Kidney

Deficiency of the Interleukin 17/23 Axis Accelerates Renal Injury in Mice With Deoxycorticosterone Acetate+Angiotensin II–Induced Hypertension


Abstract—T cells participate in angiotensin II (Ang II)–induced hypertension. However, the specific subsets of T cells that are important in the end-organ damage are unknown. T-helper 17 cells are a recently identified subset that produces interleukin 17 (IL-17) and requires interleukin 23 (IL-23) for expansion. To evaluate the role of the T-helper 17 immune response in hypertensive renal and cardiac end-organ damage, hypertension was induced with deoxycorticosterone acetate (DOCA)+Ang II in wild-type (n=39) and IL-17–deficient (n=31) mice. The injury was evaluated at day 4 and day 14. To inactivate the IL-17/IL-23 axis at a different point, DOCA+angiotensin II hypertension was also induced in IL-23p19–deficient mice. Renal infiltration by T-helper 17 cells was increased in hypertensive wild-type mice. Systolic blood pressure did not differ between hypertensive IL-17–deficient and wild-type mice. Three days after induction of hypertension, a significantly higher albuminuria was found in IL-17–deficient than in wild-type mice (196±64 versus 58±16 mg/mg albumin/creatinine). Histology revealed significantly more glomerular injury (1.04±0.06 versus 0.67±0.05) and renal infiltration of γδ T cells in IL-17–deficient than in wild-type mice after 14 days. Similarly, significantly higher albuminuria, glomerular injury, and γδ T cell infiltration were found in IL-23p19–deficient mice with DOCA+Ang II–induced hypertension. DOCA+Ang II also induced cardiac damage as assessed by heart weight, cardiac fibrosis, as well as expression of fetal genes and matrix components, but no significant differences were found among IL-17–/–, IL-23p19–/–, and wild-type mice. IL-17/IL-23 deficiency accelerates DOCA+Ang II–induced albuminuria and hypertensive renal but not cardiac end-organ damage. (Hypertension. 2014;63:565-571.) ● Online Data Supplement

Key Words: albuminuria ■ angiotensin II ■ deoxycorticosterone acetate ■ interleukin-17 ■ interleukin-23

Recent data suggest that hypertension and hypertensive end-organ damage are not only mediated by hemodynamic injury but also by innate and adaptive immune responses.1 In their seminal article, Guzik et al2 were able to show that RAG-1–/– mice that lack T and B cells have attenuated hypertension in response to angiotensin II (Ang II) infusion. This finding was confirmed in SCID mice.3 In 2005, a novel T-helper cell subset (Th17) producing interleukin 17 (IL-17) was discovered.4 IL-17 is a proinflammatory cytokine secreted by innate and adaptive immune cells. Although the source of IL-17 is restricted to hematopoietic cells, the IL-17 receptor is widely expressed. Th17 cells need interleukin 23 (IL-23) for expansion and survival. IL-23 is secreted by activated dendritic cells and macrophages. The potential function of Th17 cells in autoimmune disease was first shown in IL-23p19–deficient mice. IL-23p19 knockout animals demonstrated a substantial decrease in Th17-polarized cells and were resistant to the development of experimental autoimmune encephalomyelitis,5 experimental induction of multiple sclerosis, and rheumatoid arthritis. A new link between cardiovascular disease and IL-17 has been proposed by recent data showing that increased dietary salt intake drives autoimmune diseases through the induction of Th17 cells.6

C57black mice are resistant to hypertensive end-organ damage.7 We recently showed that combining deoxycorticosterone acetate (DOCA) salt and Ang II infusion induces substantial hypertensive renal and cardiac injury.8 This model has been successfully used to evaluate the role of chemokine receptors and ADMA (asymmetric dimethylarginine) in hypertensive end-organ damage.9,10 Therefore, we used DOCA+Ang II in IL-17–/– and IL-23p19–/– mice to elucidate the role of the IL-17/IL-23 axis

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in hypertensive end-organ damage. Our data show that deficiency of the IL-17/IL-23 axis aggravates albuminuria and renal but not cardiac damage in hypertensive mice.

Methods

Details on knockout mice and methods have been described by us. A full description of animals and methods can be found in the online-only Data Supplement.

Results

Detection of Th17 Cells in Hypertensive Mice

In a first step, we analyzed whether Th17 cells are detectable in the kidneys of hypertensive mice. Renal T cells were isolated, and intracellular cytokine staining with subsequent fluorescence-activated cell sorter analysis revealed that 0.94% of the infiltrating CD4+ cells in normotensive mice produced IL-17, whereas this was increased to 2.88% in hypertensive mice (Figure 1).

Early Renal and Cardiac Injury

DOCA+Ang II induced heavy albuminuria already 3 days after the start of Ang II infusion. The albuminuria was significantly higher in IL-17−/− mice than in wild-type mice (Figure 2A). We next examined the kidney injury at day 4 after starting Ang II infusion. No hypertensive glomerular changes were found at this early time point in 9 DOCA+Ang II–treated wild-type mice, whereas 3 of 5 IL-17−/− mice showed glomeruli with segmental sclerosis as shown in Figure 2B (arrows). Basal data of the early time point are shown in Table S1 in the online-only Data Supplement. Glomerular size was increased in both hypertensive groups compared with controls, but no difference was found between IL-17−/− and wild-type mice. No significant difference was found for urea-N at day 4. Staining for CD3 and F4/80 showed increased renal infiltration of T cells and macrophages in both hypertensive groups without a difference between IL-17−/− and wild-type mice. Furthermore, the increase in GR-1+ neutrophils in both hypertensive groups was not significant (Figure S1). Recently, increased albuminuria in response to Ang II was found in interferon γ receptor knockout mice and was explained by disturbed autophagy in podocytes. In the present study, an upregulation of the autophagosomal/lysosomal system was observed in podocytes of DOCA+Ang II mice by immunohistochemical stainings for LC3 and Limp2, but no difference between both genotypes was noted (Figure S2).

The heart is another important target of hypertension. DOCA+Ang II induced extensive cardiac fibrosis already 4 days after starting Ang II infusion as shown in Figure 2C. The area stained blue greenish was significantly larger in DOCA+Ang II mice than in normotensive controls, suggesting fibrosis with concomitant loss of cardiomyocytes. Scoring of the fibrosis revealed an increase in hypertensive mice (Figure 2D). The expression of fetal genes such as ANP (atrial natriuretic peptide) and the ratio of myosin heavy chain isoforms, as well as collagen 1, were also upregulated in hypertensive mice. However, no significant difference between IL-17−/− and wild-type mice was found for fibrosis or gene expression (Figure 2D and Figure S3).

Day 14 Renal and Cardiac Injury

DOCA+Ang II increased systolic blood pressure in IL-17−/− and wild-type mice, with no difference between the 2 groups as shown in Figure 3A. With longer duration of Ang II infusion, the initial difference in albuminuria between IL-17−/− and wild-type mice was not maintained as shown in Figure 3B. However, a higher number of proteinaceous casts and higher plasma cholesterol levels were found in IL-17−/− mice (Figure 3C and 3D). The increased cholesterol levels indicate that proteinuria was in the nephrotic range. The significantly higher cholesterol levels in IL-17−/− mice reflect the increased albuminuria found at the early time point. An increased mortality was found in hypertensive mice in both genotypes (Table S2). Semiquantitative analysis of glomerular injury by scoring revealed higher injury scores in IL-17−/− compared with wild-type mice (Figure 3E). DOCA+Ang II induced hypertensive focal and segmental glomerular injury as shown in Figure 3F. Renal function was decreased in both hypertensive groups as indicated by increased BUN (blood urea nitrogen) levels compared with normotensive controls (27±2 mg/dL). BUN was significantly higher in IL-17−/− (54±8 mg/dL) mice than in wild-type mice (39±2 mg/dL; P<0.05). The renal injury observed in hypertensive mice was accompanied by an increased infiltration of F4/80+ macrophages and GR-1+ neutrophils. Although significantly more neutrophils were found in IL-17−/− compared with wild-type mice, no significant difference was found between both genotypes for macrophage infiltration (Figure S4A and S4B). No regulation was found for the IL-17 receptors a and c in the kidney, and no difference was found for the renal regulation of vascular cell adhesion protein 1 and IL-17F between knockout and wild-type mice (Figure S4C–S4F). The enhanced renal injury in the knockout mice was accompanied by a significantly increased infiltration of CD3+ T cells (Figure 3G). The number of renal Foxp3+ cells and renal expression of Foxp3 were not statistically significantly different between both genotypes (Figure S4G and S4H). Next, we isolated lymphocytes from the kidney to analyze further the subsets infiltrating the kidney in hypertension. No significant differences were found between hypertensive wild-type and IL-17−/− mice for CD3, CD4, CD8, natural killer T, and natural killer cells as shown in Figure S4I. In contrast, an increased number of γδ T cells was found in the kidney of IL-17−/− compared with wild-type mice (Figure 3H and Figure S4J).
Figure 2. Early renal and cardiac injury. A, Three days after start of angiotensin II (Ang II) administration, heavy albuminuria was found in hypertensive mice. Albuminuria was significantly higher in interleukin 17 (IL-17)−/− than in wild-type mice. B, Early glomerular changes are shown in PAS (periodic acid schiff)-stained sections. No glomerular changes were found in hypertensive wild-type mice, whereas in IL-17−/− mice glomeruli showed already hypertensive injury (arrows). C, Masson trichrome staining (25- and 100-fold magnification) showed myocardial fibrosis in IL-17−/− and wild-type mice already after 4 days. D, Scoring revealed no significant difference between both hypertensive groups (*P<0.05). DOCA indicates deoxycorticosterone acetate.
No difference in TH1 response and virtually no TH2 response as assessed by intracellular staining of renal T cells for interferon γ and IL-13 were found (Figure S4K). In addition, we also analyzed renal macrophages and dendritic cells from the kidney. We found no differences between hypertensive wild-type and IL-17–deficient mice for the activation markers F4/80, Ly6C, and myosin heavy chain II in both cell types (Figure S5).

Relative heart weight was increased in hypertensive mice, with no significant difference between hypertensive IL-17–deficient and wild-type mice (Figure S6A). Scoring of cardiac fibrosis revealed no significant difference between IL-17–deficient and wild-type hypertensive mice (Figure S6B). Cardiac injury was also assessed by measuring the expression of ANP, myosin heavy chain isoform ration, and collagen type I. All parameters were upregulated in hypertensive mice, but no significant differences between IL-17–/– and wild-type mice were found (Figure S6C–S6E).

**Interleukin 23p19**

Because IL-23 is important for Th17 expansion and survival, we induced hypertension by DOCA+Ang II in IL-23p19

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**Figure 3.** Blood pressure and late renal injury. **A**, Deoxycorticosterone acetate (DOCA)+ angiotensin II (Ang II) increased blood pressure with no difference between the interleukin 17 (IL-17)–/– and wild-type mice. **B**, No significant difference for albuminuria was found at the end of the experiment. **C** and **D**, Histology and plasma analysis still showed a significantly higher number of proteinaceous cast and cholesterol plasma levels in IL-17–/– than in wild-type mice. **E**, Semiquantitative analysis of glomerular injury by scoring reveals significantly more injury in IL-17–/– compared with wild-type mice. **F**, Representative micrographs of the hypertensive glomerular injury are shown. **G**, Quantification of CD3+ cells by immunohistochemistry revealed a higher number in IL-17–/– mice. **H**, Quantification of intrarenal γδ T cells showed an increased number in the knockout mice (*P*<0.05, **P*<0.01, ***P*<0.001).
knockout mice. As shown in Figure 4A, significantly higher albuminuria was found in IL-23p19 knockout mice compared with wild-type mice at the early time points. Examination of the kidney revealed significantly more glomerular injury and more proteinaceous casts in knockout compared with wild-type mice (Figure 4B and 4C). Plasma cholesterol was significantly higher in knockout than in wild-type mice (Figure 4D). To evaluate the role of Foxp3+ regulatory T cells in the renal injury, the number of infiltrating Foxp3+ cells was evaluated by immunohistochemistry, and Foxp3 expression was measured by real-time polymerase chain reaction in the kidney. Micrographs of Foxp3+ cells in the kidney are shown in Figure S7A. Counting of these cells revealed a lower number in IL-23p19−/− mice compared with wild-type mice, but this difference was not significant (Figure S7B). In contrast, a significantly lower renal expression of Foxp3 was found in hypertensive IL-23p19−/− compared with wild-type mice (Figure S7C). No difference was found for IL-17F between hypertensive IL-23p19−/− and wild-type mice (Figure S7D). Similar to the IL-17−/− mice, also in hypertensive IL-23p19−/− mice the number of renal γδ T cells was significantly higher than in wild-type mice (Figure S7E). Furthermore, similar to the IL-17−/− mice, no significant difference was found for blood pressure (controls, 89±4 mm Hg; wild-type DOCA+Ang II, 125±2 mm Hg; IL-23p19−/− DOCA+Ang II, 120±4 mm Hg). No difference was found for cardiac fibrosis and injury between both genotypes (Figure S8A–S8D).

Finally, we ruled out that injury or inflammation is already present under basal conditions. Representative micrographs, scoring of glomerular changes, and immunohistochemistry data are shown in Figure S9. In addition, a detailed fluorescence-activated cell sorter analysis of lymphocytes, as well as macrophages isolated from the kidney, revealed no difference between wild-type and IL-17−/− mice under homoeostatic conditions as shown in Figures S10 and S11.

Discussion

The adaptive and innate immune systems are increasingly recognized to play a role in arterial hypertension– and hypertension-induced end-organ damage. T cells have been shown to play an essential role in the development of hypertension. The specific subsets of T cells that are important in hypertensive end-organ damage are not known. Recent data suggest that IL-17 may play an important role in hypertension and might be a therapeutic target. C57black mice serve as the background strain for most knockout mice. However, a detailed study of hypertensive end-organ damage in response to Ang II infusion is not feasible in C57black mice because these mice do not develop glomerular injury or albuminuria in response to Ang II alone. Hence, we recently developed a model of hypertensive end-organ damage by combining DOCA salt and Ang II infusion in C57black mice. This model is particularly helpful to study the role of the immune system in hypertensive end-organ damage because suppression of the immune system might be useful in patients with massive end-organ damage.

The current study provides novel insights into the role of the IL-17/IL-23 axis in hypertensive end-organ damage. In our model, we could demonstrate renal recruitment of Th17 cells. To our surprise, IL-17 deficiency increased albuminuria, and hypertensive glomerular injury was higher in the kidneys of IL-17−/− than in wild-type mice. Similar effects were observed in IL-23p19−deficient mice, which strongly supports a protective role of the IL-17/IL-23 axis in the development of hypertensive kidney injury. The precise underlying mechanisms driving the adverse effect of IL-17/IL-23 deficiency remain unidentified to date. We detected expression of the IL-17a and c receptors in the kidney. Therefore, it is likely that IL-17 may influence infiltrating and resident cells present in the kidney. IL-17 may positively affect epithelial cell survival or maintain the integrity of the glomerular filtration barrier. Autophagy in podocytes influences glomerular disease susceptibility. Although an increased autophagy was found in the present study, no difference was detected between both genotypes.

γδ T cells are a population of cells that express the T cell receptor γ-δ chains. By bridging innate and adaptive immunity, the proinflammatory function of γδ T cells has attracted...
attention only in recent years. These cells have not been examined in hypertension. Interestingly, an increased number of γδ T cells was found in IL-17− and IL-23p19−deficient mice. γδ T cells have antigen-presenting function and can secrete cytokines such as interferon-γ, IL-6, and perforin. The enhanced infiltration of γδ T cells could be one of the mechanisms causing the enhanced renal injury in the IL-17−/− mice. γδ T cells show rapid, innate-like responses that place them often in the initiation phase of immune reactions. This underpins a possible role of these cells because the difference in albuminuria between wild-type and IL-17−/− mice was pronounced in the beginning of Ang II infusion. Regulatory T cells are protective in hypertension. A lower expression of Foxp3 in IL-23p19−/− mice may suggest less inflammatory capacity of the regulatory T cells in the kidney. This significant decrease was not found in IL-17−/− mice.

The dogma that Th17 is the major pathogenic cell subtype has been challenged. IL-17 and IL-23 may have either pathogenic or protective role in diseases. For example, IL-17 deficiency aggravated experimental colitis. In atherosclerosis, aggravating and protective effects of IL-17 deficiency have been described. In glomerulonephritis, a biphasic response with protection against renal injury in the early phase and aggravation in the later phase was found in IL-17−/− mice. A protective effect of IL-17 has also been suggested in aortic aneurysm formation, and low circulating IL-17 levels were associated with a higher cardiovascular risk after myocardial infarction.

In line with renal end-organ damage, we could demonstrate cardiac injury in hypertensive mice. However, neither IL-17 nor IL-23p19 deficiency changed the degree of cardiac injury. This indicates that the enhanced hypertensive end-organ damage caused by the absence of the IL-17/IL-23 axis is specific for the kidney.

Our data are on a first view not in line with the recent data from Nguyen et al. Although these authors found that daily injection of IL-17 causes endothelial dysfunction and hypertension, as well as beneficial effect of IL-17 deficiency in response to Ang II infusion, we describe an aggravation in response to DOCA+Ang II. Madhur et al. found less vascular dysfunction in response to Ang II in IL-17−/− deficient mice. Although the initial hypertensive response to Ang II was similar in IL-17−/− and wild-type mice, blood pressure was after 4 weeks lower in IL-17−/− than in wild-type mice. The difference to the work of Nguyen et al. could be explained by the fact that IL-17 was given alone and was not examined in the combination with Ang II and DOCA salt. The difference to the study of Madhur et al. could be explained by the administration of the aldosterone analog DOCA and salt in our mice. Aldosterone promotes autoimmune damage by enhancing Th17-mediated immunity. Furthermore, recent data suggest that salt drives autoimmune disease by induction of Th17 cells. Taken together, these data suggest a mechanistic link among Ang II, aldosterone, and IL-17 production.

Perspectives

Our data underscore the complexity of the immune mechanisms in hypertensive end-organ damage. Shutting off the IL-17/IL-23 axis does not necessarily result in a protective response. IL-17 and IL-23 deficiency aggravates hypertensive renal injury, whereas the cardiac damage is not influenced. Future studies are needed to determine whether targeting the IL-17/IL-23 axis will be useful or harmful to treat cardiovascular disease. In addition, patients enrolled in clinical trials testing inhibitors of IL-17 pathways should be closely monitored for change in renal function.

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Disclosures

None.

References


**Novelty and Significance**

**What Is New?**

- The current study provides novel and unexpected insights into the role of the interleukin 17 (IL-17)/interleukin 23 (IL-23) axis during hypertensive end-organ damage. IL-17 and IL-23p19 knockout mice have an increased renal injury in response to deoxycorticosterone acetate+angiotensin II. In contrast, neither IL-17 nor IL-23p19 deficiency affects the cardiac injury found in the deoxycorticosterone acetate+angiotensin II-induced hypertension.

**What Is Relevant?**

- These observations suggest a protective role for the IL-17/IL-23 axis in this model of arterial hypertension. Future studies are needed to determine whether targeting the IL-17/IL-23 axis will be useful to treat cardiovascular disease.

**Summary**

Our data underscore the complexity of the immune mechanisms in hypertensive end-organ damage. Shuttling off the IL-17/IL-23 axis does not necessarily result in a protective response. IL-17 and IL-23 deficiency aggravate hypertensive renal injury, whereas the cardiac damage is not influenced.
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