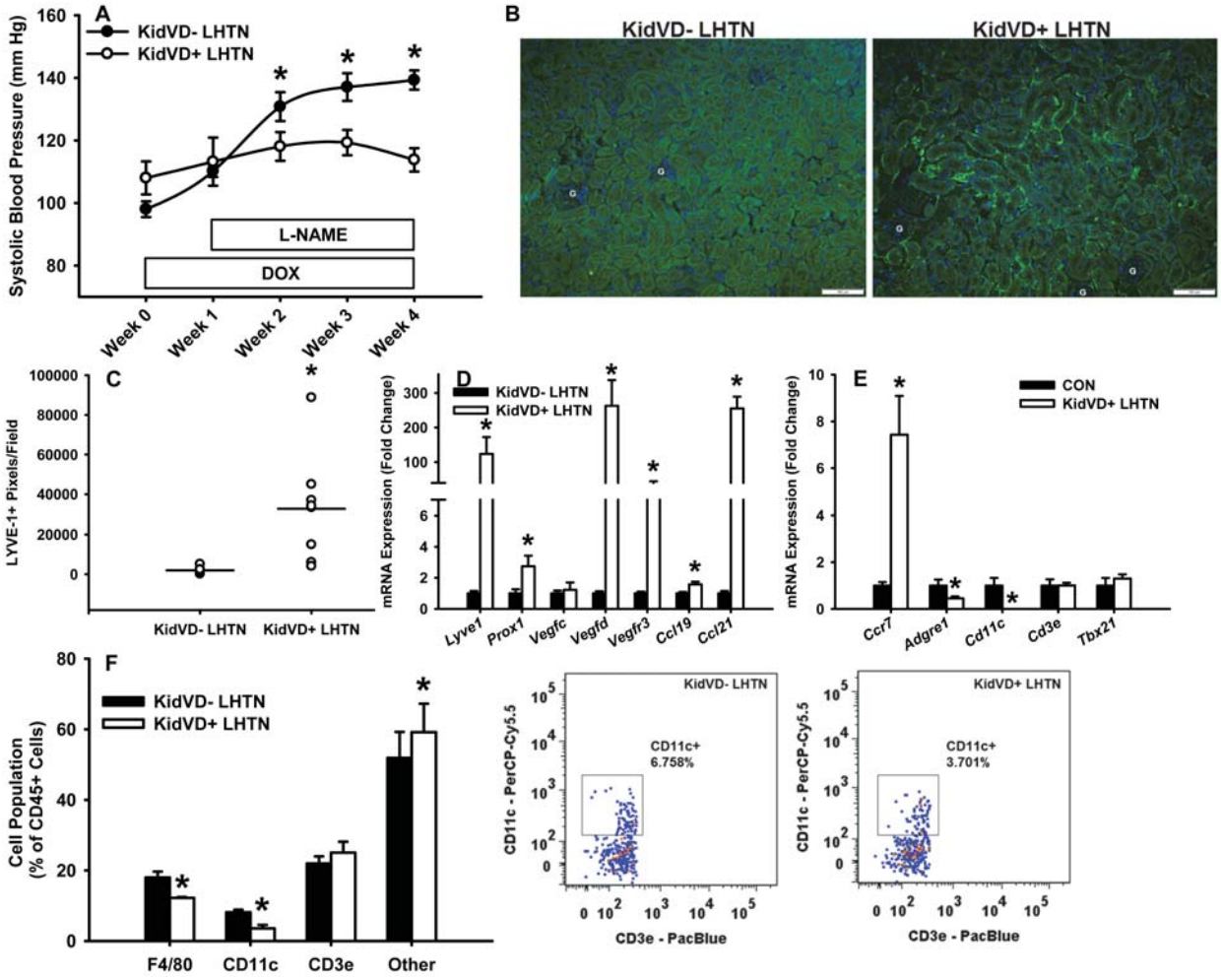
A
KidVD- LHTN
KidVD+ LHTN
Systolic Blood Pressure (mm Hg)
L-NAME
DOX
Week 0
Week 1
Week 2
Week 3
Week 4
B
KidVD- LHTN
KidVD+ LHTN
C
LYVE-1+ Pixels/Field
KidVD- LHTN
KidVD+ LHTN
D
KidVD- LHTN
KidVD+ LHTN
mRNA Expression (Fold Change)
Lyve1
Prox1
Vegfc
Vegfd
Vegfr3
Ccl19
Ccl21
E
CON
KidVD+ LHTN
mRNA Expression (Fold Change)
Ccr7
Adgre1
Cd11c
Cd3e
Tbx21
F
KidVD- LHTN
KidVD+ LHTN
Cell Population (% of CD45+ Cells)
F4/80
CD11c
CD3e
Other
KidVD- LHTN
CD11c+ 6.758%
CD11c - PerCP-Cy5.5
CD3e - PacBlue
KidVD+ LHTN
CD11c+ 3.701%
CD11c - PerCP-Cy5.5
CD3e - PacBlue

Figure 4