Myeloid CD11c^{+} Antigen-Presenting Cells Ablation Prevents Hypertension in Response to Angiotensin II Plus High-Salt Diet

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See Editorial Commentary, pp 557–558

Abstract —Increasing evidence shows that antigen-presenting cells (APCs) are involved in the development of inflammation associated to hypertension. However, the potential role of APCs in the modulation of renal sodium transport has not been addressed. We hypothesized that APCs participate in renal sodium transport and, thus, development of high blood pressure in response to angiotensin II plus a high-salt diet. Using transgenic mice that allow the ablation of CD11c^{high} APCs, we studied renal sodium transport, the intrarenal renin–angiotensin system components, blood pressure, and cardiac/renal tissue damage in response to angiotensin II plus a high-salt diet. Strikingly, we found that APCs are required for the development of hypertension and that the ablation/restitution of APCs produces rapid changes in the blood pressure in mice with angiotensin II plus a high-salt diet. Moreover, APCs were necessary for the induction of intrarenal renin–angiotensin system components and affected the modulation of natriuresis and tubular sodium transporters. Consistent with the prevention of hypertension, the ablation of APCs also prevented cardiac hypertrophy and the induction of several indicators of renal and cardiac damage. Thus, our findings indicate a prominent role of APCs as modulators of blood pressure by mechanisms including renal sodium handling, with kinetics that suggest the involvement of tubular cell functions in addition to the modulation of inflammation and adaptive immune response. **(*Hypertension*. 2018;71: 709-718. DOI: 10.1161/HYPERTENSIONAHA.117.10145.)** • **Online Data Supplement**

Key Words: angiotensin II ■ antigen-presenting cells ■ epithelial sodium channel ■ hypertension ■ inflammation

Studies during the past years have shown that inflammation contributes to the elevation of blood pressure and end-organ damage in hypertension.[1–4] The activation of adaptive immunity[5–9] in hypertension caused by angiotensin II (AngII), mineralocorticoids, or high-salt diet[5,6] suggests that antigen-presenting cells (APCs) might play a pathogenic role.

Dendritic cells (DCs) are professional APC characterized by their expression of CD11c (α_X integrin), which prime/activate naive T lymphocytes, thus triggering adaptive immunity. Of all APCs, DCs are particularly abundant in the kidney, forming a cellular network confined to the tubule interstitium.[10] Recent studies addressing the role of DCs in hypertension have shown that AngII infusion in mice increased activated splenic DCs. Accordingly, the same authors showed that blocking antigen presentation by DCs ameliorated hypertension in response to AngII or mineralocorticoids plus a high-salt diet.[11] In addition, neoantigens generated on hypertension may activate DCs obtained from hypertensive mice,[12,13] inducing the production of proinflammatory cytokines and reactive oxygen species.[3,4,12,13] Thus, the available evidence indicates that DCs contribute to the inflammatory response associated to hypertension. However, the potential role of DCs and other APCs in the modulation of natriuresis and the increase of blood pressure has not been addressed.

Increased dietary salt intake or arterial pressure leads to a rapid elevation of urinary sodium excretion, allowing the homeostatic control of arterial pressure.[14,15] This homeostatic response depends on the renin–angiotensin system (RAS); when the RAS activity is fixed, for example, by the infusion of AngII, renal sodium excretion is hampered and a new steady state characterized by high blood pressure is established. We hypothesized that, in addition to their role orchestrating the

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immune response and inflammation in RAS-dependent hypertension, APCs may influence urinary sodium excretion.

The goal of the present study was to test whether the ablation of CD11c$^+$ APCs changes blood pressure, renal sodium reabsorption, intrarenal RAS (iRAS), cytokines, tissue damage, and inflammation in mice given AngII infusion plus a high-salt diet (AngII+salt). We compared control wild-type (WT) mice with transgenic mice in which the diphtheria toxin (DT) receptor is expressed under the control of the CD11c gene promoter (CD11c.DOG).[4,16] The cell-specific expression of the DT receptor allowed the depletion of CD11c$^+$ APCs over prolonged periods of time by multiple injections of DT. To confirm the role of CD11c$^+$ APCs, we interrupted DT injections, which resulted in replenishing of CD11c$^+$ APCs in AngII+salt CD11c.DOG mice. A complementary approach to analyze the effect of recovering CD11c$^+$ APCs function was the adoptive transfer of CD11c$^+$ APCs from WT animals into CD11c.DOG mice under AngII+salt+DT treatment. Using these gain- and loss-of-function approaches, we analyzed the relevance of CD11c$^+$ APCs ablation on blood pressure, heart hypertrophy, markers of renal and cardiac oxidative stress, inflammation, and fibrosis after AngII+salt treatment. We also performed physiological studies of renal sodium handling and analyzed whether expression of renal sodium transporters and components of the iRAS modulated by the AngII+salt treatment was somehow affected by the ablation of CD11c$^+$ APCs.

Methods

The data and materials that support the findings of this study are available from the corresponding author on reasonable request.

Animals

The Bioethics Committee on Animal Research of Facultad de Medicina, Universidad de Chile, approved the protocols for animal experimentation according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (protocol CBA-079-FMUCH). Ten- to 12-week-old male mice were used for all experiments. CD11c.DOG mice were kindly donated by Drs Natalio Garbi and Günter Hämmerling (Deutsches

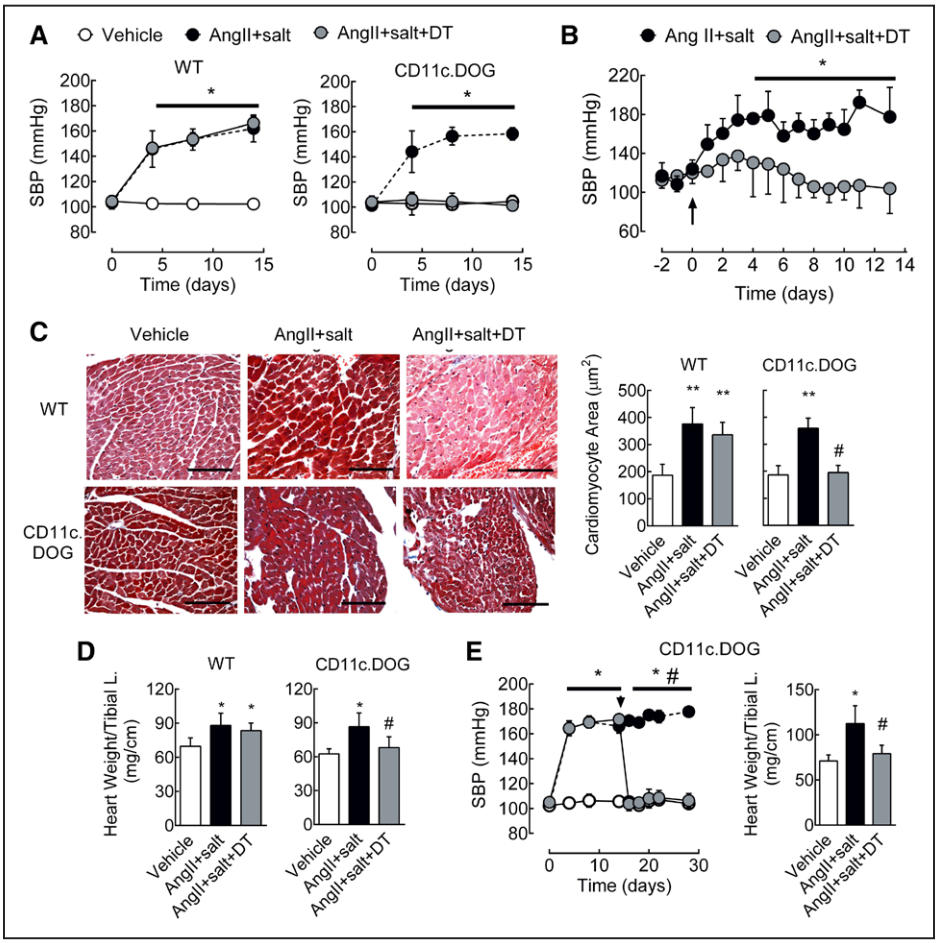

Figure 1. CD11c$^+$ antigen-presenting cells (CD11c$^+$ APCs) ablation in CD11c.DOG mice prevented the development of hypertension and cardiac hypertrophy. **A**, Time course of systolic blood pressure (SBP) determined by tail-cuff method in wild-type (WT, **left**) and CD11c.DOG (**right**) mice treated with vehicle (open circles), angiotensin II (AngII)+salt (black circles) or AngII+salt+diphtheria toxin (DT; gray circles). **B**, Time course of morning SBP (telemetric recordings) of CD11c.DOG mice before and after treatment with AngII+salt (black circles) or AngII+salt+DT (gray circles). The arrow indicates the beginning of treatments. **C**, Cardiomyocyte cross-sectional area in the 6 groups of mice, hematoxylin–eosin staining; magnification=×400; scale bar 100 μm (**left**). Cardiomyocyte area values measured in hearts of WT (**left** graph) or CD11c.DOG mice (**right** graph) presented as mean±SD. **D**, Cardiac hypertrophy of WT (**left** graph) and CD11c.DOG (**right** graph) mice treated with vehicle (white bar), AngII+salt (black bar), or AngII+salt+DT (gray bar). **E**, Time course of SBP of CD11c.DOG mice after treatment with vehicle (open circles), AngII+salt (black circles), or AngII+salt+DT (gray circles) by 28 d, and cardiac hypertrophy of CD11c.DOG (right graph) mice treated with vehicle (white bar), AngII+salt (black bar), or AngII+salt+DT (gray bar) after 28 d. For all experiments, n=4 animals per group. *P<0.05 vs vehicle; **P<0.01 vs vehicle, #P<0.05 vs AngII+salt.

Krebsforschungszentrum, Heidelberg, Germany).[17] WT and OVA-specific OT-I transgenic mice expressing specific T-cell receptors for H-2K^b/OVA$_{257-264}$, were purchased from The Jackson Laboratory (Bar Harbor, ME). CD11c.DOG, OT-I, and WT mice were in the C57BL/6 genetic background and were housed according to institutional guidelines at the specific pathogen-free animal facility of the Fundación Ciencia & Vida.

Statistical Analyses

Results are expressed as mean±SD or mean±SEM for each experimental group. Comparisons between 2 groups were made by Student *t* test, whereas comparisons between ≥3 experimental groups were made using 1-way ANOVA and Tukey post hoc test, all tests were 2-tailed, and statistical significance was considered with a *P* value <5% ($P<0.05$). All statistical analyses were done using GraphPad Prism 6.0 software (GraphPad Software Inc, La Jolla, CA).

A detailed Materials and Methods section is given in the online-only Data Supplement.

Results

CD11c+ APCs Ablation in CD11c.DOG Mice Prevented the Development of Hypertension in Response to AngII+Salt

AngII+salt caused a rapid and sustained increase of systolic blood pressure (SBP) both in WT and in CD11c.DOG mice (Figure 1A). Simultaneous administration of DT with AngII+salt to WT mice did not modify the increase of SBP. However, DT prevented the increase of SBP in AngII+salt-treated CD11c.DOG mice, as confirmed by radiotelemetric blood pressure monitoring (Figures 1B; Figure S1A in the online-only Data Supplement). AngII+salt treatment induced cardiac hypertrophy and increased the cardiomyocyte cross-sectional area, both in WT and in CD11c.DOG mice (Figure 1C and 1D). Consistent with the effect on blood pressure, DT prevented cardiac hypertrophy in CD11c.DOG mice only. Also, we evaluated the effect of CD11c+ APCs ablation after 14 days of AngII+salt treatment, when hypertension was established for up to 28 days. In line with the results observed in the AngII+salt model at 14 days, the ablation of CD11c+ APCs reverted the increase of both SBP and cardiac hypertrophy (Figure 1E).

In addition, we evaluated the effect of the ablation of CD11c+ APCs in uninephrectomized mice infused with aldosterone and fed a high-salt diet (NAS). In line with the results observed in the AngII+salt model, the increase of both SBP and cardiac hypertrophy were prevented by the depletion of CD11c+ APCs (Figure S1).

AngII+Salt Treatment Did Not Modify Abundance but Induced a Proinflammatory Phenotype in CD11c+ APCs; DT Reduced CD11c+ APCs in CD11c.DOG Mice

The treatment with AngII+salt did not modify the abundance of splenic or renal CD11c+ APCs (CD45+ and CD11c^{hi}; Figure 2). DT administration to AngII+salt CD11c.DOG mice decreased CD11c^{hi} cells by >95% (Figure 2). Splenic CD11c+ APCs from AngII+salt mice showed a significant increase of CD86, a costimulatory molecule, with no significant changes in MHC-I (major histocompatibility complex class I) or in MCH-II (Figure S2A and S2B). Resident

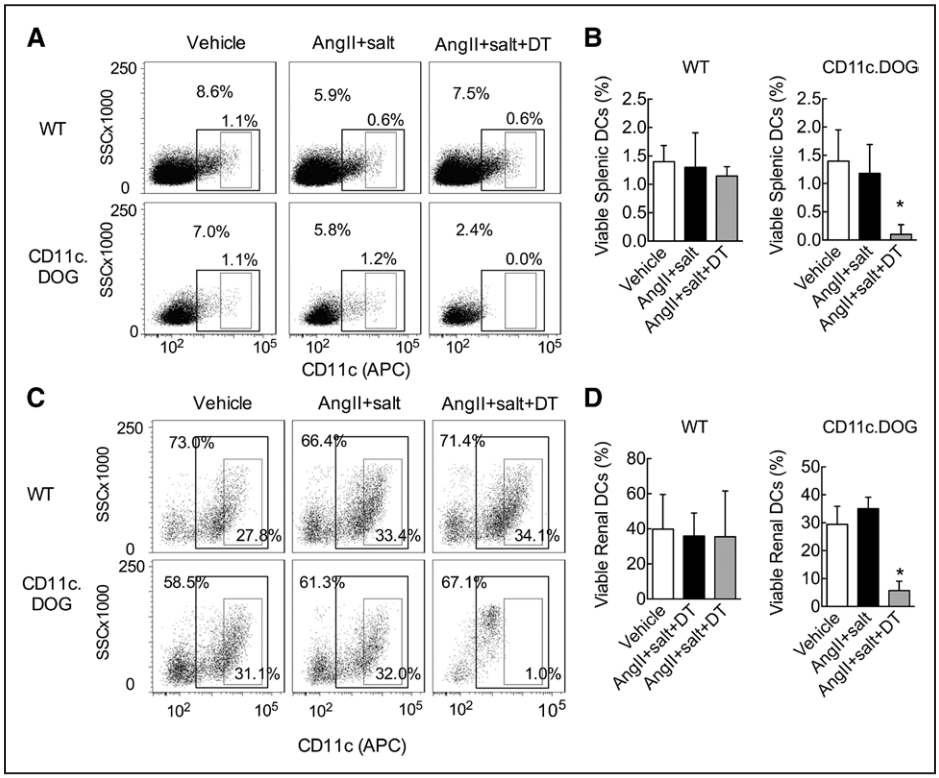

Figure 2. Angiotensin II (AngII)+salt did not modify the abundance of CD11c+ antigen-presenting cells (CD11c+ APCs), and diphtheria toxin (DT) injection eliminated CD11c^{hi} APCs in CD11c.DOG mice. Viable CD11c+ APCs were obtained from spleen (**A**, **B**) and kidney (**C**, **D**) of vehicle, AngII+salt, or AngII+salt+DT-treated mice (wild type [WT] and CD11c.DOG) at day 14. Representative dot plots of CD11c+ and CD11c^{hi} frequency from CD45+ viable cells. Values represent mean±SD, n=4 animals per group. *$P<0.05$ vs vehicle. DC indicates dendritic cell.

renal CD11c$^+$ APCs of AngII+salt-treated mice showed a small but significant increase in the expression of MHC-I, MHC-II, and CD86 (Figure S2C and S2D). The study of renal CD11c$^+$ APCs function in cocultures with naive T lymphocytes obtained from OT-I mice showed that renal CD11c$^+$ APCs obtained from AngII+salt mice caused a greater, when loaded with the OT-I peptide ($OVA_{257-264}$), increase in lymphocyte proliferation when compared with CD11c$^+$ APCs from control mice (Figure S3A). In addition, renal CD11c$^+$ APCs from AngII+salt mice increased the percentage of CD8$^+$/IFN-γ$^+$ (interferon-γ) cells (Figure S3B).

Consistent with the results described above, the mRNA of the anti-inflammatory cytokine IL-10 decreased in hearts and kidneys from AngII+salt WT and CD11c.DOG mice (Figure S4A). Similarly, AngII+salt decreased cardiac and renal transcription of FoxP3 (forkhead box P3), the master transcription factor that controls the suppressive activity of regulatory CD4$^+$ T cells (Tregs). However, administration of DT to CD11c.DOG mice prevented the decrease in IL-10 and FoxP3 mRNAs in response to AngII+salt (Figure S4A and S4B). Moreover, AngII+salt treatment decreased CD4$^+$/FoxP3$^+$ cells in kidney draining lymph nodes of WT mice; DT prevented the decrease of CD4$^+$/FoxP3$^+$ cells in CD11c.DOG mice only (Figure S4C and S4D).

CD11c$^+$ APCs Restitution Reestablished Hypertension in Response to AngII+Salt Treatment

We used 2 approaches to test whether the presence of CD11c$^+$ APCs was necessary for the development of high blood pressure and cardiac hypertrophy in mice treated with AngII+salt. In a first set of experiments, the administration of DT started along with the AngII+salt treatment; in one group of mice, we interrupted DT injections at the fourth day, whereas in another group, the DT injection continued during 14 days.

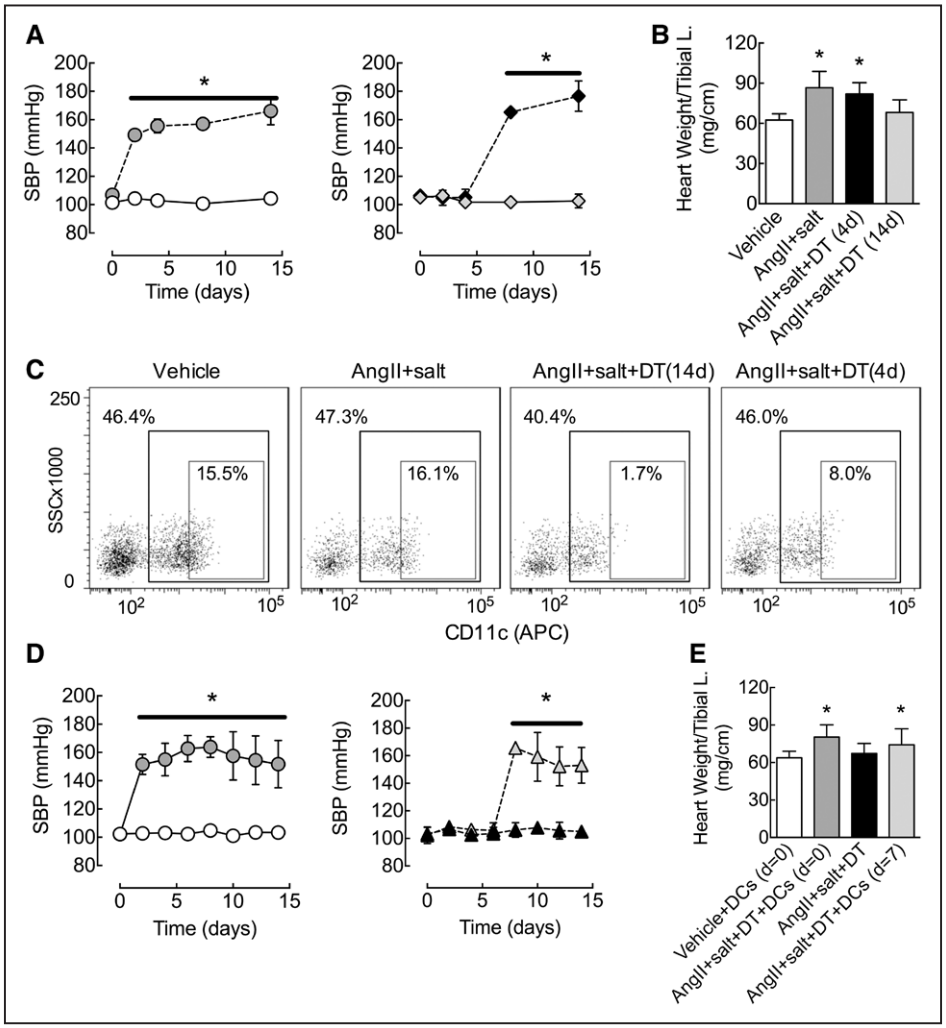

Figure 3. Restitution of CD11c$^+$ antigen-presenting cells (CD11c$^+$ APCs) reestablished hypertensive response to angiotensin II (AngII)+salt. **A**, Time course of systolic blood pressure (SBP) determined by tail-cuff method in CD11c.DOG mice treated with vehicle (open circles) or AngII+salt (gray circles). The graph on the right shows SBP of CD11c.DOG mice-treated AngII+salt+DT for 14 d (gray diamonds) or AngII+salt during 14 d and with the coinjection of diphtheria toxin (DT) until day 4 (black diamonds). **B**, Cardiac hypertrophy of CD11c.DOG mice groups of **A**. **C**, Representative dot plots of side scatter (SCC) versus CD11c$^+$ and CD11c^{hi} frequency from renal CD45$^+$ viable cells from CD11c.DOG mice groups. **D**, **Left**, SBP of AngII+salt+DT (14 d) CD11c.DOG mice (open circles) or treated with AngII+salt+DT (14 d) plus the injection of 10^6 wild type (WT) CD11c$^+$ APCs intravenously after the first injection of DT (gray circles). **Right**, SBP of CD11c.DOG mice-treated AngII+salt+DT (black triangles) or with AngII+salt+DT plus WT CD11c$^+$ APCs intravenous injection after 7 d (gray triangles). **E**, Cardiac hypertrophy of CD11c.DOG mice groups of **D**. Values represent mean±SD, n=4 animals per group. *P<0.05 vs vehicle. DC indicates dendritic cell.

The continuous administration of DT prevented the development of hypertension in response to AngII+salt (Figure 3A, left), whereas the interruption of DT injections at day 4 was followed by a rapid increase in SBP (Figure 3A, right), similar to AngII+salt mice injected with vehicle (Figure 3A, closed circles). Consistent with these results, cardiac hypertrophy was present in AngII+salt mice that received DT injections until day 4 (Figure 3B). The interruption of DT injections to AngII+salt CD11c.DOG mice reestablished renal CD11c+ APCs (Figure 3C). Of note, it has been described that the population of CD11c+ cells had completely recovered in CD11c.DOG mice 72 hours after a single injection of DT.[17] In a second set of experiments, we tested the effect of the adoptive transfer of CD11c+ APCs isolated from the spleen of WT mice to CD11c.DOG mice injected with DT. We selected 2 time points to perform the transfer of CD11c+ APCs: day 0 (Figure 3D) and day 7 (Figure 3E). The adoptive transfer of WT CD11c+ APCs to AngII+salt+DT CD11c.DOG mice at day 0 resulted in the development of high SBP, similar to control mice (Figure 3D), whereas the injection of WT CD11c+ APCs to vehicle-treated CD11c.DOG mice had no effect on SBP (Figure 3D). Moreover, the adoptive transfer of WT CD11c+ APCs to AngII+salt+DT CD11c.DOG mice at day 7 caused a rapid increase in SBP (Figure 3E). The adoptive transfer of WT CD11c+ APCs promoted cardiac hypertrophy in AngII+salt CD11c.DOG mice that received DT (Figure 3E). Thus, we concluded that CD11c+ APCs, which were recovered after DT suppression (Figure 3C), are required for the development of hypertension in response to AngII plus a high-salt diet.

CD11c+ APCs Ablation Enhanced Natriuresis

We evaluated whether CD11c+ APCs ablation improved renal sodium balance and excretion in AngII+salt mice. When compared with the control group, AngII+salt CD11c.DOG mice showed increased sodium balance, sodium intake, and natriuresis after 2 and 3 days of treatment (Figure 4A; Figure S5). The depletion of CD11c+ APCs in CD11.cDOG mice caused a less positive sodium balance, sodium intake, and further increase of natriuresis at day 3 (Figure 4A; Figure S5). We performed a saline load test (injection of 0.9% saline solution, 10% of body weight, i.p.) at 0 (basal), 4, and 14 days to evaluate further changes in natriuresis. AngII+salt WT mice presented increased excretion of the sodium load at days 4 and 14 (Figure 4B). The simultaneous administration of DT with AngII+salt to WT mice did not modify the increment of natriuresis. CD11c.DOG mice treated with AngII+salt presented a similar increase in renal sodium excretion at 4 and 14 days (Figure 4B). Interestingly, the coadministration of DT to CD11c.DOG mice caused a further increase in the renal excretion of the saline load (Figure 4B). The SBP versus natriuresis plot showed that DT injection resulted in an enhanced natriuretic response attained at lower SBP values in CD11c.DOG mice only (Figure 4C). In contrast, the injection of DT to AngII+salt WT mice did not modify natriuresis–SBP.

We also evaluated the protein abundance of renal sodium transport proteins known to respond to high AngII/iRAS activation. In WT mice, AngII+salt treatment induced a significant increase of NHE3 (sodium-proton exchanger type-3) protein

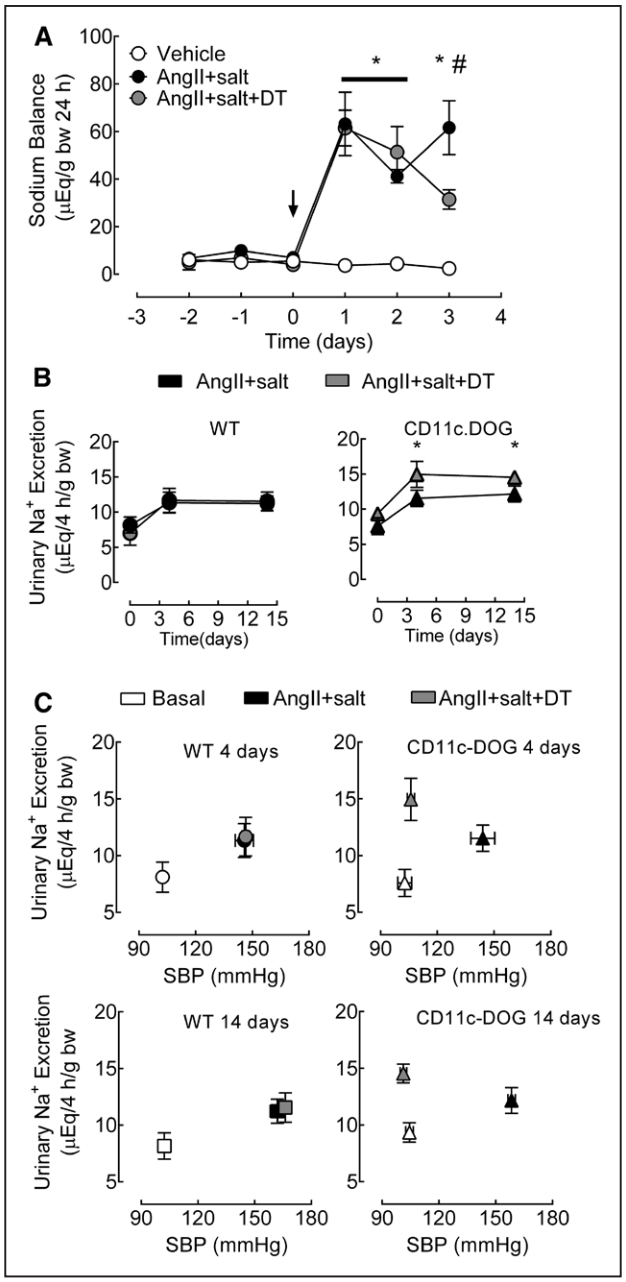

Figure 4. CD11c+ antigen-presenting cells (CD11c+ APCs) ablation enhanced natriuresis. Mice were housed individually in metabolic cages with free access to food and water before and during the angiotensin II (AngII)+salt treatment. **A**, Sodium balance (expressed as µEq Na^+ per body weight [g]/d) was measured daily 2 d before and 3 d after AngII+salt treatment. Values represent mean±SEM (n=4–6 animals per group); *P<0.05 vs vehicle; #P<0.05 vs AngII+salt. **B**, Wild-type (WT) and CD11c.DOG mice of vehicle AngII+salt and AngII+salt+diphtheria toxin (DT) groups were challenged with an intraperitoneal bolus of warmed saline equivalent to 10% of their body weight and placed in metabolic cages for urine collection at baseline, 4, and 14 d after treatment. Urinary sodium excretions are expressed as µEq Na^+ per body weight (g), excreted during a 4-h collection period. Values represent mean±SD (n=5 animals per group) *P<0.05 vs vehicle. **C**, Pressure–natriuresis relationship, calculated separately for WT and CD11c.DOG mice of all groups at 4 and 14 d after treatment. Values represent mean±SD (n=5 animals per group). SBP indicates systolic blood pressure.

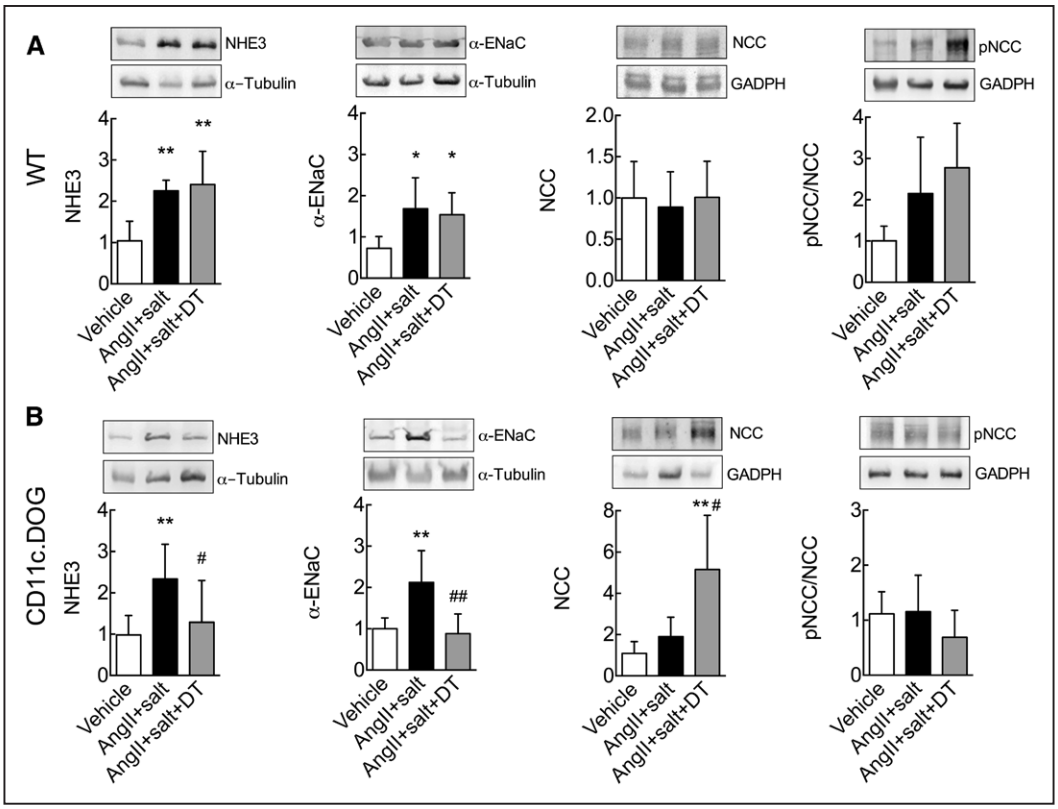

Figure 5. The ablation of CD11c⁺ antigen-presenting cells (CD11c⁺ APCs) prevented the induction of renal sodium transporters by angiotensin II (AngII)+salt in CD11c.DOG mice. Protein abundance of NHE3 (sodium-proton exchanger type-3), α-subunit of the epithelial sodium channel (α-ENaC), and total sodium chloride cotransporter (NCC) and phosphorylated NCC (pNCC) was analyzed by Western blot in whole-renal tissue homogenates from wild type (WT; **A**), and CD11c.DOG (**B**) mice after 14 d of treatment. For NHE3 and α-ENaC, α-tubulin was used as loading control, whereas NCC was normalized to GAPDH and then normalized to controls. **Upper**, Representative immunoblots, and relative abundance for each group is plotted **lower**. Data are expressed as mean±SD (n=4 animals per group); **P*<0.05 vs vehicle; ***P*<0.01 vs vehicle; #*P*<0.05 vs AngII+salt; ##*P*<0.01 vs AngII+salt. DT indicates diphtheria toxin.

(Figure 5A). Similarly, we observed a significant increase in the abundance of the α-subunit of the epithelial sodium channel (α-ENaC). AngII+salt treatment did not cause significant changes in total sodium chloride cotransporter (NCC) or phosphorylated NCC. The injection of DT did not affect the induction of NHE3 or α-ENaC in the kidney of WT mice (Figure 5A). In CD11c.DOG mice, AngII+salt treatment caused a similar increase of NHE3 and α-ENaC and also in NCC abundance. Coadministration of DT with AngII+salt ameliorated the induction of NHE3 and α-ENaC but further increased NCC. However, AngII+salt treatment alone or with DT did not affect phosphorylated NCC:NCC ratio.

Ablation of CD11c⁺ APCs Prevented the Induction of the iRAS by AngII+Salt

We evaluated next iRAS modulation in WT and CD11c.DOG mice treated with AngII+salt with/without DT injection. The transcripts of AngII receptor type-1a and the (pro)renin receptor increased their abundance in response to the AngII+salt treatment in both WT and CD11c.DOG mice (Figure 6A and 6B). However, DT injection to CD11c.DOG mice prevented the increase in the abundance of AngII receptor type-1a mRNA (Figure 6A), but had no effect on the induction of (pro)renin receptor (Figure 6B). The abundance of the renal ACE (angiotensin-converting enzyme) and AGT (angiotensinogen) protein showed an increase in response to AngII+salt treatment that was also prevented by the injection of DT in CD11c.DOG mice, but not in WT mice. These results show that AngII receptor type-1a, ACE, and AGT induction in AngII+salt mice require CD11c⁺ APCs.

Ablation of CD11c⁺ APCs Prevented the Induction of Inflammation Mediators

Previous studies have shown the increase in reactive oxygen species production and the NADPH oxidase subunits Nox2 and Nox4 by AngII and high-salt diet treatment.[18–20] Also, reactive oxygen species could be implicated in the modulation of sodium transport, iRAS activity, and inflammation. Therefore, we evaluated whether the ablation of CD11c⁺ APCs modified the profile of renal Nox2, Nox4, and inflammatory cytokines. AngII+salt treatment increased Nox2, Nox4, IL-1β, IL-6, and TNF-α (tumor necrosis factor-α) transcripts (Figure S6). The administration of DT in AngII+salt CD11c.DOG mice prevented the increase in the abundance of all these transcripts, but had no significant effects in AngII+salt WT mice (Figure S6).

Discussion

The results showed that CD11c⁺ APCs are necessary for the development of hypertension and cardiac hypertrophy in response to AngII+salt, and after nephrectomy plus aldosterone+salt treatment. In AngII+salt-treated mice, the

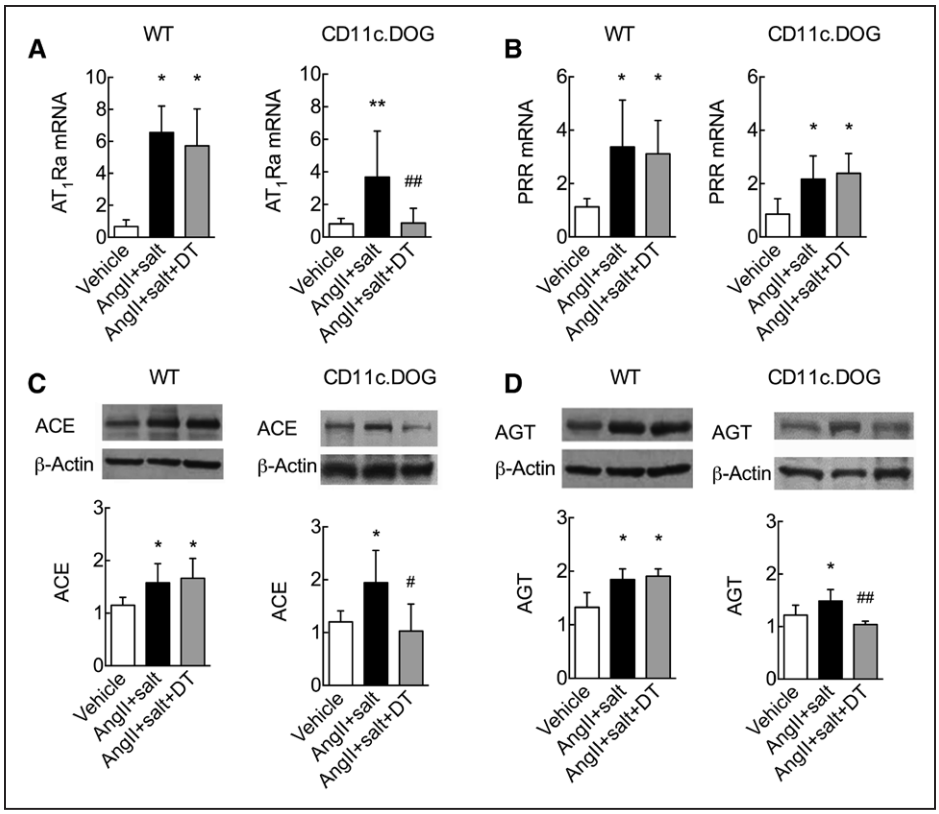

Figure 6. The ablation of CD11c+ antigen-presenting cells (CD11c+ APCs) prevented the induction of the intrarenal renin–angiotensin system (iRAS) components by angiotensin II (AngII)+salt in CD11c.DOG mice. Angiotensin II receptor type-1a (AT_1Ra; **A**) and (pro)renin receptor (PRR; **B**) mRNA abundance in wild-type (WT) and CD11c.DOG mice. Transcripts were measured by qRT-PCR; 18S rRNA was used as housekeeping gene. Data are expressed as mean±SD (n=6–8 animals per group); **P*<0.05 vs control. Angiotensin-converting enzyme (ACE; **C**) and AGT (angiotensinogen; **D**) protein abundance was measured by Western blot, β-actin was used as loading control. **Upper**, Representative immunoblots, and relative abundance for each group is plotted **lower**. Data are expressed as mean±SD (n=4 animals per group); **P*<0.05 vs vehicle; ***P*<0.01 vs vehicle; #*P*<0.05 vs AngII+salt; ##*P*<0.01 vs AngII+salt. DT indicates diphtheria toxin.

ablation/restitution of CD11c+ APCs induced rapid changes in blood pressure. Moreover, the absence of CD11c+ APCs not only prevented inflammation but also improved natriuresis and modified the expression of tubular sodium transporters of AngII+salt-treated mice. Thus, our findings show a new role of CD11c+ APCs in the physiopathology of hypertension. Previous studies showed that depletion of LysM+ cells using the LysM-CreiDT receptor mice model prevented the development of hypertension in AngII-infused mice[21,22] and that reconstitution of monocytes (CD11b+/Gr-1+) was sufficient to restore the hypertension. In this model, DT treatment eliminates a significant number of CD11c^{high} cells that may represent DCs of myelomonocytic origin.[23] Therefore, our data complement these studies by describing a population of immune cells contributing to the development of hypertension as CD11c^{high} cells of the myeloid LysM+CD11b+ origin, for example, CD11b+ DCs or a subset of monocyte-derived/inflammatory APCs.[24]

In line with previous studies,[4,12] we observed that ablation of CD11c+ APCs in AngII+salt CD11c.DOG mice prevented the induction of proinflammatory cytokines. The reduction in the abundance of CD4+FoxP3+ T cells and IL (interleukin)-10 caused by AngII+salt treatment was also prevented by CD11c+ APCs depletion in CD11c.DOG mice. These results are consistent with recent reports demonstrating the decrease of regulatory Tregs[7,16,25] and the activation of subsets of proinflammatory T cells[7,8] in response to AngII or mineralocorticoids in rodents. Surprisingly, we did not observe changes in the abundance of renal and splenic CD11c+ APCs but a marked increase in proinflammatory differentiation markers (MHC-II and CD86). Thus, we hypothesize that differentiated CD11c+ APCs may participate in neoantigens presentation and T-cell polarization during AngII+salt-induced hypertension.[12] One limitation of our study is that we did not measure changes in CD11c+ APCs in vascular tissue; therefore, their role in the development of high blood pressure and tissue damage remains to be tested. The induction of Nox2 and Nox4 caused by AngII+salt was also dependent on the presence of CD11c+ APCs. Increased reactive oxygen species production and the induction of NADPH oxidase activity are observed in the kidney of rodents in a high-salt diet,[18] and the activation of AT_1R by AngII induces Nox2 and Nox4.[19,20] Therefore, NADPH induction would be a downstream effect that requires or is potentiated by CD11c+ APCs. However, CD11c+ APCs ablation prevented the increase of blood pressure, and it is plausible that the hemodynamic effects of high blood pressure are required for increased oxidative stress and the generation of neoantigens. Thus, further studies are needed to evaluate whether other APCs could substitute neoantigens generation/presentation by CD11c+ APCs.

We observed that CD11c$^+$ APCs ablation not only prevented but also reverted hypertension, showing that CD11c$^+$ APCs have an essential pathogenic role in the elevation of blood pressure. The fast kinetics of blood pressure changes caused by the ablation or adoptive transfer of CD11c$^+$ APCs lead us to find that CD11c$^+$ APCs ablation improved natriuresis of AngII+salt mice. Cytokines and chemokines produced by macrophages/DCs[24,26] could modulate tubular sodium transport. AngII infusion induced renal IFN-γ, IL-6, and IL-17.[27–30] Mice lacking IL-6[31] or IFN-γ[32] showed ameliorated hypertension after AngII infusion and IL-17A$^{-/-}$ mice that additionally displayed preserved natriuresis in response to a saline load and decreased renal NHE3 expression after AngII infusion.[29] Recent studies showed that both IFN-γ and IL-17A production interfere with the pressure natriuretic response after AngII infusion and that IFN-γ production is necessary to activate distal sodium reabsorption.[32] Furthermore, a study using IFN-γ$^{-/-}$ mice showed an improvement of endothelial function, infiltration of proinflammatory cells, and oxidative stress compare to WT mice treated with AngII.[33] In contrast, another study using IFN-γR$^{-/-}$ mice showed no difference in the development of hypertension in AngII-infused mice compared with WT mice, although a decreased infiltration by proinflammatory cells of cardiovascular and renal tissues and diminished damage of these tissues was observed.[34] The discrepancy between these studies may arise from the fact that IFN-γ$^{-/-}$ and IFN-γR$^{-/-}$ mice exhibit differences in their inflammatory responses probably to phenotypic differences because of previously unsuspected redundancies between IFN-γ and its R1 and R2 mutations induced by the gene disruption protocol, or background genetic differences.[35,36] Our study shows that IL-1β, TNF-α, and IL-6 inductions were dependent on CD11c$^+$ APCs. In agreement with these studies, we observed that ablation of CD11c$^+$ APCs in CD11c.DOG mice prevented the upregulation of NHE3 and α-ENaC leading to a decreased capacity for tubular sodium reabsorption. Thus, we speculate that the rapid kinetics of the changes in blood pressure after elimination/restitution of CD11c$^+$ APCs could imply the prevention of the induction of cytokines that can modulate the expression/activity of sodium transport proteins in tubular cells and thus natriuresis. However, although increased protein expression of NHE3 and α-ENaC suggests increased capacity for sodium reabsorption, other regulatory mechanisms such as protein phosphorylation, proteolytic cleavage, and traffic to plasma membrane must be considered. Also, renal hemodynamic parameters such as glomerular filtration rate, renal blood flow, renal vascular resistance, and tubuloglomerular feedback that determine sodium filtered load and glomerulotubular balance may be influenced by CD11c$^+$ APCs ablation and should be addressed. Finally, osmotic balance in response to high salt intake involves a complex regulatory mechanism that is modulated by hormone fluctuation, metabolism, food consumption, water intake, and renal salt and water excretion. Therefore, the analysis of sodium and water balance in this animal model is complex and more than often difficult to interpret as recently demonstrated.[37]

It is known that plasma volume decrease or a low-sodium diet can cause a marked increase of plasma AngII, which is not associated to the upregulation of iACE or AGT expression.[18,19] In contrast, we observed that AngII plus high-salt diet induced the expression of AngII receptor type-1a, ACE, and AGT in kidney tissue. This inadequate iRAS activation was prevented by the elimination of CD11c$^+$ APCs in CD11c.DOG mice. Thus, our results suggest that inadequate systemic RAS activation and high-salt diet activate prohypertensive functions in CD11c$^+$ APCs, which may be necessary for iRAS activation. IL-6 is required for the increase in AGT production in response to AngII in proximal tubule cells,[38] and IFN-γ induced AGT production in proximal tubule cells.[39] Thus, the prevention of the induction of renal cytokines may also be implicated. Previous studies demonstrated that the expression of renal ACE is necessary to generate sufficiently high intrarenal AngII to stimulate epithelial sodium transporters. Indeed, the absence of renal ACE expression blunts the increase of blood pressure after AngII infusion or high-salt diet.[40,41] Moreover, the chronic infusion of AngII in mice that express ACE only in the kidneys caused hypertension, with blood pressure levels similar to those observed in WT mice infused with AngI.[27] Thus, the lack of renal ACE induction in AngII+salt mice with reduced CD11c$^+$ APCs may be an important factor that contributed to the protective effect of CD11c$^+$ APCs ablation.

Also, we observed that the absence of CD11c$^+$ APCs prevented the increase in the renal abundance of AGT. Previous studies have shown that the infusion of AngII stimulates intrarenal AGT expression[42] and rodents with overexpression of AGT in proximal tubule cells presented hypertension.[43] Thus, the lack of AGT upregulation in mice with reduced CD11c$^+$ APCs is a second factor contributing to the prevention of hypertension. The activation of intrarenal AT_1R by circulating and renal AngII is also a key factor for the development of hypertension in response to AngII. Mice with kidney-specific AT_1R knockout subjected to AngII infusion showed an early increase of blood pressure that was followed by a subsequent decrease of blood pressure.[20] These results demonstrated that the activation of intrarenal AT_1R is necessary to sustain hypertension in mice infused with AngII. Therefore, the prevention of the increase of renal AT_1R in CD11c.DOG mice with reduced CD11c$^+$ APCs is a third factor that contributes to the prevention of hypertension in response to AngII+salt. In summary, we hypothesize that CD11c$^+$ APCs are required to the upregulation of iRAS and inflammatory cytokines production that in turns increases sodium transporters activity leading to sodium and fluid retention and thus high blood pressure. Further studies will be necessary to clarify the causal relationship among CD11c$^+$ APCs function, iRAS activation, and the modulation of sodium transport.

Perspectives

We found that ablation of CD11c$^+$ APCs prevented the increase of blood pressure in response to AngII+salt treatment. The effect of CD11c$^+$ APCs ablation resulted from improved renal sodium excretion. According to Guyton hypothesis, salt and water retention are initial steps for the development of hypertension, and the shifting of the pressure–natriuresis curve to the left determines a new steady state where sodium balance is attained at an elevated blood pressure set point. Our results indicate that CD11c$^+$ APCs may be modulators of the blood pressure set point for renal salt and water handling through

the modulation of tubular salt transport. In the present study, we selected an experimental model that allowed testing the role of CD11c+ APCs in severe and salt-dependent hypertension. However, AngII+salt is a complex pathophysiological model, and further studies are needed to better clarify the mechanisms that mediate the action of CD11c+ APCs on renal salt and water handling and hypertension and to better clarify the role of CD11c+ APCs in other hypertensive models and in human hypertension. Recent studies have shown that inflammatory cytokines secreted by innate and adaptive immune cells, as well as renal epithelial cells, can modulate the expression and activity of sodium transporters along the nephron.[44] Our results show that ablation of CD11c+ APCs prevented the upregulation of some of these cytokines, thus providing a putative mechanism to be explored. Also, the mechanism mediating the functional change of CD11c+ APCs in vivo after the initiation of the prohypertensive treatment will need further evaluation.

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Disclosures

None.

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Novelty and Significance

What Is New?

- The ablation of CD11c$^+$ antigen-presenting cells (APCs) in CD11c.DOG mice prevented the development of hypertension, cardiac hypertrophy, and the induction of proinflammatory cytokines after angiotensin II+salt and Aldo+salt+uninephrectomy treatment. CD11c$^+$ APCs ablation prevented the induction of renal sodium transporters expression and improved natriuresis.

What Is Relevant?

- CD11c$^+$ APCs ablation was associated to a better pressure–natriuresis, preventing the shifting of the renal blood pressure set point after angiotensin II+salt treatment.

Summary

The ablation/restitution of CD11c$^+$ APCs produced rapid changes in blood pressure of mice with angiotensin II plus a high-salt diet. CD11c$^+$ APCs were required for the induction of intrarenal renin–angiotensin system components and affected the modulation of tubular sodium transporters and natriuresis. The ablation of CD11c$^+$ APCs also prevented cardiac hypertrophy and the induction of proinflammatory cytokines in response to angiotensin II plus a high-salt diet. The results show a novel role of CD11c$^+$ APCs in the modulation of renal sodium handling and indicate that CD11c$^+$ APCs are implicated in the reduction of natriuresis that characterizes hypertension.