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

Allison E. Norlander, Mohamed A. Saleh, Nikhil V. Kamat, Benjamin Ko, Juan Gnecco, Linjue Zhu, Bethany L. Dale, Yoichiro Iwakura, Robert S. Hoover, Alicia A. McDonough, Meena S. Madhur

Abstract—Angiotensin 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 expression after 2 weeks of angiotensin II infusion compared with wild-type 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, the 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 and the epithelial sodium channel and protected mice from glomerular and tubular injury. In human proximal tubule (HK-2) cells, IL-17A increased sodium hydrogen exchanger 3 expression through a serum and glucocorticoid-regulated kinase 1–dependent pathway. In mouse distal convoluted tubule cells, IL-17A increased sodium–chloride cotransporter activity in a serum and glucocorticoid-regulated kinase 1/Nedd4-2–dependent pathway. In both cell types, acute treatment with IL-17A induced phosphorylation of serum and glucocorticoid-regulated kinase 1 at serine 78, and treatment with a serum and glucocorticoid-regulated kinase 1 inhibitor blocked the effects of IL-17A on sodium hydrogen exchanger 3 and sodium–chloride cotransporter. Interestingly, both HK-2 and mouse distal convoluted tubule 15 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. (Hypertension. 2016;68:167-174. DOI: 10.1161/HYPERTENSIONAHA.116.07493.) ● Online Data Supplement

Key Words: angiotensin II ■ blood pressure ■ hypertension ■ interleukin 17 ■ kidney

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 (BP) 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.3,4 Interleukin-17A (IL-17A), a proinflammatory cytokine produced predominantly by CD4+ T-helper 17 cells as well as γδ T cells, plays an important role in numerous autoimmune diseases.5 We have shown that in response to interleukin II (Ang II) infusion, mice deficient in IL-17A develop an initial increase in BP that is similar to wild-type (WT) mice but are unable to sustain these elevated pressures. BP starts to decline after 2 weeks and is ≈30 mm Hg 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 with WT mice.6 In keeping with this, Amador et al7 reported a marked increase in T-helper 17 cells in deoxycorticosterone acetate–salt treated rats and observed that treating deoxycorticosterone acetate–salt rats with an antibody against IL-17A reduced BP and collagen deposition in the heart and kidneys. 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 sodium hydrogen exchanger 3 (NHE3) protein abundance was reduced

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by 40% in IL-17A−/− mice but not in 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. The role of IL-17F in hypertension is previously unknown. The goal of this study was to determine the effect of IL-17A and IL-17F on renal sodium transporters after a prolonged (4 weeks) period of Ang II infusion, a time when the BP blunting in IL-17A−/− mice is most prominent. In 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 sodium–chloride cotransporter (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, BP.

Methods

Animals, Ang II Infusion, and BP Measurement

Wild-type (C57Bl/6J) mice were purchased from Jackson Laboratories, Bar Harbor, ME. IL-17A−/− and IL-17F−/− mice were generated as previously described. Male mice =10 to 12 weeks of age were used. Mice were anesthetized with ketamine/xylazine (90–120 mg/kg+10 mg/kg; 1:1 volume ratio) and 2- or 4-week osmotic mini-pumps containing Ang II (490 ng/kg per minute) or vehicle (0.08 mol/L sodium chloride/1% acetic acid solution) were inserted subcutaneously. BP was measured noninvasively using tail cuff as previously described. At the end of the experiment, mice were euthanized using CO2 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, 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 γ epithelial sodium channel (ENaC) were performed as previously described. Albumin was measured in the urine using an ELISA kit (Albuwell, Bar Harbor, ME) that works in both species because of sequence conservation. 

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.

Reagents and Cell Lines

Cytokines IL-17A, IL-17F, and tumor necrosis factor-α were obtained from R&D systems. The SGK1 inhibitor, GSK 650394, was obtained from TOCRIS Bioscience. Mouse distal convoluted tubule (mDCT15) cells were purchased from ATCC. 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 confluency during the ensuing days before experiments were performed. HK-2 cells were cultured in Keratinocyte SFM (Invitrogen, Carlsbad, CA) media in which the epidermal growth factor and bovine pituitary extract were added. In addition, 1% penicillin/streptomycin was added to the media. mDCT15 cells were grown in Dulbecco’s modified Eagle medium/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, 5 mmol/L histidine/imidazole, 0.5 mmol/L EDTA with protease and phosphates inhibitors) to isolate protein. Protein was quantified using a Bradford Assay Kit. Samples (30 μg 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, Billerica, MA) or p78SGK1 (Cell Signaling Technologies, Danvers, MA). Membranes were then labeled using a BioRad HRP Conjugate and detected with BioRad ECL Chemiluminescence solution.

Transporter Profiling

Transporter Profiling

NCC Activity Assay and Lentiviral Transduction

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, Valencia, CA). 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 were measured using a spectrophotometer. cDNA was made using a High Capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA). Samples were evaluated for mouse or human SGK1 (Taqman), mouse or human IL-17A (SA Biosciences, Valencia, CA), mouse or human GAPDH (Taqman, Foster City, CA), and a primer designed against 18s (Sigma, St. Louis, MO) that works in both species because of sequence conservation. Samples were normalized to GAPDH and then normalized again to one of the control samples. The relative quantification 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, 100 ng/mL IL-17A, 100 ng/mL IL-17F, 100 mmol/L GSK 650394, or a combination of the agents. Thirty minutes before uptake, 0.1 mmol/L 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 0.1 mmol/L 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 mmol/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 by Arroyo et al.

Statistics

Data are expressed as mean±SEM. T tests, 1-way ANOVA, and 2-way ANOVA were used as appropriate. A P value of ≤0.05 was considered significant.

Results

IL-17A−/− Mice Fail to Sustain the Upregulation of Distal Sodium Transporters in Response to Chronic (4 Weeks) Ang II Infusion

After 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 mm Hg lower than Ang II–infused WT mice by 4 weeks (Figure S1 in the online-only Data Supplement). 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 time point, 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 γ 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, these data show 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 BP.

To determine if this effect was specific to the IL-17A isoform, we investigated the effect of IL-17F on BP and renal sodium transporters using IL-17F−/− deficient mice. BP in IL-17F−/− mice during chronic Ang II infusion was not blunted (Figure S1) 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 BP and distal renal sodium transporter abundance are specific to the IL-17A isoform.

**IL-17A Regulates Renal SGK1 Expression**

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.17,18 We hypothesized that SGK1 mediates the effects of IL-17A on renal sodium transporters. To test this hypothesis, we performed quantitative real-time polymerase chain reaction 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 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 mDCT15 cell line that has been previously characterized and shown to recapitulate many features of in vivo distal convoluted tubule cells.14 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 increase in NCC activity was abrogated by cotreatment with the SGK1 inhibitor, GSK 650394.

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**Figure 1.** Interleukin-17A (IL-17A) deficiency blunts the angiotensin II (Ang II)–induced increase in distal sodium transporters. Abundance of total and phosphorylated sodium–chloride cotransporter (t-NCC and p-NCC) as well as full-length (FL) and cleaved (CL) γ epithelial sodium channel (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 Ang II infusion. Fold change in transporter abundance in Ang II–infused mice compared with vehicle (Sham)-infused mice is shown below. Values are plotted as mean±SEM. Student 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).
transduction of a short-hairpin RNA targeting Nedd4-2 or a nontargeting short-hairpin RNA as control. As shown in Figure S2, the Nedd4-2 short-hairpin RNA–transduced cells expressed ≈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 time point (2 weeks) after Ang II infusion compared with WT mice. In this 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 (Figure S3). Thus, although 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

(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
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 BP and Ang II. Interestingly, IL-17A directly upregulated NHE3 protein expression by >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-α for 24 hours. Interestingly, only IL-17A caused a dose-dependent increase in SGK1 expression by quantitative real-time polymerase chain reaction (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 (Figure 4C). 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 before 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 Ang 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-hour 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, whereas 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 hours urine samples from

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**Figure 4.** Interleukin-17A (IL-17A) upregulates sodium hydrogen exchanger 3 (NHE3) in cultured human proximal tubule (HK-2) cells via a serum and glucocorticoid-regulated kinase 1 (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 glyceraldehyde 3-phosphate dehydrogenase (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 to 100 ng/mL) or tumor necrosis factor-α (TNF-α, 1 or 10 ng/mL) for 24 hours. Quantitative real-time polymerase chain reaction 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 minutes of treatment with recombinant IL-17A (100 ng/mL). Quantitative 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 minutes with vehicle or the SGK1 inhibitor, GSK 650394, at the indicated concentrations and subsequently treated without or with 100 ng/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 B and D, and a paired Student t test was used for A and C. *P<0.05, **P<0.01, ***P<0.001.
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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.3,4 However, there is also evidence that renal epithelial cells may produce their own cytokines such as IL-6, IL-8, and tumor necrosis factor-α.19–21 By real-time polymerase chain reaction, we confirmed the previously reported observation22 that HK-2 cells produce IL-17A. Interestingly, we also found that mDCT15 cells express IL-17A (Figure S4). This was not observed in mouse pulmonary artery smooth muscle cells or human aortic endothelial cells. RNA from mouse CD4+ T cells polarized toward the T-helper 17 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 BP.

Discussion

A major cause and complication of hypertension is renal dysfunction leading to increased sodium and water retention, which promotes a rise in BP and compensatory pressure natriuresis. Here, we show that the proinflammatory 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 BP 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 downregulated NHE3, whereas 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).8 BP starts to normalize in the IL-17A−/− mice after 2 weeks of Ang II infusion, reaching pressures 30 mm Hg lower than Ang II–infused WT mice by 4 weeks (Figure S1). At this 4 week time point, 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 BP 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.23 Moreover, many of the Mendelian forms of hypertension are because of mutations that influence the activity of NCC and the amiloride-sensitive ENaC. Thus, the BP reduction observed after 4 weeks of Ang II infusion in IL-17A−/− mice is probably because of 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.

Figure 5. Interleukin-17A (IL-17A)−/− mice are protected from glomerular and tubular injury in response to angiotensin II (Ang II) infusion. Wild-type (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 A. Student t test was used in 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).
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 al25 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 renin–angiotensin system.25 It is conceivable that IL-17A may be functioning in a similar manner. However, we did not see an elevation of angiotensinogen by quantitative real-time polymerase chain reaction 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 renin–angiotensin system 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.5 Limitations of this study include the use of one specific mouse strain and one model of hypertension, namely Ang 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 the IL-17A or the IL-17RA receptor have recently been developed for human use. In 2015, the Food and drug Administration 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 Food and drug Administration approval for the treatment of moderate to severe psoriasis. Our data suggest that targeting the IL-17A or the IL-17RA/RC receptor complex may be a novel therapeutic strategy for the treatment of the hypertension and the associated renal dysfunction.

Perspectives

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 in 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.

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Disclosures

None.

References

Novelty and Significance

What Is New?

- Deficiency of interleukin-17A (IL-17A) blunts activation of renal distal tubule sodium transporters, namely sodium–chloride cotransporter and epithelial sodium channel, in response to chronic angiotensin II infusion.
- Deficiency of IL-17A protects against angiotensin II-induced glomerular and tubular injury.
- IL-17A induces renal serum and glucocorticoid-regulated kinase 1 expression and phosphorylation.
- IL-17A regulates sodium hydrogen exchanger 3 in the proximal tubule and sodium–chloride cotransporter in the distal tubule in a serum and glucocorticoid-regulated kinase 1–dependent pathway.

What Is Relevant?

- Regulation of sodium transporters in the kidney by IL-17A seems 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 serum and glucocorticoid-regulated kinase 1 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.
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INTERLEUKIN 17A REGULATES RENAL SODIUM TRANSPORTERS AND RENAL INJURY IN ANGIOTENSIN II-INDUCED HYPERTENSION

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Running Title: IL-17A modulates renal sodium transporters
METHODS

**Nedd4-2 immunoblotting** - Proteins were transferred electrophoretically to PVDF membranes. After blocking with 3% BSA, the membranes were probed with corresponding primary antibodies (anti Nedd4-2 antibody (Abcam, Cambridge, UK, 1:500), anti-actin (Sigma, 1:1000)) and incubated overnight. The blots were washed in TBST. Signal detection for Nedd4-2 and actin was done using IRDye800 rabbit anti-goat IgG antibody, (Rockland immunochemicals, dilution 1:10000) and subsequent scanning of the membrane by the Odyssey Infrared Imager. Intensity of the protein bands was analyzed by using Odyssey Infrared Imaging Software (Li-Cor Biosciences).
SUPPLEMENTAL FIGURES

**S1:** Tail cuff blood pressures at baseline and during 28 days of Ang II infusion (490 ng/kg/min) in WT, IL-17A\(^{-/-}\), and IL-17F\(^{-/-}\) mice. Two-way ANOVA followed by the Holm-Sidak post-hoc test was performed. **\(P<0.01\) vs WT (n=5-13 per group).

**S2:** Representative immunoblot showing amount of NEDD4-2 protein in mDCT15 control cells (C), mDCT15 cells transducted with a non-targeting shRNA (NT), and mDCT15 cells transducted with an shRNA targeting NEDD4-2 (N4). Actin was used as a loading control. Quantification of all blots relative to actin and normalized to control is shown on the right. Repeated measures one-way ANOVA followed by the Newman-Keuls post-hoc test was used. ***\(P<0.001\) (n=4).
**S3**: Abundance of NHE3 in WT and IL-17A−/− mice after 4 weeks of vehicle (Sham) or Ang II infusion was analyzed by immunoblotting in whole renal tissue homogenates. Quantification data are reported under the blots 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).

**S4**: Representative RT-PCR gel for IL-17A in mDCT15 and HK-2 cells. Mouse Th17 polarized CD4 T cells were used a positive control. IL-17A mRNA was not detected in 2 other cell lines, mouse pulmonary artery smooth muscle cells (SMCs) and human aortic endothelial cells. 18s was used as a control for RNA integrity.