Interleukin-17A Regulates Renal Sodium Transporters and Renal Injury in Angiotensin II-Induced Hypertension

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Journal Title: HYPERTENSION
Volume: Volume 68, Number 1
Publisher: LIPPINCOTT WILLIAMS & WILKINS | 2016-07-01, Pages 167-+
Type of Work: Article
Publisher DOI: 10.1161/HYPERTENSIONAHA.116.07493
Permanent URL: https://pid.emory.edu/ark:/25593/vkd8c

Final published version:
http://dx.doi.org/10.1161/HYPERTENSIONAHA.116.07493

Accessed November 14, 2021 11:36 PM EST
INTERLEUKIN 17A REGULATES RENAL SODIUM TRANSPORTERS AND RENAL INJURY IN ANGIOTENSIN II-INDUCED HYPERTENSION

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Abstract

Angiotensin II (Ang II)-induced hypertension is associated with an increase in T cell production of interleukin 17A (IL-17A). Recently, we reported that IL-17A−/− mice exhibit blunted hypertension, preserved natriuresis in response to a saline challenge, and decreased renal sodium hydrogen exchanger 3 (NHE3) expression after 2 weeks of Ang II infusion compared to wild type (WT) mice. In the current study, we performed renal transporter profiling in mice deficient in IL-17A or the related isoform, IL-17F, after 4 weeks of Ang II infusion, a time when the blood pressure reduction in IL-17A−/− mice is most prominent. Deficiency of IL-17A abolished the activation of distal tubule transporters, specifically the sodium-chloride cotransporter (NCC) and the epithelial sodium channel (ENaC) and protected mice from glomerular and tubular injury. In human proximal tubule (HK-2) cells, IL-17A increased NHE3 expression through a serum and glucocorticoid regulated kinase 1 (SGK1) dependent pathway. In mouse distal convoluted tubule

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Disclosures: The authors have no conflicts of interest to disclose.
(mDCT15) cells, IL-17A increased NCC activity in an SGK1/Nedd4-2 dependent pathway. In both cell types, acute treatment with IL-17A induced phosphorylation of SGK1 at serine 78, and treatment with an SGK1 inhibitor blocked the effects of IL-17A on NHE3 and NCC. Interestingly, both HK-2 and mDCT15 cells produce endogenous IL-17A. IL17F had little or no effect on blood pressure or renal sodium transporter abundance. These studies provide a mechanistic link by which IL-17A modulates renal sodium transport and suggest that IL-17A inhibition may improve renal function in hypertension and other autoimmune disorders.

Keywords
Hypertension; kidney; NHE3; NCC; interleukin 17; angiotensin II

INTRODUCTION

Hypertension is a leading cause of cardiovascular disease morbidity and mortality\(^1\). Yet, the pathogenesis of hypertension is still poorly understood, and despite conventional treatment, blood pressure remains uncontrolled in nearly half of the hypertensive population\(^2\). Emerging evidence from our group and others implicates innate and adaptive immune cells and the cytokines that they produce as pathogenic mediators of this disease and its attendant end-organ damage\(^3\)\(^\cdot\)\(^4\).

Interleukin 17A (IL-17A), a pro-inflammatory cytokine produced predominantly by CD4\(^+\) T helper 17 (Th17) cells as well as gamma delta T cells, plays an important role in numerous autoimmune diseases\(^5\). We have shown that in response to angiotensin II (Ang II) infusion, mice deficient in IL-17A develop an initial increase in blood pressure that is similar to wild type (WT) mice but are unable to sustain these elevated pressures. Blood pressure starts to decline after 2 weeks and is approximately 30 mmHg lower than Ang II infused WT mice by 4 weeks. Moreover, IL-17A\(^{-/-}\) mice exhibit reduced vascular inflammation and preserved vascular function in response to Ang II infusion compared to WT mice\(^6\). In keeping with this, Amador et al. reported a marked increase in Th17 cells in DOCA-salt treated rats and observed that treating DOCA-salt rats with an antibody against IL-17A reduced blood pressure and collagen deposition in the heart and kidneys\(^7\). We recently showed that Ang II treated IL-17A\(^{-/-}\) mice have preserved diuresis and natriuresis in response to an acute saline challenge unlike Ang II treated WT mice which retain salt and water\(^8\). Consistent with this finding, proximal tubule NHE3 protein abundance was reduced by 40% in IL-17A\(^{-/-}\) mice but not WT mice after 2 weeks of Ang II infusion, suggesting a mechanism for enhanced pressure natriuresis in the IL-17A\(^{-/-}\) mice.

There are 6 isoforms of IL-17: A through F. IL-17A shares 50% sequence similarity with IL-17F, and both can bind as homo- or heterodimers to the same receptor complex composed of IL-17RA and IL-17RC subunits\(^9\). The role of IL-17F in hypertension is previously unknown. The goal of the present study was to determine the effect of IL-17A and IL-17F on renal sodium transporters after a prolonged (4 week) period of Ang II infusion, a time when the blood pressure blunting in IL-17A\(^{-/-}\) mice is most prominent. In

__Hypertension. Author manuscript; available in PMC 2017 July 01.\__
addition, we investigated whether the effects of IL-17A or F are direct effects of these cytokines acting on renal sodium transporters in renal epithelial cells.

Our results demonstrate that IL-17A (but not IL-17F) mediates Ang II induced renal injury and regulates renal sodium transporters, namely NHE3 and NCC, through a serum and glucocorticoid regulated kinase 1 (SGK1) dependent pathway. Moreover, we found that cultured renal proximal tubule and distal convoluted tubule cells produce endogenous IL-17A. This study provides mechanistic insight into how inflammatory cytokines can regulate sodium and water balance and therefore blood pressure.

METHODS

Animals, angiotensin II infusion, and blood pressure measurement

Wild-Type (C57Bl/6J) mice were purchased from Jackson Laboratories. IL-17A−/− and IL-17F−/− mice were generated as previously described \(^\text{10, 11}\). Male mice approximately 10–12 weeks of age were used. Mice were anesthetized with ketamine/xylazine (90–120 mg/kg + 10mg/kg) (1:1 volume ratio) and 2 or 4 week osmotic mini-pumps containing angiotensin II (490 ng/kg/min) or vehicle (0.08 M sodium chloride/1% acetic acid solution) were inserted subcutaneously. Blood pressure was measured non-invasively using tail cuff as previously described \(^\text{6}\). At the end of the experiment, mice were sacrificed using CO\(_2\) inhalation. Mice were perfused with saline until all blood was cleared from the circulation and then kidneys were extracted and flash frozen. All animal procedures were approved by Vanderbilt University Institutional Animal Care and Use Committee (IACUC), and mice were housed and cared for in accordance with the Guide for the Care and Use of Laboratory Animals.

Transporter profiling

Immunoblotting for NHE3, NCC-total, phosphorylated NCC, and gamma ENaC were performed as previously described \(^\text{8, 12, 13}\).

Urine analysis

Mice were placed in metabolic cages for 24 hour urine collection. Albumin was measured in the urine using an ELISA kit (Albuwell, Exocell). Immunoblotting for albumin and angiotensinogen was performed on urine samples as previously described \(^\text{12, 13}\).

Reagents and cell lines

Cytokines IL-17A, IL-17F, and TNF\(\alpha\) were obtained from R&D systems. The SGK1 inhibitor, GSK 650394, was obtained from TOCRIS Bioscience. Mouse distal convoluted tubule (mDCT15) cells were cultured as previously described \(^\text{14}\). NEDD4-2 deficient mDCT15 cells were generated as previously described \(^\text{15}\). HK-2 cells were purchased from ATCC.

Cell culture

HK-2 cells or mDCT15 cells were plated in 6 well dishes at a density of 100,000 cells per well and allowed to grow to confluence over the ensuing days before experiments were performed.
performed. HK-2 cells were cultured in Keratinocyte SFM (Invitrogen) media in which the Epidermal Growth Factor and Bovine Pituitary Extract were added. Additionally 1% Penicillin/Streptomycin was added to the media. mDCT15 cells were grown in DMEM/F-12 50/50 media in which 5% Fetal Bovine Serum and 1% Penicillin/Streptomycin were added. mDCT15 cells were serum starved overnight before experiments were performed.

**Immunoblotting**

HK-2 cells or mDCT15 cells plated in 6 well dishes were scraped in cold sorbitol buffer (5% sorbitol, 5mM histidine/imidazole, 0.5mM EDTA with protease and phosphates inhibitors) to isolate protein. Protein was quantified using a Bradford Assay Kit. Samples (30μg of protein) were separated by SDS-PAGE. Samples were then transferred to a nitrocellulose membrane and blocked with either 5% BSA or 5% milk. Membranes were probed using either NHE3 (Millipore) or p78SGK1 (Cell Signaling Technologies). Membranes were then labelled using a BioRad HRP Conjugate and detected with BioRad ECL Chemiluminescence solution. Membranes were imaged with a BioRad ChemiDoc Imager. Blots were then stripped for 15 minutes and re-probed using an antibody to GAPDH (Santa Cruz Biotech). Densitometry was performed using BioRad Image Lab software, and bands of interest were normalized to GAPDH.

**Quantitative RT-PCR**

Whole kidneys were homogenized in Trizol using a Bead Beater, and lysates were then subjected to phenol-chloroform extraction. RNA was isolated using an RNeasy Mini Kit (Qiagen). HK-2 cells or mDCT15 cells plated on a 6 well dish were washed and then lysed in RLT Buffer and RNA was extracted using an RNeasy Mini Kit (Qiagen). RNA quantity and purity was measured using a spectrophotometer. cDNA was made using a High Capacity cDNA reverse transcriptase kit (Applied Biosystems). Samples were evaluated for mouse or human SGK1 (Taqman), mouse or human IL-17A (SABiosciences), mouse or human GAPDH (Taqman), and a primer designed against 18s (Sigma) that works in both species due to sequence conservation. Samples were normalized to GAPDH and then normalized again to one of the control samples. The Relative Quantification (RQ) values are plotted.

**NCC activity assay and lentiviral transduction**

NCC activity was measured as previously described. Briefly, mDCT15 were incubated at 37°C with vehicle, 100ng/mL IL-17A, 100ng/mL IL-17F, 100nM GSK 650394, or a combination of the agents. Thirty minutes before uptake, 0.1 mM metolazone (an inhibitor of NCC) or vehicle (DMSO) was added to the media. The media was then changed to a 22Na+ containing medium with or without 0.1mM metolazone and incubated for 20 minutes. Cells were lysed and radioactivity was measured by liquid scintillation. Protein concentrations were determined by a BCA assay. Uptakes were normalized to nmol/mg protein. NCC activity was defined as the thiazide-sensitive uptake which is the difference in Na uptake with or without metolazone. Inhibition of Nedd4-2 in the mDCT15 cells was performed using lentiviral transduction as described in Arroyo et al.
**Statistics**

Data are expressed as mean ± standard error of the mean. T tests, one-way ANOVA, and two-way ANOVA were used as appropriate. A p-value ≤0.05 was considered significant.

**RESULTS**

**IL-17A−/− mice fail to sustain the upregulation of distal sodium transporters in response to chronic (4 weeks) angiotensin II infusion**

Following Ang II infusion, IL-17A−/− mice exhibit an initial rise in BP similar to WT mice but are unable to sustain these pressures. BP starts to decline around 2 weeks and reaches pressures 30 mmHg lower than Ang II infused WT mice by 4 weeks (Madhur et al. and Supplemental Figure 1). Therefore, we performed renal transporter profiling in whole kidneys of WT and IL-17A−/− mice after 4 weeks of Ang II or vehicle (Sham) infusion. Interestingly, at this timepoint, there was a marked difference in the activation of distal sodium transporters between WT and IL-17A−/− mice. As expected, Ang II increased abundance and phosphorylation of NCC and cleavage (activation) of the gamma subunit of ENaC in WT mice, but this was abolished in IL-17A−/− mice (Figure 1). We previously showed that after 2 weeks of Ang II infusion, distal transporters are activated in IL-17A−/− mice but to a lesser degree than in WT mice. Taken together, this data shows that IL-17A−/− mice fail to sustain the Ang II induced increases in distal convoluted tubule and collecting duct sodium channels, coincident with their decline in blood pressure.

To determine if this effect was specific to the IL-17A isoform, we investigated the effect of IL-17F on blood pressure and renal sodium transporters using IL-17F deficient mice. Blood pressure in IL-17F−/− mice during chronic Ang II infusion was not blunted (Supplemental Figure 1) and distal sodium transporter abundance was similar to that observed in WT mice with the exception of a reduction in cleaved γENaC (Figure 1). Thus, the reductions in blood pressure and distal renal sodium transporter abundance are specific to the IL-17A isoform.

**IL-17A regulates renal SGK1 expression**

Serum and glucocorticoid regulated kinase 1 (SGK1) is an important mediator of salt and water retention in the kidney through inhibition of Nedd4-2 mediated ubiquitination and degradation of NCC and ENaC in the distal convoluted tubule, thereby enhancing the expression of these transporters on the cell surface. We hypothesized that SGK1 mediates the effects of IL-17A on renal sodium transporters. To test this hypothesis, we performed quantitative RT-PCR on whole kidney lysates from WT and IL-17A−/− mice infused with 2 or 4 weeks of Ang II or vehicle (Sham). SGK1 expression was upregulated 2-fold in kidneys from WT mice infused for 2 or 4 weeks with Ang II (Figure 2). In contrast, kidneys from IL-17A−/− mice had no increase in SGK1 expression after either 2 or 4 weeks of Ang II infusion (Figure 2). This suggests that SGK1 is a potential mediator of the effects of IL-17A in the kidney.
IL-17A, but not IL-17F, directly upregulates NCC activity in cultured mouse distal convoluted tubule (mDCT15) cells via an SGK1/Nedd4-2 dependent pathway

To determine if the effect of IL-17A on distal tubule sodium transporters was a direct and specific effect, we measured NCC activity in a cultured mouse distal convoluted tubule cell line (mDCT15) that has been previously characterized and shown to recapitulate many features of in vivo distal convoluted tubule cells. NCC activity was defined as the metallozone inhibited increase in radioactive sodium uptake. IL-17A, but not IL-17F, significantly increased NCC activity in these cells (Figure 3A).

We did not detect an increase in SGK1 mRNA with IL-17A treatment of mDCT15 cells (data not shown), but phosphorylation of SGK1 at Serine 78 was increased 1.5 fold after 15 minutes of IL-17A treatment (Figure 3B). Importantly, the IL-17A induced increased in NCC activity was abrogated by co-treatment with the SGK1 inhibitor, GSK 650394 (Figure 3C). To test the hypothesis that the effect of IL-17A and SGK1 on NCC activity is mediated via Nedd4-2, we used mDCT15 cells in which Nedd4-2 was silenced by lentiviral transduction of a short hairpin RNA (shRNA) targeting Nedd4-2 or a non-targeting shRNA as control. As shown in Supplemental Figure 2, the Nedd4-2 shRNA transducted cells expressed approximately 60% less Nedd4-2 protein by immunoblotting. In these cells, the basal level of NCC activity was increased, and there was virtually no additional effect of IL-17A on NCC activity (Figure 3D). Taken together, these results suggest that IL-17A induces an increase in NCC activity through phosphorylation of SGK1 and inhibition of Nedd4-2 mediated ubiquitination and degradation of NCC.

IL-17A upregulates NHE3 in cultured human proximal tubule (HK-2) cells via an SGK1 dependent pathway

Downregulation of NHE3 is an important mechanism for pressure natriuresis. Our previous study demonstrated that IL-17A−/− mice exhibit suppressed NHE3 abundance at an earlier timepoint (2 weeks) after Ang II infusion compared to WT mice. In the current study, we found that IL-17A−/− mice maintained this decrease in NHE3 abundance, and that WT mice exhibited a similar decrease in NHE3 abundance by 4 weeks of Ang II infusion (Supplemental Figure 3). Thus, while pressure natriuresis eventually occurs in both WT and IL-17A−/− mice, it is accelerated in the absence of IL-17A. Thus, we hypothesized that IL-17A has a direct effect on NHE3 regulation in proximal tubule cells. To test this hypothesis, we quantified NHE3 abundance by immunoblotting in cultured human proximal tubule (HK-2) cells treated with or without recombinant IL-17A for 72 hours. Using this in vitro system, we were able to remove the confounding effects of blood pressure and angiotensin II. Interestingly, IL-17A directly upregulated NHE3 protein expression by greater than 2-fold in these cultured cells (Figure 4A).

To determine whether IL-17A regulates SGK1 in the proximal tubule, we treated HK-2 cells with increasing doses of recombinant IL-17A, IL-17F, or tumor necrosis factor alpha (TNFα) for 24 hours. Interestingly, only IL-17A caused a dose-dependent increase in SGK1 expression by quantitative RT-PCR (Figure 4B). We then investigated whether IL-17A treatment also increased phosphorylation of SGK1. We found that phosphorylation of SGK1 at Serine-78 increased 2-fold in HK-2 cells after 15 minutes of treatment with IL-17A.
To determine whether the IL-17A dependent increase in NHE3 was dependent on SGK1, we treated HK-2 cells with increasing doses of the SGK1 inhibitor, GSK 650394, starting 30 minutes prior to IL-17A treatment. The SGK1 inhibitor reduced the basal expression of NHE3 and caused a dose-dependent decrease in the IL-17A induced NHE3 expression (Figure 4D) indicating that the effect of IL-17A on NHE3 in proximal tubule cells is mediated by SGK1. Taken together, these data demonstrate that IL-17A increases expression and phosphorylation of SGK1 in the proximal tubule leading to increased abundance of NHE3.

**IL-17A−/− mice are protected from glomerular and tubular injury in response to angiotensin II infusion**

Albuminuria is a marker of glomerular injury and is known to increase in response to Ang II induced hypertension. To determine the effect of IL-17A and IL-17F on glomerular injury, we measured urinary albumin by ELISA on 24-hr urine samples from WT, IL-17A−/−, and IL-17F−/− mice after 4 weeks of vehicle (Sham) or Ang II infusion. WT and IL-17F−/− mice exhibited a marked increase in albuminuria while IL-17A−/− mice were completely protected from glomerular injury during Ang II infusion (Figure 5A). We then performed immunoblotting to confirm this finding and to quantify urinary angiotensinogen (a marker of tubular injury) in these 24 hr urine samples from WT and IL-17A−/− mice. Interestingly, Ang II infusion induced a marked increase in urinary angiotensinogen in WT mice and this was absent in IL-17A−/− mice (Figure 5B). Thus IL-17A, but not IL-17F, contributes to the renal end-organ damage that occurs as a result of Ang II induced hypertension.

**Renal proximal and distal convoluted tubule cells produce endogenous IL-17A**

We showed that IL-17A plays an important role in the regulation of renal sodium transporters both in vivo and in vitro. However, a key question is what is the source of IL-17A in the kidney? We and others have observed an accumulation of immune cells, particularly T lymphocytes, in the kidneys of animals and humans with hypertension. However, there is also evidence that renal epithelial cells may produce their own cytokines such as IL-6, IL-8, and TNF-α. By RT-PCR, we confirmed the previously reported observation that HK-2 cells produce IL-17A. Interestingly, we also found that mDCT15 cells express IL-17A (Supplemental Figure 4). This was not observed in mouse pulmonary artery smooth muscle cells or human aortic endothelial cells. RNA from mouse CD4+ T cells polarized towards the Th17 lineage served as a positive control. Thus, IL-17A produced by renal tubular cells themselves or from infiltrated T cells can regulate sodium absorption, renal injury, and blood pressure.

**DISCUSSION**

A major cause and complication of hypertension is renal dysfunction leading to increased sodium and water retention which promotes a rise in blood pressure and compensatory pressure natriuresis. Here we show that the pro-inflammatory cytokine, IL-17A, produced by immune cells as well as renal epithelial cells, can induce the expression and activity of both proximal and distal sodium transporters through an SGK1 dependent pathway, thus counteracting pressure natriuresis and contributing to sodium retention (Figure 6). Moreover,
loss of IL-17A protects against glomerular and tubular injury in response to Ang II induced hypertension as evidenced by attenuation of albuminuria and urinary angiotensinogen levels in the IL-17A−/− mice. In contrast, deficiency of the related cytokine, IL-17F, has little or no effect on blood pressure and renal transporter expression.

Interestingly, the effect of IL-17A on renal transporters is dependent on the duration of Ang II infusion. We previously reported that after 2 weeks of Ang II infusion, IL-17A−/− mice down-regulated NHE3, while NHE3 expression was unchanged in WT mice. In both groups, distal sodium transporters such as NCC and ENaC were activated (although to a lesser extent in the IL-17A−/− mice). Blood pressure starts to normalize in the IL-17A−/− mice after 2 weeks of Ang II infusion, reaching pressures 30 mmHg lower than Ang II infused WT mice by 4 weeks (Madhur et al. and Supplemental Figure 1). At this 4 week timepoint, the major differences between WT and IL-17A−/− mice are seen in the distal tubule. IL-17A−/− (but not WT) mice have significant blunting of the activation of distal sodium transporters. These are key transporters in the regulation of blood pressure as evidenced by the fact that thiazide diuretics, pharmacological inhibitors of NCC, are still one of the most effective drugs in the treatment of hypertension. Moreover, many of the Mendelian forms of hypertension are due to mutations that influence the activity of NCC and/or the amiloride-sensitive ENaC. Thus, the blood pressure reduction observed after 4 weeks of Ang II infusion in IL-17A−/− mice is likely due to decreased activity of distal sodium transporters. In fact, our data suggests that IL-17A inhibition may be as or more effective than the use of thiazide diuretics or amiloride in the treatment of hypertension and the resultant glomerular and tubular injury.

Urinary angiotensinogen is a biomarker of tubular injury as well as an indicator of the intrarenal renin-angiotensin system (RAS) activity. It is interesting to note that Ang II infusion failed to induce an increase in urinary angiotensinogen in the IL-17A−/− mice. In response to Ang II, proximal tubule cells produce greater levels of angiotensinogen which leads to increased Ang I release into the tubule lumen and subsequently conversion to Ang II by local angiotensin converting enzyme. Ang II then acts on the distal tubule and collecting duct to increase expression of NCC and ENaC. Satou et al. showed that prolonged treatment of renal proximal tubule cells with interferon-γ results in increased angiotensinogen production in these cells. Interferon-γ may thus indirectly activate distal sodium transporters via stimulation of the intrarenal RAS system. It is conceivable that IL-17A may be functioning in a similar manner. However, we did not see an elevation of angiotensinogen by qRT-PCR after IL-17A treatment of HK-2 cells (data not shown). Moreover, our in vitro studies show that IL-17A can stimulate NCC activity in cultured cells. Nevertheless, we cannot rule out a potential effect of IL-17A on the intrarenal RAS in vivo.

Of note are the differential effects seen between IL-17A versus IL-17F. Although they share 50% sequence similarity and bind the same receptor complex, our results indicate that IL-17A (but not IL-17F) regulates NCC activity, renal injury, and hypertension. Limitations of the present study include the use of one specific mouse strain and one model of hypertension, namely angiotensin II infusion. In addition, we have only tested the effects of IL-17 isoforms on one proximal tubule and one distal tubule cell line. Thus, additional
studies using other animal strains, cell lines, and different models of hypertension are necessary to generalize these findings.

Monoclonal antibodies to IL-17A or the IL-17RA receptor have recently been developed for human use. In 2015, the FDA approved the first IL-17A antagonist Secukinumab (a human monoclonal antibody that selectively binds to IL-17A) for the treatment of moderate to severe plaque psoriasis. Also in development are drugs such as Brodalumab, a monoclonal antibody that targets the IL-17RA receptor subunit. This drug is awaiting FDA approval for the treatment of moderate to severe psoriasis. Our data suggests that targeting IL-17A or the IL-17RA/RC receptor complex may be a novel therapeutic strategy for the treatment of hypertension and the associated renal dysfunction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

SOURCES OF FUNDING

This work was supported by a training grant from the National Institutes of Health (NIH T32 HL069765-11A1) to AEN, an American Heart Association (AHA) Post-doctoral Fellowship Award (14POST20420025) to MAS, an AHA GIA (15GRNT21660003) and an NIH NIDDK R01 (DK083785) to AMcD, an NIH NHLBI K08 award (HL121671) to MSM, and support from the National Center for Research Resources, Grant UL1 RR024975-01, now at the National Center for Advancing Translational Sciences, Grant 2 UL1 TR000445-06. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or AHA.

References


This study is the first to elucidate a potential mechanism by which IL-17A contributes to renal dysfunction in hypertension. Sources of IL-17A in the kidney include renal infiltrating T lymphocytes as well as renal epithelial cells. Mice deficient in IL-17A (but not IL-17F) have blunted hypertension and virtually no glomerular injury (as detected by albuminuria and urinary angiotensinogen) in response to Ang II infusion. IL-17A primarily affects proximal tubule sodium transport in the early phase of Ang II infusion and ultimately regulates distal sodium transporters during chronic Ang II infusion through an SGK1 dependent pathway. Thus, IL17A inhibition may have beneficial effects in hypertension and slow the progression to renal failure.
**NOVELTY AND SIGNIFICANCE: 1) WHAT IS NEW? 2) WHAT IS RELEVANT? and SUMMARY**

**What is new**
- Deficiency of IL-17A blunts activation of renal distal tubule sodium transporters, namely NCC and ENaC, in response to chronic Ang II infusion.
- Deficiency of IL-17A protects against Ang II induced glomerular and tubular injury.
- IL-17A induces renal SGK1 expression and phosphorylation.
- IL-17A regulates NHE3 in the proximal tubule and NCC in the distal tubule in an SGK1 dependent pathway.

**What is relevant?**
- Regulation of sodium transporters in the kidney by IL-17A appears to be biphasic, affecting proximal tubule transporters early and distal tubule transporters chronically in hypertension.
- These studies further support the therapeutic potential of IL-17A inhibition in reducing blood pressure and renal injury in hypertension.

**Summary**
Our results identify a mechanism by which IL-17A stimulates SGK1 in the kidney resulting in maintenance of renal sodium transporters on the surface leading to enhanced salt and water reabsorption and elevated blood pressure. Targeting IL-17A or its receptor may be a novel therapeutic strategy for the treatment of hypertension.
Figure 1. IL-17A deficiency blunts the Ang II induced increase in distal sodium transporters

Abundance of total and phosphorylated Na-Cl cotransporter (t-NCC and p-NCC) as well as full length (FL) and cleaved (CL) gamma ENaC were analyzed in whole renal tissue homogenates from wild-type (WT), IL-17A−/−, and IL-17F−/− mice after 4 weeks of vehicle (Sham) or angiotensin II (Ang II) infusion. Fold change in transporter abundance in Ang II infused mice compared to vehicle (Sham) infused mice is shown below. Values are plotted as mean±SEM. Student’s t-Test was used to compare transporter abundance in Ang II infused animals to the corresponding vehicle infused animals for each genotype. *P≤0.05 (n=5–6 per group).
Figure 2. IL-17A regulates renal SGK1 expression

WT and IL-17A\(^{-/-}\) mice were infused with vehicle (Sham) or Ang II for two or four weeks as indicated. Quantitative real time PCR was performed on RNA extracted from whole renal tissue homogenates and normalized to GAPDH. Relative quantification values are plotted as mean±SEM. Two-way ANOVA followed by Bonferroni post-hoc test was used. *\(P<0.05\) (n=5–10 per group).
Figure 3. IL-17A increases NCC activity in cultured mouse distal convoluted tubule (mDCT15) cells via an SGK1/Nedd4-2 dependent pathway

A) NCC activity in mDCT15 cells with or without treatment with 100ng/mL of IL-17A or IL-17F (n=6 per group).

B) Representative immunoblot for phosphorylated SGK1 on serine 78 in mDCT15 cells with or without 15 min of treatment with recombinant IL-17A (100ng/ml). Quantification of all blots relative to GAPDH and normalized to untreated is shown on the right (n=5 per group).

C) NCC activity in mDCT15 cells after treatment with IL-17A or IL-17A plus 100nM of the SGK1 inhibitor, GSK 650394 (n=6 per group).

D) NCC activity with or without IL-17A treatment in mDCT15 cells lentivirally transduced with a short hairpin RNA (shRNA) to silence NEDD4-2 or a non-targeting shRNA (n=4 per group). All data are plotted as mean±SEM. One-way ANOVA followed by Newman-Keuls post-hoc test was used for panels A, C, and D, and a paired Student’s t-Test was used for panel B.

**P<0.01, ***P<0.001, ****P<0.0001.
Figure 4. IL-17A upregulates NHE3 in cultured human proximal tubule (HK-2) cells via an SGK1 dependent pathway

A) HK-2 cells were incubated with or without 100 ng/mL of recombinant IL-17A for 72 hours. Representative immunoblot for NHE3 and GAPDH is shown with quantification of all blots relative to GAPDH and normalized to untreated shown below (n=7 per group).

B) HK-2 cells were treated with IL-17A or IL-17F (increasing doses from 1 ng/ml to 100 ng/ml) or TNFα (1ng/ml or 10ng/mL) for 24 hours. Quantitative real time PCR for SGK1 was performed on the cell lysates. Relative quantification normalized to GAPDH is shown (n=4 per group).

C) Representative immunoblot for phosphorylated SGK1 on serine 78 in HK-2 cells with or without 15 min of treatment with recombinant IL-17A (100ng/ml). Quantification of all blots relative to GAPDH and normalized to untreated is shown below (n=4 per group).

D) HK-2 cells were pretreated for 30 min with vehicle or the SGK1 inhibitor, GSK 650394, at the indicated concentrations and subsequently treated with or without 100ng/mL of IL-17A for 72 hours. A representative immunoblot for NHE3 and GAPDH is shown. Quantification of all blots relative to GAPDH and normalized to untreated is shown below (n=3 per group). All data are plotted as mean±SEM. One-way
ANOVA followed by Bonferroni post-hoc test was used for panels B and D, and a paired Student’s t-Test was used for panels A and C. *$P<0.05$, **$P<0.01$, ***$P<0.001$. 
Figure 5. IL-17A−/− mice are protected from glomerular and tubular injury in response to angiotensin II infusion

WT, IL-17A−/−, or IL-17F−/− mice were infused with vehicle (Sham) or Ang II for 4 weeks and then placed in metabolic cages for 24 hours for urine collection. A) Albuminuria was measured via ELISA and corrected for urine volume. B) Urinary albumin and angiotensinogen were measured by immunoblot and relative abundance for each group is plotted below. Data are expressed as mean±SEM. Two-way ANOVA followed by Newman-Keuls post-hoc test was used in panel A. Student’s t-Test was used in panel B to compare protein levels in Ang II infused animals to the corresponding vehicle infused animals for each genotype. *P<0.05, **P<0.01, ****P<0.0001 (n=5–6 per group).
Figure 6.
Schematic illustrating the effects of IL-17A (endogenous and T cell derived) on both proximal and distal tubule cells in the kidney.