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ORIGINAL RESEARCH

# CD1d-Dependent Natural Killer T Cells Mediate Hypertension and Vascular Injury Through Interleukin-17A

Xue Xiao, MD; Cui-Liu Hou, PhD; Yun-Long Zhang, PhD; Hui Yang, PhD; Xiao-Xiao Wang, MD; Hong-Xia Wang, PhD

**BACKGROUND:** Different T-lymphocyte subsets, including CD1d-dependent natural killer T (NKT) cells, play distinct roles in hypertension, highlighting the importance of identifying key immune cells for its treatment. This study aimed to determine the unknown effects of CD1d-dependent NKT cells on hypertension and vascular injury.

**METHODS AND RESULTS:** Hypertension models were induced in male CD1d knockout (CD1dko), wild-type, and adoptive bone marrow transfer mice by angiotensin II (Ang II) or deoxycorticosterone acetate salt. Blood pressure was measured by the tail-cuff system and radiotelemetry. Vascular injury was assessed by histologic studies or aortic ring assay. Inflammation was detected by flow cytometry, quantitative real-time polymerase chain reaction, or ELISA. Results showed that Ang II infusion significantly reduced CD1d expression and NKT cell numbers in the aorta of mice. CD1dko mice exhibited worsened blood pressure elevation, vascular injury, and inflammatory response induced by Ang II or deoxycorticosterone acetate salt. However, these effects were markedly reversed in wild-type mice treated with NKT cell–specific activator. Adoptive transfer of CD1dko bone marrow cells to wild-type mice also significantly worsened Ang II–induced responses. Mechanistically, CD1dko increased Ang II–induced interleukin-6 production and activated signal transducer and activator of transcription 3 and orphan nuclear receptor γ, subsequently inducing interleukin-17A production. Neutralizing interleukin-17A partially reversed Ang II–induced hypertension and vascular injury in CD1dko mice. In addition, levels of NKT cells were lower in the blood of patients with hypertension (n=57) compared with normotensive individuals (n=87).

**CONCLUSIONS:** These findings reveal a previously unknown role for CD1d-dependent NKT cells in hypertension and vascular injury, indicating that NKT cell activation could be a promising therapeutic target for hypertension.

**Key Words:** CD1d-dependent natural killer T cells ■ hypertension ■ interleukin-17A ■ vascular injury

Hypertension is a leading risk factor worldwide for cardiovascular disease and finally contributes to target organ damage, including heart, kidney, brain, eye, and peripheral vascular diseases. There are several underlying events in the pathogenesis of hypertension, including attenuated endothelium-dependent vasodilatation, blood vessel remodeling, and imbalanced prooxidant and antioxidant systems. However, the cause and mechanism of hypertension remain unknown, and it is now widely recognized that abnormal immune system activation and inflammation have a mechanistic effect in initiating and sustaining elevated blood pressure.[1–3] Many innate and adaptive immune cells have been defined to play a critical role in the development of hypertension.[4–8] For example, both innate immune cells, including monocytes, macrophages, dendritic cells, and myeloid-derived suppressor cells,[9,10] and adaptive immune cells, including $CD8^+$ T cells, $CD4^+$ cells (T-helper

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## RESEARCH PERSPECTIVE

### What Is New?

- Levels of natural killer T cells (a small population of innate-like T cells) were decreased in the blood of patients with hypertension, which indicated that natural killer T cell inactivation aggravates blood pressure elevation, vascular remodeling, inflammatory response, vascular dysfunction, and oxidative stress.
- Mechanistically, natural killer T cell inactivation increased interleukin-17A production by inducing interleukin-6 production and subsequently activating signal transducer and activator of transcription 3 and orphan nuclear receptor γ, whereas neutralizing interleukin-17A in CD1d knockout mice partially reversed angiotensin II–induced hypertension and vascular injury.

### What Question Should be Addressed Next?

- According to this study, the effect and prognosis of activating natural killer T cells in the treatment of essential hypertension can be verified clinically next in the future.

### Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **Ang II** | angiotensin II |
| **CD1dko** | CD1d knockout |
| **DOCA** | deoxycorticosterone acetate |
| **MCP-1** | monocyte chemoattractant protein-1; NADPH, nicotinamide adenine dinucleotide phosphate |
| **NKT** | natural killer T |
| **RORγ** | orphan nuclear receptor γ |
| **STAT3** | signal transducer and activator of transcription 3 |
| **Th** | T-helper cell |
| **Treg** | T regulatory |
| **WT** | wild type |

cells [Th] 1, Th2, Th17, and T-regulatory [Treg] cells), and B cells can promote or inhibit hypertension.[11,12] In addition, a small subset of innate-like T cells that express the γδ T-cell receptor rather than the αβ T-cell receptor is involved in angiotensin II (Ang II)–induced hypertension, vascular injury, and T-cell activation.[13] CD1d-dependent natural killer T (NKT) cells are a specialized subset of γδ T cells that are characterized by coexpression of invariant T-cell receptors and natural killer receptors; however, it is unknown whether CD1d-dependent NKT cells contribute to the development of hypertension and vascular dysfunction.

CD1d-dependent NKT cells are activated by recognizing self and foreign lipids presented on CD1d, a major histocompatibility complex class 1–like molecule primarily expressed on antigen-presenting cells.[14] Unlike natural killer cells, which are considered as innate response cells, CD1d-dependent NKT cells are classified as innate-like T cells. α-Galactosylceramide was the first identified CD1d-presented lipid antigen for CD1d-dependent NKT cells. On activation by α-galactosylceramide, CD1d-dependent NKT cells rapidly secrete large amount of various cytokine, including Th1 cytokines (tumor necrosis factor-α [TNF-α], interferon-γ, and interleukin-2), Th2 cytokines (interleukin-4, interleukin-5, and interleukin-10), and Th17 cytokines (interleukin-17 and TNF-α).[15,16] The ability of CD1d-dependent NKT cells to produce these cytokines, thereby activating other immunes cells, underscores their unique regulatory function that bridges innate and adaptive immunity.[17] CD1d-dependent NKT cells have been shown to have protective roles against pathogens and contribute to tissue homeostasis, but they also cause pathologic tissue inflammation and damage. Recently, a study showed that an alteration in the distribution of CD1d molecule, resulting from actin cytoskeletal reorganization by endoplasmic reticulum, contributes to the enhancement of CD1d-dependent NKT cell activation.[18] The selective absence of CD1d-dependent NKT cells results in an increase in plasma cholesterol level.[19] CD1d-deficient mice are more susceptible to certain bacteria, viruses, and protozoa, and they have a lower survival rate following influenza virus infection.[20,21] In addition, infiltration of CD1d-dependent NKT cells increases in the heart after ischemia/reperfusion or infarction, and activation of CD1d-dependent NKT cells improves myocardial ischemia/reperfusion injury, postinfarct cardiac remodeling, and failure.[22,23] Our previous study also found that CD1d-dependent NKT cells have a protective role against Ang II–induced cardiac remodeling.[24] Therefore, we hypothesized that CD1d-dependent NKT cells may play a crucial role in the development of hypertension, vascular injury, and inflammation.

In this study, CD1d knockout (CD1dko) mice, wild-type (WT) mice treated with NKT-specific activators (α-galactosylceramide), and chimeric mice that lack CD1d only in hematopoietic cells were analyzed. Here, we demonstrate that Ang II– or deoxycorticosterone acetate (DOCA) salt–induced arterial hypertension, vascular dysfunction, and oxidative stress were exacerbated in CD1dko and chimeric mice, but activation of NKT cells significantly alleviated these changes. Furthermore, CD1dko markedly increased Ang II–induced interleukin-6 production, leading to activation of signal transducer and activator of transcription 3 (STAT3) and

orphan nuclear receptor γ (RORγ) and, subsequently, interleukin-17A production. Administration of interleukin-17 neutralizing antibody to CD1dko mice partially reversed Ang II–induced hypertension and vascular injury. In addition, we analyzed serum NKT cell number in humans with and without hypertension. Collectively, our results provide novel evidence supporting that activation of CD1d-dependent NKT cells plays a protective role against Ang II–induced hypertension and vascular dysfunction.

## METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. Detailed Materials and Methods are presented in Data S1 (Supplemental Materials and Methods).

### Models and Animals

Hypertension was induced in male CD1dko mice and matched C57BL/6 WT mice (aged 8 weeks; weight, 25–30 g) by subcutaneous infusion of 490 ng/kg per minute Ang II (Sigma-Aldrich, St Louis, MO) using minipumps (Alzet model 1007D) for 14 days. Another hypertension model was created using DOCA salt, as described previously. All animal studies were approved by the Animal Care and Use Committee of Capital Medical University and were in accordance with US National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

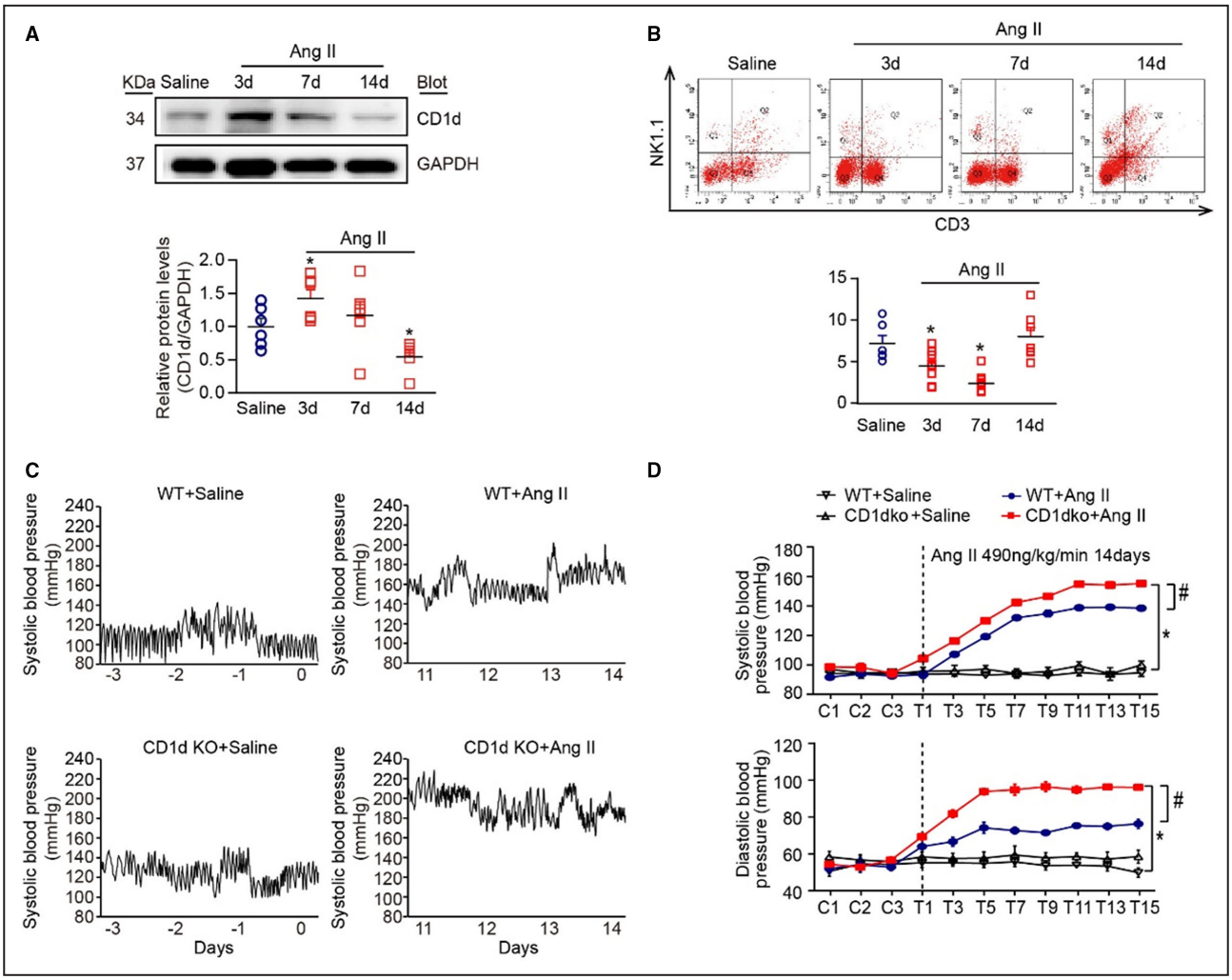


**Figure 1. Ang II decreases CD1d expression and NKT cell number. CD1d deficiency exacerbates Ang II–induced hypertension.** **A**, Western blot analysis of CD1d protein levels in the aorta tissues (n=6). **B**, Flow cytometry analysis of NKT cells ($CD45^{+}CD^{3+}NK1.1^{+}$) in the aorta tissues (n=6). **C**, SBP in WT and CD1dko mice (n=6). **D**, The measurement of SBP by noninvasive tail-cuff method (n=6). Data are expressed as mean±SEM. *$P$<0.05 compared with WT + saline group; #$P$<0.05 compared with WT + Ang II group. Ang II indicates angiotensin II; CD1dko, CD1d knockout; GAPDH, glyceraldehyde 3–phosphate dehydrogenase;NKT, natural killer T; SBP, systolic blood pressure; and WT, wild type.

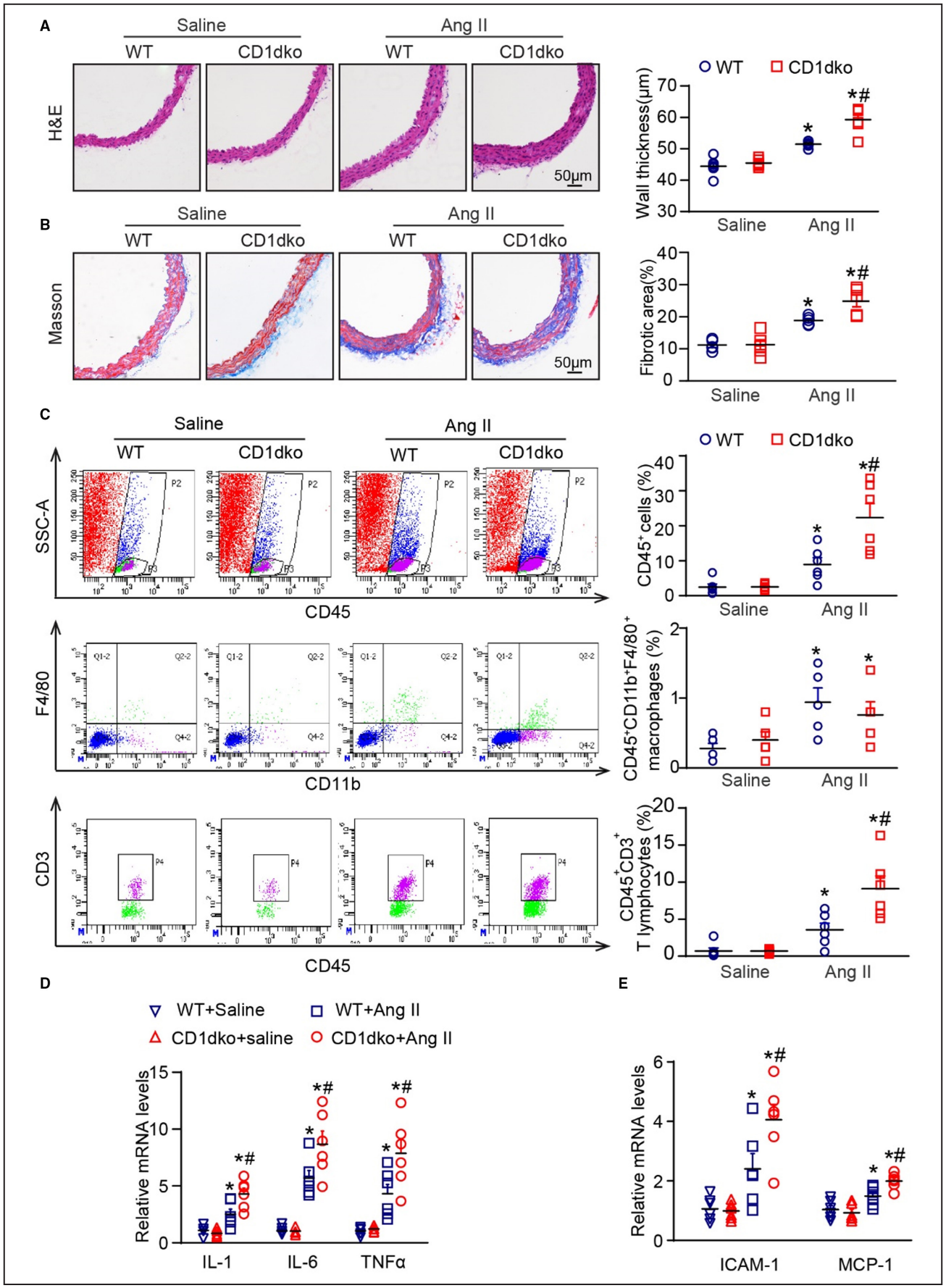
A
Saline
Ang II
WT
CD1dko
H&E
50μm
Wall thickness(μm)
B
Masson
Fibrotic area(%)
C
SSC-A
CD45
CD45+ cells (%)
F4/80
CD11b
CD45+CD11b+F4/80+ macrophages (%)
CD3
CD45+CD3+ T lymphocytes (%)
D
WT+Saline
WT+Ang II
CD1dko+saline
CD1dko+Ang II
Relative mRNA levels
IL-1
IL-6
TNFα
E
ICAM-1
MCP-1

**Figure 2. CD1d deficiency exacerbates Ang II–induced vascular remodeling and inflammation.**
**A**, Representative images of H&E staining from thoracic aorta (left) and the quantitative analysis of the wall thickness (right; n=6). Bar=50 μm. **B**, Representative images of Masson staining from thoracic aorta (left) and the quantitative analysis of the wall thickness (right; n=6). **C**, Flow cytometry analysis of macrophages ($CD45^+CD11b^+F4/80^+$) and T lymphocytes ($CD45^+CD3^+$) in the aorta tissues (left) with histograms showing the percentage of gated cells (right; n=5–6). **D**, qPCR analysis of IL-1β, IL-6, and TNF-α mRNA level in the aorta tissues (n=6). **E**, qPCR analysis of ICAM-1 and MCP-1 mRNA level in the aorta tissues (n=6). Data are expressed as mean±SEM. $^*P<0.05$ compared with WT+saline group; $^\#P<0.05$ compared with CD1dko + Ang II group. Ang II indicates angiotensin II; CD1dko, CD1d knockout; H&E, H&E, hematoxylin and eosin; ICAM–1, intercellular cell adhesion molecule-1; IL, interleukin; MCP–1, monocyte chemoattractant protein-1; qPCR, quantitative real-time polymerase chain reaction; SSC, side scatter; TNF, tumor necrosis factor; and WT, wild type.

## Histologic Study

Vessel tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and analyzed histologically.

## Flow Cytometry

Flow cytometry was used to analyze the inflammatory cells in vessel tissues.

## Bone Marrow Chimeric Mice

Chimeric mice were used to examine the function of bone marrow–derived CD1dko cells for vessel remolding and inflammation.

## Human Study

We explored the patients with essential hypertension (n=57; systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg) and normotensive control subjects (n=87) between June and July 2021. The baseline characteristics of control subjects and patients are indicated in Table S1. Each subject provided written informed consent before participating. This study was approved by the Ethics Committees of the First Affiliated Hospital of Dalian Medical University (No. LCKY2016-31).

## Statistical Analysis

All the results were analyzed using GraphPad Prism 9 software and SPSS version 25.0 and expressed as mean±SEM. The Shapiro-Wilk normality test was used to test if data are normally distributed. If the results were distributed normally, the difference between 2 groups was tested using a Student *t* test; if not, the Mann-Whitney test was used. One-way ANOVA was used for comparison between multiple groups, and the Welch correction was applied when variances were not equal. Repeated-measures ANOVA was used to analyze the blood pressure and acetylcholine or sodium nitroprusside–induced vasodilation tests in aortic rings. Two-way ANOVA was used for comparison among the different treatment groups (eg, CD1dko mice, WT mice, and WT mice with NKT cell activator administration). $P<0.05$ was considered statistically significant.

# RESULTS

## Ang II Infusion Decreases the Expression of CD1d and the Number of NKT Cells in the Aorta, and CD1dko Aggravates the Elevation of Blood Pressure Induced by Ang II Infusion

We first determined whether Ang II infusion could change CD1d expression and NKT cell number. Western blotting analysis revealed a significant decrease in CD1d expression in the aorta after Ang II infusion on day 14, although there was a slight increase on days 3 and 7 (Figure 1A). Flow cytometry analysis showed that, although the number of NKT cells was restored on day 14 after Ang II infusion, it was also decreased on days 3 and 7 (Figure 1B). These results suggest CD1d-dependent NKT cells may play a role in the development of hypertension. We then investigated the effect of CD1dko on Ang II–induced hypertension. The results showed Ang II infusion caused a significant increase in systolic blood pressure in WT mice that was further elevated in CD1dko mice, measured by invasive radiotelemetry and noninvasive tail-cuff method (Figure 1C and 1D). There are no changes in baseline blood pressure.

## CD1dko Aggravates Ang II–Induced Vascular Remodeling, Infiltration of Vascular T Lymphocytes, and Gene Expression of Inflammatory Markers

Hypertension is associated with vascular remodeling, including increased vascular smooth muscle cell hypertrophy, aortic collagen deposition, and inflammatory response. To determine the effect of CD1dko on vascular remodeling, WT and CD1dko mice were infused with vehicle or Ang II for 2 weeks. The results showed Ang II infusion induces aortic wall thickening and collagen deposition in WT animals, and this effect was significantly accelerated in CD1dko mice (Figure 2A and 2B). It has been shown that macrophages and T lymphocytes are involved in the development of hypertension. Here, we qualified total leukocytes ($CD45^+$ cells), macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ cells), and total T lymphocytes ($CD45^+$ $CD3^+$ cells) on single-cell

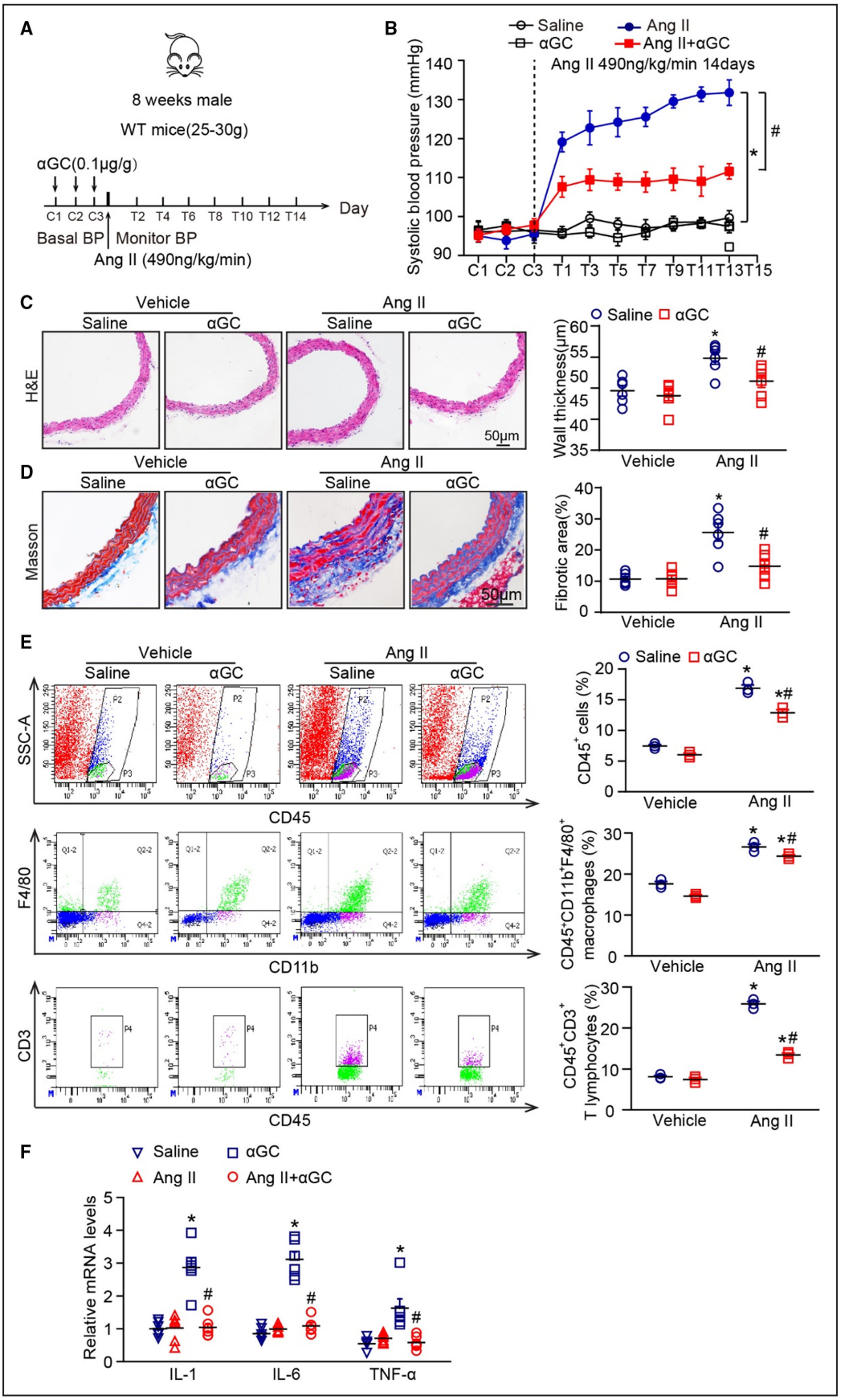
A
8 weeks male
WT mice(25-30g)
αGC(0.1μg/g)
C1 C2 C3 T2 T4 T6 T8 T10 T12 T14 Day
Basal BP Monitor BP
Ang II (490ng/kg/min)
B
Saline
αGC
Ang II
Ang II+αGC
Ang II 490ng/kg/min 14days
Systolic blood pressure (mmHg)
C1 C2 C3 T1 T3 T5 T7 T9 T11 T13 T15
C
Vehicle
Ang II
Saline
αGC
H&E
50μm
Wall thickness(μm)
D
Masson
Fibrotic area(%)
E
SSC-A
CD45
F4/80
CD11b
CD3
CD45+ cells (%)
CD45+CD11b+F4/80+ macrophages (%)
CD45+CD3+ T lymphocytes (%)
F
Relative mRNA levels
IL-1
IL-6
TNF-α

**Figure 3. α-GC treatment attenuates Ang II–induced hypertensive response.**
**A**, Experimental schematic diagram of WT mice subjected to α-GC and Ang II. **B**, SBP measured by noninvasive tail-cuff method (n=6). **C**, Representative images of H&E staining from thoracic aorta (left) and the quantitation of the wall thickness (right; n=6). Bar=50 μm. **D**, Representative images of Masson staining from thoracic aorta (left) and the quantitation of the wall thickness (right; n=6). **E**, Flow cytometry analysis of macrophages ($CD45^+CD11b^+F4/80^+$) and T lymphocytes ($CD45^+CD3^+$) in the aorta tissues (left), with histograms indicating the percentage of gated cells (right; n=4–5). **F**, Quantitative real-time polymerase chain reaction analysis of IL-1β, IL-6, and TNF-α mRNA level in the aorta tissues (n=6). Data are expressed as mean±SEM. $^*P<0.05$ compared with WT + saline group; $^{\#}P<0.05$ compared with Ang II + α-GC group. α-GC indicates α–galactosylceramide; Ang II, angiotensin II; H&E, H&E, hematoxylin and eosin; IL, interleukin; SBP, systolic blood pressure; TNF, tumor necrosis factor; SSC, side scatter; and WT, wild type.

suspensions of thoracic aortas by flow cytometry. The result found Ang II–induced infiltration of $CD45^+$ myelomonocytes, especially T lymphocytes in the aorta, was markedly increased in CD1dko mice. Depletion of CD1d did not change the number of macrophages (Figure 2C). In addition, Ang II–induced expression of several proinflammatory (interleukin-1β, interleukin-6, and TNF-α) and adhesion molecule genes (intercellular cell adhesion molecule-1 and MCP-1 [monocyte chemoattractant protein-1]) in aortic tissue was also increased in CD1dko mice (Figure 2D and 2E). These results suggest that CD1d-dependent NKT cell inactivation contributes to the genesis of Ang II–induced hypertension and its associated vascular damage.

## Administration of an NKT Cell Activator Attenuates Ang II–Induced Hypertension, Vascular Remodeling, and Inflammation

α-Galactosylceramide is known as a specific activator of NKT cells. WT mice were systemically treated with α-galactosylceramide and Ang II for 14 days to further investigate the role of NKT cells in hypertension. The results showed α-galactosylceramide partly reversed Ang II–induced hypertension, thickening of aortic wall, collagen deposition, and accumulation of $CD45^+$ myeloid-derived inflammatory cells, mainly T cells. The expression of proinflammatory genes was also reduced, which was consistent with the results obtained from CD1dko mice (Figure 3A through 3F). The baseline vascular remodeling and inflammatory responses were not statistically different between saline- and α-galactosylceramide–treated mice. This further confirms the role of NKT cells in hypertension.

## Inactivation of NKT Cells Aggravates Ang II–Induced Vascular Dysfunction and Oxidative Stress, Whereas Activation Attenuates These Effects

Aortic ring test was performed to investigate whether the change of blood pressure mediated by NKT cells is associated with vascular tone by measuring endothelium-dependent and endothelium-independent relaxation. As previously reported, Ang II infusion impaired endothelium-dependent vasodilatation to acetylcholine, but not endothelium-independent vasodilatation to sodium nitroprusside in WT mice compared with saline-treated control group, and this impairment became more serious in CD1dko mice (Figure 4A). Conversely, α-galactosylceramide administration mitigated this effect (Figure 4B). Because superoxide levels are considered a hallmark of hypertension and play a role in mediating vascular reactivity, we further evaluated superoxide production and the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The results showed that CD1dko mice had increased aortic superoxide, as indicated by dihydroethidium staining, whereas α-galactosylceramide treatment reduced this level (Figure 4C and 4D). These findings were supported by the results of quantitative real-time polymerase chain reaction analysis, which showed that CD1dko mice had increased NADPH oxidase 1/2 catalytic subunits and $p22^{phox}$ mRNA levels, whereas α-galactosylceramide treatment reduced these levels (Figure 4E and 4F), as well as NADPH oxidase activity (Figure 4G and 4H). These results suggest that NKT cells play a role in Ang II–induced endothelial dysfunction by mediating superoxide production.

## CD1dko Aggravates DOCA Salt–Induced Hypertension

DOCA salt–induced hypertension is characterized by low levels of circulating Ang II, so we also used this model to further determine that the hypertensive response of NKT cells was not specific to Ang II. Similar to the results shown in Figure 2, CD1dko aggravated DOCA salt–induced elevation of blood pressure, as well as vascular remodeling, superoxide production, the expression of inflammatory and chemokine cytokines (interleukin-1β, TNF-α, MCP-1, and intercellular cell adhesion molecule-1), and NADPH oxidase subunits (NADPH oxidase 1/2) mRNA (Figure 5A through 5G).

## Selective Deletion of CD1d in Bone Marrow–Derived Cells Aggravates Ang II–Induced Hypertension, Vascular Remodeling and Dysfunction, and Oxidative Stress

Given recent findings that the bone marrow is critical in regulating peripheral inflammation in hypertension,

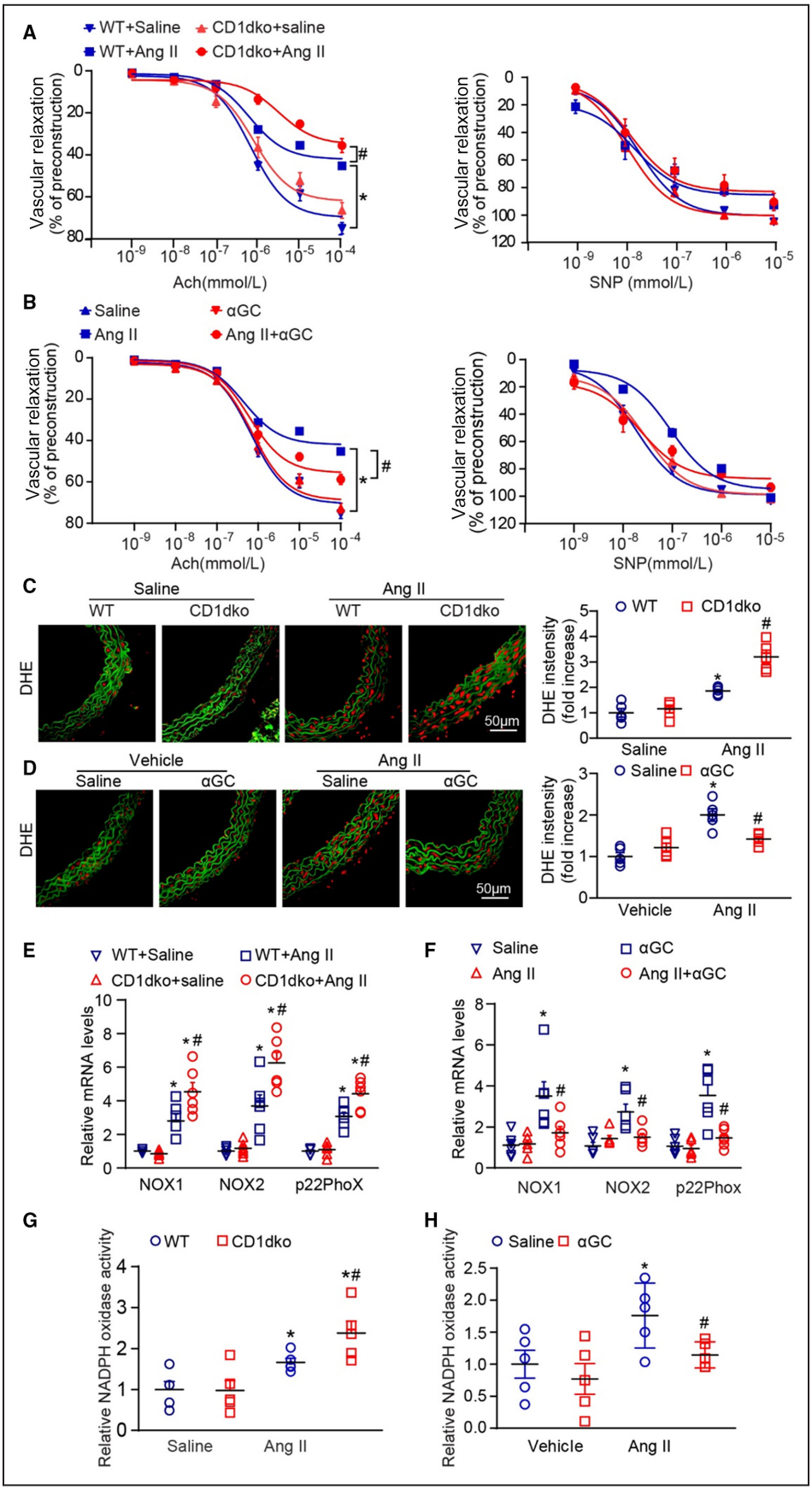
A
WT+Saline
CD1dko+saline
WT+Ang II
CD1dko+Ang II
Vascular relaxation (% of preconstruction)
Ach(mmol/L)
SNP (mmol/L)
B
Saline
αGC
Ang II
Ang II+αGC
Ach(mmol/L)
SNP(mmol/L)
C
Saline
Ang II
WT
CD1dko
DHE
50μm
DHE instensity (fold increase)
D
Vehicle
Ang II
Saline
αGC
E
Relative mRNA levels
NOX1
NOX2
p22PhoX
F
Saline
αGC
Ang II
Ang II+αGC
p22Phox
G
Relative NADPH oxidase activity
H
Vehicle

**Figure 4. CD1d deficiency exacerbates, and α-GC treatment improves, Ang II–induced vascular dysfunction and oxidative stress response.**
**A** and **B**, Concentration-relaxation curves of endothelium-dependent (ACh) and endothelium-independent (SNP) vasodilators (A: n=4–8; B: n=6–7). **C** and **D**, Representative images of DHE staining from thoracic aorta (left) and the quantitative analysis of DHE fluorescence intensity (right; n=5). Red fluorescence means the superoxide production, and green fluorescence represents the laminae. Bar=50 μm. **E** and **F**, Quantitative real-time polymerase chain reaction analysis of NOX1/NOX2 and $p22^{Phox}$ mRNA level of different groups in the aorta tissues (n=6). **G** and **H**, NADPH oxidase activity measurement in the aorta tissues (n=5). Data are expressed as mean±SEM. $^{*}P<0.05$ compared with WT + saline group; $^{\#}P<0.05$ compared with CD1dko + Ang II or WT + Ang II + α-GC group. α-GC indicates α–galactosylceramide; ACh, acetylcholine; Ang II, angiotensin II; CD1dko, CD1d knockout; DHE, dihydroethidium; NADPH, nicotinamide adenine dinucleotide phosphate; NOX1, nicotinamide adenine dinucleotide phosphate oxidase 1; SNP, sodium nitroprusside; and WT, wild type.

we aimed to determine whether the effect of NKT cells was mediated by myeloid cell CD1d expression. To do this, we generated chimeric mice by transplanting bone marrow–derived cells from CD1dko and WT mice into lethally irradiated CD1dko and WT mice. After 4 weeks, these mice were infused with either Ang II or a vehicle for an additional 4 weeks. WT mice transplanted with CD1dko bone marrow showed significantly elevated systolic pressure, aortic thickening, collagen deposition, vascular dysfunction, aortic superoxide production, and NADPH oxidase activity, and elevated mRNA expression of proinflammatory cytokines (interleukin-1β and interleukin-6) and NADPH oxidase (NADPH oxidase 1/2 and $p22^{phox}$) (Figure 6A through 6I). Similar effects were observed in CD1dko mice transplanted with CD1dko bone marrow (CD1dko-CD1dko). In contrast, CD1dko mice transplanted with WT bone marrow (WT-CD1dko) reversed these pathologic features of vascular functional alterations and remodeling compared with the CD1dko-WT and CD1dko-CD1dko groups (Figure 6A through 6I). These results indicate that the deletion of CD1d in myeloid cells exacerbates Ang II–induced vascular hypertension, vascular remodeling, dysfunction, and superoxide production, although the effect of other immune cells cannot be ruled out.

## CD1dko Aggravates Ang II Infusion-Induced Interleukin-17 Expression

It has been proven that activation of NKT cells can rapidly produce Th1 (interferon-γ, interleukin-2, and TNF-α), Th2 (interleukin-4, interleukin-5, and interleukin-13), Tregs (interleukin-10), and Th17 (interleukin-17 and interleukin-22) cytokines, serving as a bridge between innate and adaptive immune responses. Th1 and Th17 effectors contribute to inflammation, leading to increased blood pressure, whereas Tregs suppress immune response activation. Therefore, we examined the effect of CD1dko on Th1, Th2, Tregs, and Th17. The results showed that Ang II infusion upregulated the expression of interleukin-17A and interleukin-22 mRNA in the aorta of WT mice compared with saline controls, as determined by quantitative real-time polymerase chain reaction analysis, and this effect was significantly increased in CD1dko mice (Figure 7A). The change in interleukin-17A protein was further verified by ELISA and flow cytometry (Figure 7B and 7C). However, CD1dko did not affect the increase in Th1/Th2/Treg cytokine mRNA and protein levels (Figure S1A and S1B). These results indicate that CD1dko NKT cells are capable of promoting Th17 subtype differentiation.

## CD1dko Increases Ang II–Induced Interleukin-6 Production, Thus Activating STAT3 and RORγ

Th0 cells differentiate into Th17 cells on activation with antigen in the presence of transforming growth factor-β, interleukin-6, and interleukin-23. STAT3 and RORγ then drive Th17 cell differentiation. Our study evaluated changes in transforming growth factor-β, interleukin-6, and interleukin-23 cytokines and the expression of STAT3 and RORγ. Results showed that CD1dko significantly increased Ang II–induced interleukin-6 expression, as demonstrated by quantitative real-time polymerase chain reaction (mRNA) and cytokine antibody array (protein). ELISA analysis confirmed these changes (Figure 8A through 8C). CD1dko also elevated the expression of RORγ and the phosphorylation of STAT3 induced by Ang II infusion. α-Galactosylceramide treatment reduced this effect (Figure 8D and 8E). These findings suggest that CD1dko increases interleukin-6 production, possibly activating STAT3 and RORγ and regulating Th17 differentiation.

## Administration of Monoclonal Antibodies Targeting Interleukin-17A Alleviates Ang II–Induced Hypertension and Vascular Remodeling

We next determined whether interleukin-17A blockade can reverse Ang II–induced hypertension and vascular remodeling in WT and CD1dko mice. Ang II was infused for a 2-week period, and monoclonal antibodies against interleukin-17A or control IgG1 antibodies were injected intraperitoneally on days 3, 7, and 10 after the infusion. The results showed that compared with mice treated with control IgG1 antibodies, those treated with monoclonal antibodies against interleukin-17A

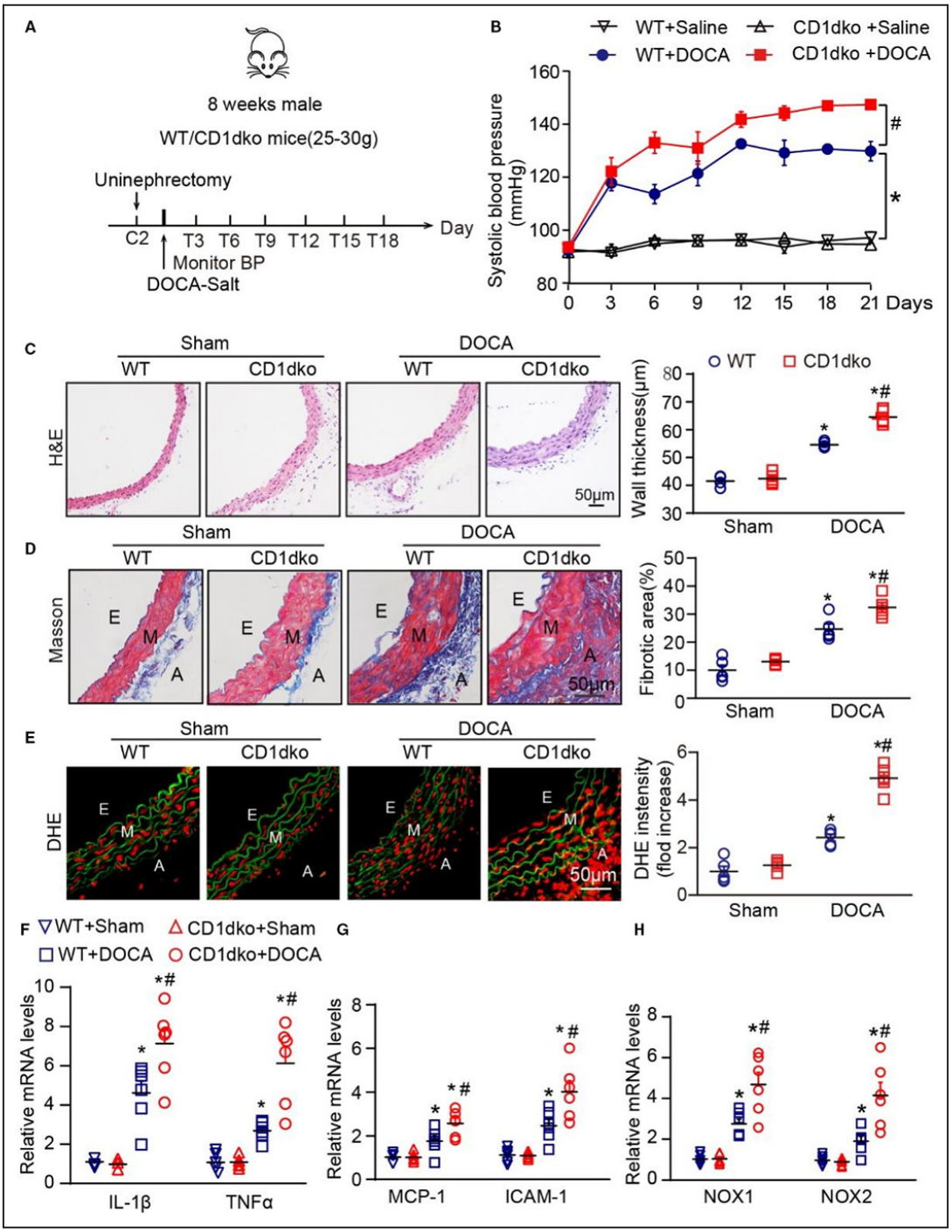


**Figure 5. CD1d deficiency exacerbates DOCA salt–induced hypertension.**
**A**, Experimental schematic diagram of WT mice subjected to DOCA salt. **B**, SBP measured by noninvasive tail-cuff method (n=5). **C**, Representative images of H&E staining from thoracic aorta (left) and quantitative analysis of the wall thickness (right; n=5). **D**, Representative images of Masson staining from thoracic aorta (left) and quantitative analysis of the wall thickness (right; n=5). **E**, Representative images of DHE staining from thoracic aorta (left) and quantitative analysis of DHE fluorescence intensity (right; n=5). **F**, qPCR analysis of IL-1β and TNF-α mRNA level in aorta tissues (n=6). **G**, qPCR analysis of MCP-1 and ICAM-1 mRNA level in the aorta tissues (n=6). **H**, qPCR analysis of NOX1/2 mRNA level in aorta tissues (n=6). Data are expressed as mean±SEM. *$P$<0.05 compared with WT + saline group; #$P$<0.05 compared with CD1dko + DOCA group. Bar=50 μm. Ang II indicates angiotensin II; CD1dko, CD1d knockout; DHE, DHE, dihydroethidium; DOCA, deoxycorticosterone acetate; H&E, hematoxylin and eosin; ICAM–1, intercellular cell adhesion molecule-1; IL, interleukin; MCP–1, monocyte chemoattractant protein-1; NOX1/2, nicotinamide adenine dinucleotide phosphate oxidase 1/2; qPCR, quantitative real-time polymerase chain reaction; SBP, systolic blood pressure; TNF, tumor necrosis factor; and WT, wild type.

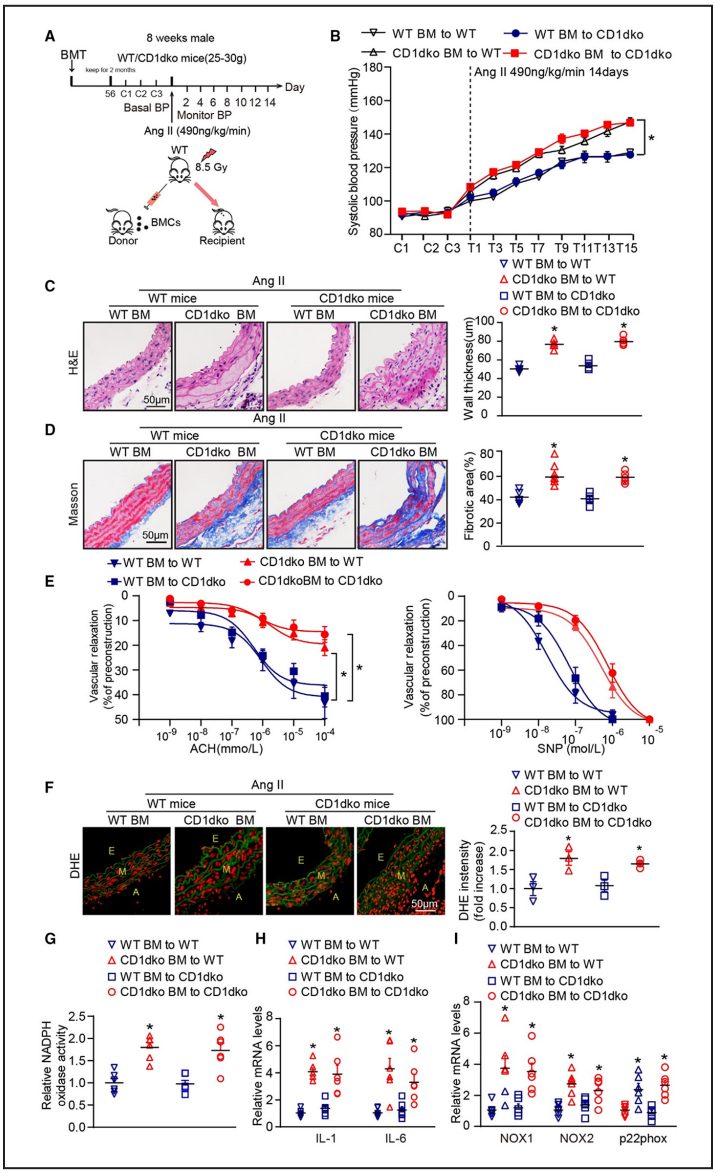


**Figure 6. CD1d deficiency in bone marrow–derived cells exacerbates Ang II–induced hypertension, vascular remodeling and dysfunction, and oxidative stress.**

**A**, Experimental schematic diagram of WT and CD1dko mice subjected to BMT and Ang II. **B**, SBP measured by noninvasive tail-cuff method (n=3–6). **C**, Representative images of H&E staining from thoracic aorta (left) and quantification of the wall thickness (right; n=5). **D**, Representative images of Masson staining from thoracic aorta (left) and quantification of the wall thickness (right; n=5). **E**, Concentration-relaxation curves of endothelium-dependent (left; n=8) and endothelium-independent (right; n=4) vasodilators. **F**, Representative images of DHE staining from thoracic aorta (left) and quantification of DHE fluorescence intensity (right; n=3). **G**, NADPH oxidase activity measured in aorta tissues (n=6). **H**, qPCR analysis of IL-1β and IL-6 mRNA level in aorta tissues (n=6). **I**, qPCR analysis of NOX1/2 and $p22^{phox}$ mRNA level in aorta tissues (n=6). Data are expressed as mean±SEM. *$P$<0.05 compared with WT BM to WT. Bar=50 μm. Ang II indicates angiotensin II; BM, bone marrow; BMT, bone marrow transplantation; BP, blood pressure; CD1dko, CD1d knockout; DHE, DHE, dihydroethidium; H&E, hematoxylin and eosin; ICAM–1, intercellular cell adhesion molecule-1; IL, interleukin; MCP–1, monocyte chemoattractant protein-1; NADPH, nicotinamide adenine dinucleotide phosphate; NOX1/2, nicotinamide adenine dinucleotide phosphate oxidase 1/2; qPCR, quantitative real-time polymerase chain reaction; SBP, systolic blood pressure; and WT, wild type.

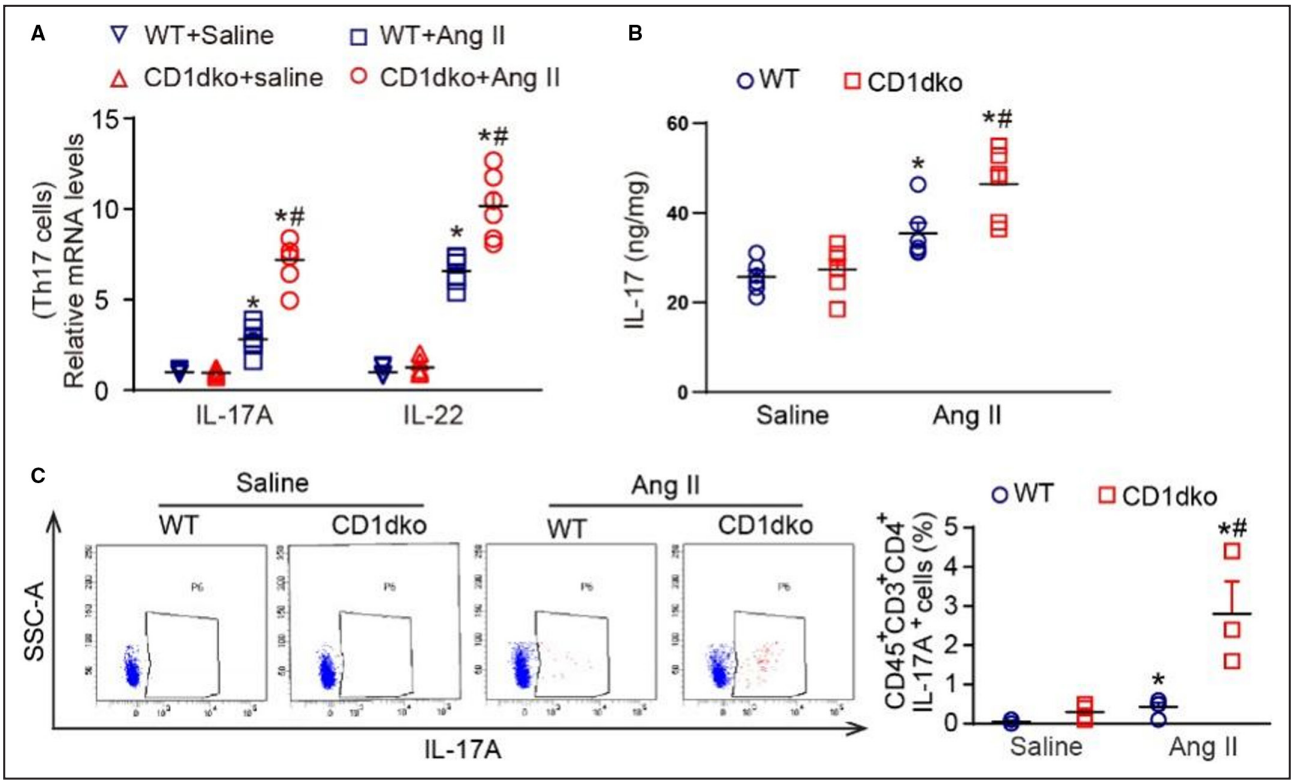


**Figure 7. CD1d deficiency exacerbates Ang II infusion-induced IL-17 expression.**
**A**, Quantitative real-time polymerase chain reaction analysis of IL-17A and IL-22 mRNA level in the aorta tissues (n=6). **B**, IL-17A levels measured by ELISA in aorta tissues (n=6). **C**, Flow cytometry analysis of IL-17A–producing cells ($CD45^+CD3^+CD4^+IL17A^+$) in the aorta tissues (left), with histograms showing the percentage of gated cells (right; n=3–4). Data are expressed as mean±SEM. $^*P<0.05$ compared with WT + saline; $^\#P<0.05$ compared with WT + Ang II. Ang II indicates angiotensin II; IL, interleukin; SSC, side scatter; and WT, wild type.

experienced a significant reduction in systolic blood pressure elevation, as well as decreased vascular hypertrophy and fibrosis (Figure 9A through 9C). These findings suggest that blocking interleukin-17A can effectively reverse hypertension and vascular remodeling in CD1d-deficient mice.

## NKT Cells Are Decreased in Human Hypertension

To examine the role of NKT cells in human hypertension, we quantified their number in blood samples collected from 57 patients with hypertension and 87 normotensive individuals (Table S1) using flow cytometry analysis. The results showed that, compared with normotensive individuals, patients with hypertension had higher systolic and diastolic blood pressure, heart rate, fasting blood glucose, and white blood cell count, as well as lower left ventricular ejection fraction, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol concentrations. Flow cytometry analysis revealed that the number of NKT cells ($CD45^+$ $CD3^+$ T-cell receptor $\gamma\delta^-$ $CD56^+$) in patients with hypertension was significantly lower than in normotensive individuals (Figure 10). To determine the association between NKT cell numbers and hypertension, we performed multivariable logistic regression analysis. After adjusting for other indicators of hypertension, the number of NKT cells still showed a statistically significant association with hypertension (odds ratio, 0.906).

# DISCUSSION

In this study, we first demonstrated that CD1d expression and the number of NKT cells was significantly decreased in Ang II–infused mice and in patients with hypertension compared with control mice and individuals without hypertension, respectively. We then evaluated the regulatory role of CD1d-dependent NKT cells in the development of hypertension. The results indicated that CD1d deficiency significantly aggravated hypertension, vascular remodeling, inflammatory response, vascular superoxide production, and vascular endothelial dysfunction of blood vessels in response to Ang II or DOCA salt. In addition, administration of α-galactosylceramide improved these effects in WT mice infused with Ang II. Transplanting

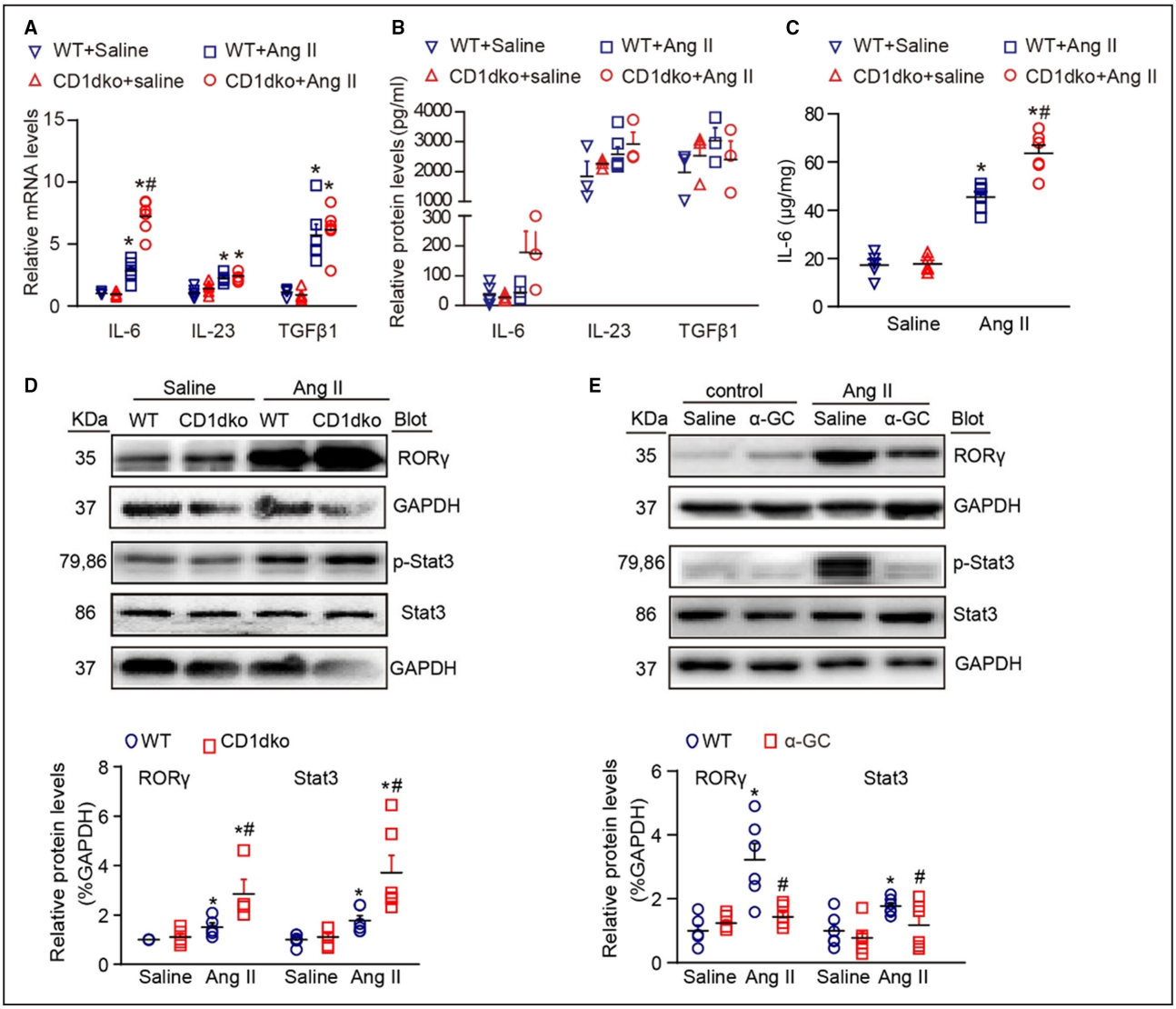


**Figure 8. CD1d deficiency increases IL-6 production, subsequently activating transcription factors STAT3 and RORγ.**
**A**, Quantitative real-time polymerase chain reaction analysis of IL-6, IL-23, and TGF-β mRNA level in the aorta tissues (n=6). **B**, Cytokine antibody array of IL-6, IL-23, and TGF-β content in aorta tissues using the RayBio Mouse Cytokine Array kit (n=3–6). **C**, ELISA measurement of IL-6 concentration in aorta tissues (n=6). **D** and **E**, Representative images of RORγ and STAT3 by Western blot (top) and quantification (bottom) in the CD1dko mice or the mice administrated with α-GC (bottom; n=6). Data are expressed as mean±SEM. *$P$<0.05 compared with WT + saline; #$P$<0.05 compared with WT + Ang II. α-GC indicates α-galactosylceramide; Ang II, angiotensin II; CD1dko, CD1d knockout; GAPDH, glyceraldehyde 3–phosphate dehydrogenase; IL, interleukin; RORγ, orphan nuclear receptor γ; STAT3, signal transducer and activator of transcription 3; TGF, transforming growth factor; and WT, wild type.

bone marrow–derived cells from WT mice into CD1d-deficient mice also reversed the changes induced by Ang II, demonstrating a specific role for leukocyte CD1d expression in the development of hypertension. CD1d deficiency also increased the production of interleukin-17, potentially through the activation of STAT3 and RORγ via interleukin-6, and neutralizing interleukin-17 with an antibody alleviated hypertension and vascular remodeling caused by CD1d deficiency. These results suggest that activating NKT cells may be a potential treatment for hypertension and vascular dysfunction (Figure 11).

Increasing evidence has now demonstrated that hypertension is an inflammatory process that may trigger the transmigration and accumulation of immune cells into the interstitium of vascular tissues.[25,26] Immune cells, both innate (monocytes, macrophages, neutrophils, and dendritic cells) and adaptive (T and B cells), are involved in the initiation and progression of hypertension, with T cells being considered critical modulators.[4] Different T-cell subsets, such as $CD4^+$ and $CD8^+$ T cells and γδ T cells, promote hypertension, whereas Tregs control its development.[12,13,27,28] The study extends previous findings and shows that

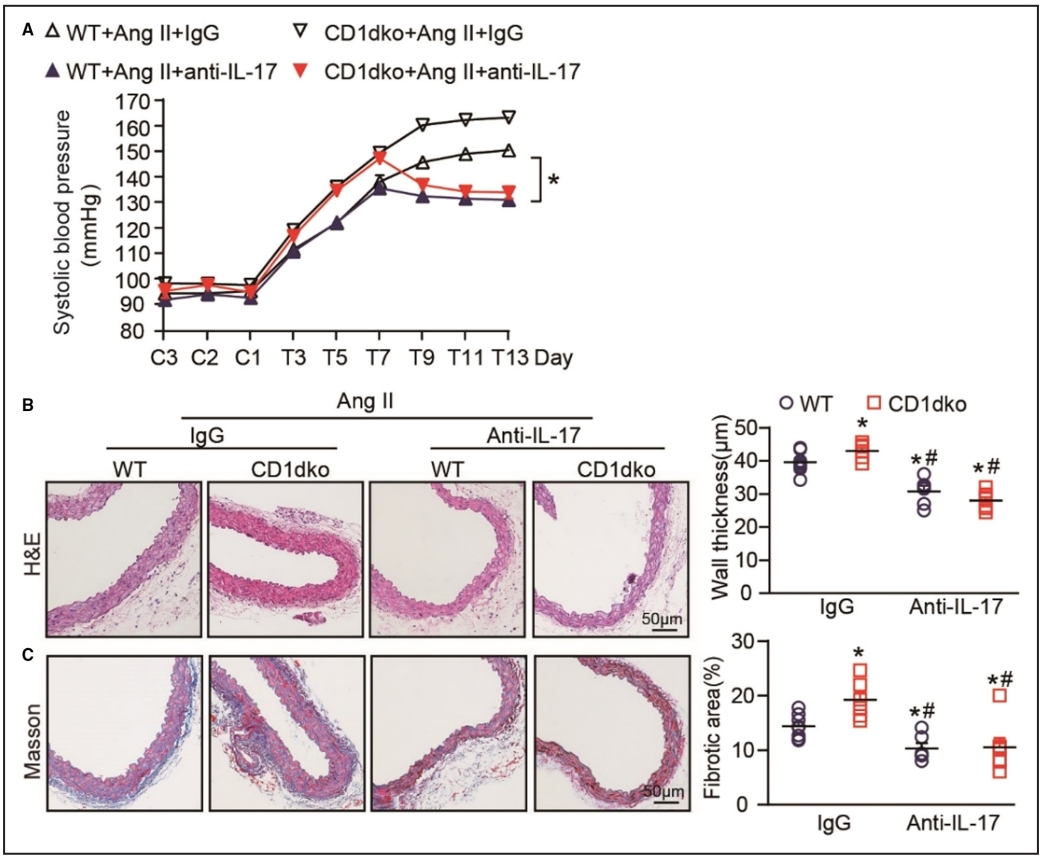


**Figure 9. IL-17A neutralizing antibody alleviates Ang II–induced hypertension and vascular remodeling.** **A**, SBP measured by noninvasive tail-cuff method (n=6). **B**, Representative images of H&E staining from thoracic aorta (left) and quantification of the wall thickness (right; n=6–8). **C**, Representative images of Masson staining from thoracic aorta (left) and the quantification of the wall thickness (right; n=6–8). Data are expressed as mean±SEM. *$P$<0.05 compared with WT + Ang II + IgG; #$P$<0.05 compared with CD1dko + Ang II + IgG. Ang II indicates angiotensin II; CD1dko, CD1d knockout; H&E, hematoxylin and eosin; IgG, immunoglobulin G; IL, interleukin; SBP, systolic blood pressure; and WT, wild type.

CD1d-dependent NKT cells, another subset of T cells, mitigate blood pressure response. CD1d deficiency exacerbates Ang II– or DOCA salt–induced elevation of systolic blood pressure, vascular endothelial dysfunction, and oxidative stress, and the extent of vascular medial hypertrophy and collagen deposition

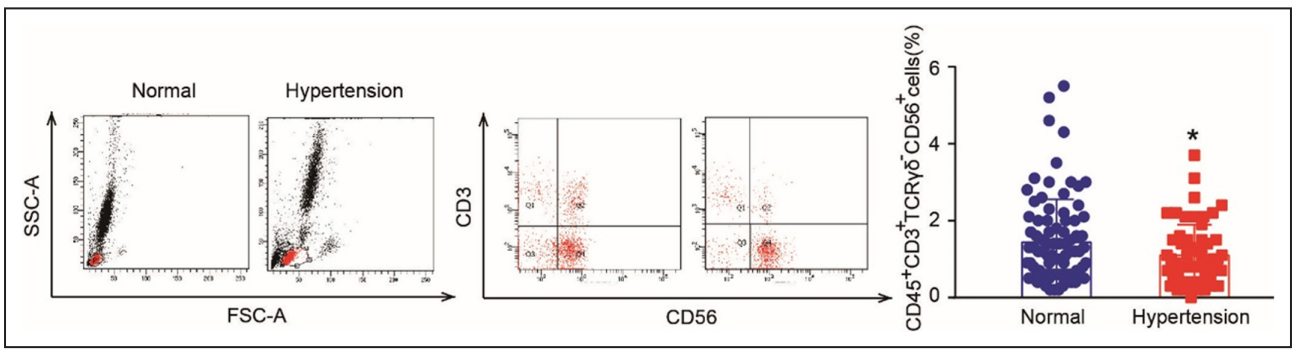


**Figure 10. Blood CD1d-positive cells and NKT cells are decreased in patients with hypertension.** Data from 87 normotensive individuals and 57 patients with essential hypertension (systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg) were analyzed. Peripheral blood was analyzed using flow cytometry for NKT cells ($CD45^+$ $CD3^+$ TCR $\gamma\delta^-$ $CD56^+$), and each point represents the number of NKT cells present in the blood of an individual patient. The histograms show the percentage of gated cells. FSC indicates forward scatter; NKT, natural killer T; SSC, side scatter; and TCR, T-cell receptor. *$P$<0.05 compared with normal control.

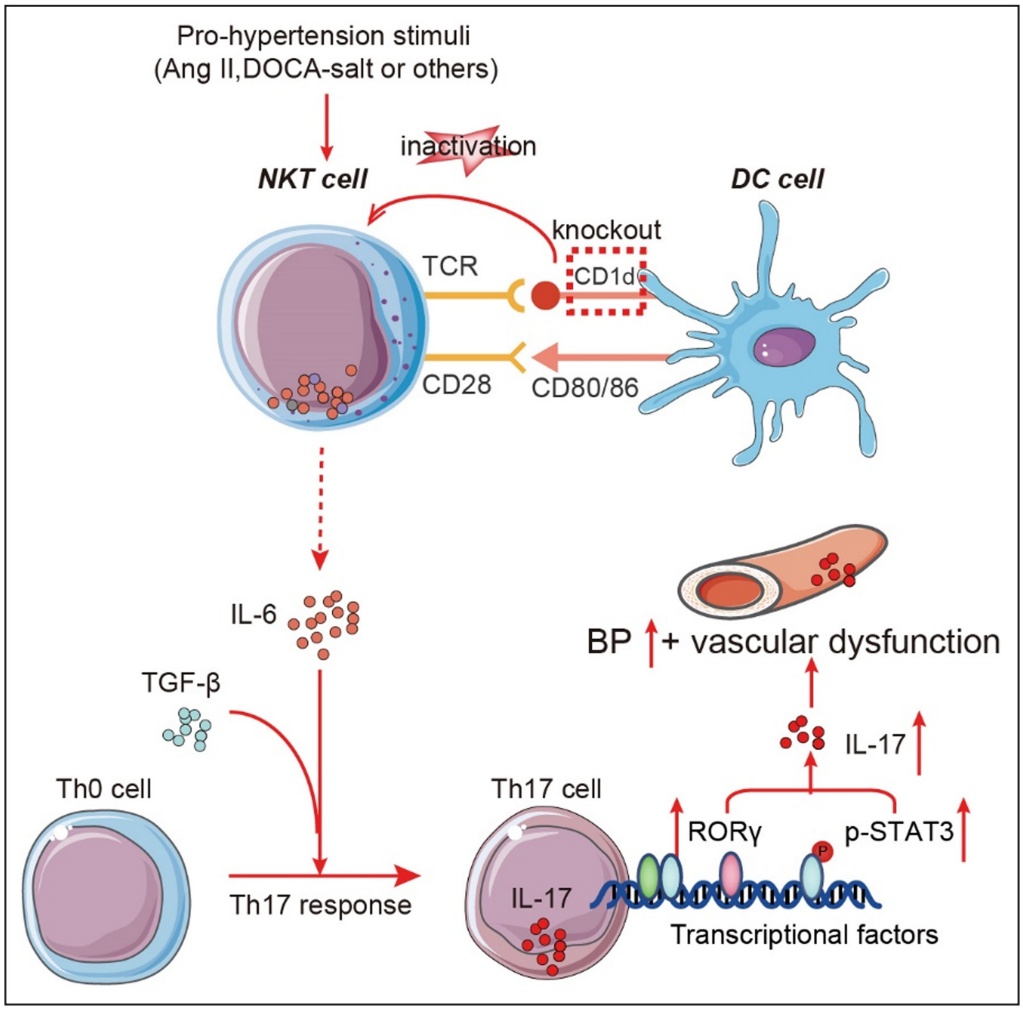


**Figure 11. Schematic of NKT cell–mediated regulation of IL-17 production during hypertension.** Ang II indicates angiotensin II; BP, blood pressure; DC, dendritic cell; DOCA, deoxycorticosterone acetate; IL, interleukin; NKT, natural killer T; p-STAT3, phosphorylated signal transducer and activator of transcription 3; RORγ, orphan nuclear receptor γ; TCR, T-cell receptor; TGF-β, transforming growth factor-β; and Th, T-helper cell.

(Figure 2–5). Administration of α-galactosylceramide, however, blunts this response. These results suggest that NKT cells play a crucial role in hypertension and vascular remodeling.

A distinctive characteristic of NKT cells is their ability to quickly release substantial quantities of cytokines, including Th1, Th2, Th17, and Treg cytokines, which shapes subsequent immune responses on activation.[29–31] Gomez-Lopez et al found that activation of NKT cells induced systemic and local alterations in T-cell subsets, including Th17, before preterm birth.[32] Oh et al showed that invariant NKT cells directly inhibited the differentiation of Th17 and Th1.[33] Grajewski et al found that activation of NKT cells improves experimental ocular autoimmunity by reducing the adaptive Th1 and Th17 responses.[34] Lennart et al showed NKT cells play a crucial role in limiting the development of the Th17 lineage and suggest that NKT cells serve as a natural barrier against Th17 responses.[35] Th17 cells produce the signature proinflammatory cytokine interleukin-17A. In the vasculature, interleukin-17A acts on endothelial cells, smooth muscle cells, and fibroblasts to contribute to hypertension through vascular dysfunction.[36–38] $CD4^+$ Th17 cells are a major source of interleukin-17A, and other innate and adaptive immune cells can also release it. For instance, γδ T cells, innate lymphoid cells, and natural killer cells can produce interleukin-17A, and in some conditions, these cells may be the primary source of interleukin-17A.[39–41] Our results found that CD1d deficiency further increased Ang II–induced Th17 cell response, as indicated by interleukin-17A expression and production, and did not affect Th1, Th2, and Treg cytokines. These results demonstrate that CD1d deficiency aggravates hypertension and vascular remodeling by promoting Th17 cell differentiation.

Th17 cell differentiation is controlled by RORγ and STAT3 and induced by transforming growth factor-β and interleukin-6.[42,43] Interleukin-6, in particular, interacts with its receptor to initiate downstream signaling, which subsequently leads to the activation of STAT3 and RORγ, the main regulators of the differentiation and function of Th17 cells.[44] It is known that NKT cell activation can secrete diverse cytokines and influence many other types of immune responses.[29] The present study found that CD1d deficiency significantly increased the expression and production of interleukin-6, and Ang II induced the expression of RORγ and the phosphorylation of STAT3. These data support the hypothesis that CD1d deficiency increased the production of interleukin-6, activating transcription factors such as STAT3 and RORγ, which regulate Th17 differentiation, and thus aggravate hypertension and vascular dysfunction.

Taken together, the present study provides novel insights into the detrimental role of CD1d-dependent NKT cell inactivation in Ang II– or DOCA salt–induced hypertension and vascular dysfunction. The mechanism involves CD1d deficiency producing interleukin-6, which enhances Th17 differentiation through STAT3 and RORγ, resulting in interleukin-17A production. These results suggest that CD1d-dependent NKT cells may play a significant role in limiting Th17 differentiation, making them a potential target for therapy in hypertension development.

## ARTICLE INFORMATION

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### Disclosures

None.

### Supplemental Material

Data S1
Table S1
Figure S1
References[45–48]

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# SUPPLEMENTAL MATERIAL

**Data S1. Supplemental Materials and Methods**

**Animal Experiments**

CD1d knockout mice (CD1dko) were obtained from the Jackson Laboratory (Sacramento, CA). Hypertension was induced in male CD1dko mice and matched C57BL/6 WT (8-weeks-old, 25-30 gram) by subcutaneous infusion of 490ng/kg/min angiotensin Ⅱ (Ang Ⅱ, Sigma-Aldrich, St Louis, MO, USA) using mini-pumps (Alzet MODEL1007D) for 14 days. Another hypertension model was created using Deoxycorticosterone acetate (DOCA)-salt as described previously[45]. The α-GC agonist (Funakoshi, 0.1μg/g) was administrated intraperitoneally, starting 3 days before Ang Ⅱ infusion and continuing for 2 weeks. On days 3,7 and 10 after Ang Ⅱ infusion, Mice were injected intraperitoneally with either IL-17 neutralizing antibody (R&D Systems, 100μg/mouse) or isotype-control antibody (R&D Systems, MAB421-500, 100μg/mouse). At the end of the protocol, mice were weighed and then anesthetized with oversaturated tribromoethanol (0.4mg/g intraperitoneally). Animals were kept on a 12 h:12 h light cycle and provided with food and water at will.

**Blood Pressure Measurement**

Blood pressure was measured non-invasively using the tail-cuff system (softron BP-2010A; Softron, Tokyo, Japan) and invasively using telemetric blood pressure system (TA-PA11C10, Data Science International, Tilburg, The Netherlands) as described previously[46,47]. Briefly, mice were anesthetized with isoflurane and a radio-telemetry probe catheter was introduced inside the thoracic aorta through the left carotid artery. The transducer was then implanted subcutaneously in the abdomen. After 14 days of recovery, continuous blood pressure recording was taken before and during angiotensin II treatment in freely moving animals using receiver platforms (DSI), data was taken using the Data Quest system (DSI).

**Histological analysis**

Mice (n=6 per group) were anesthetized with 1.5% isoflurane after Ang Ⅱ infusion for several days. Vessel tissues were then extracted, fixed in 4% paraformaldehyde,

embedded in paraffin, and analyzed histologically. Tissue sections (5μm) were stained by haematoxylin & eosin and Masson trichrome staining kit (from Sigma-Aldrich). For cryosections, the vessel tissues were stained with dihydroethidium (1μmol/L in PBS) for 30 minutes at 37℃ and fluorescence was detected using a Labophot 2 microscope (Nikon, Tokyo, Japan).

**Quantitative Real-Time PCR Analysis**

Mice (n=6 per group) were lightly anesthetized with 1.5% isoflurane after Ang Ⅱ infusion for 14 days. Total mRNA was extracted by Trizol Reagent method, and cDNA was synthesized using Moloney murine leukemia virus transcriptase (Promega, Southampton, UK). A 7500 Real Time PCR system was used for quantitative real-time polymerase chain reaction (qPCR). The primer sequences are as follows:

| Gene | Forward Primers (5'-3') | Reverse Primers (5'-3') |
|---|---|---|
| GAPDH | CATCACTGCCACCCAGAAGACTG | ATGCCAGTGAGCTTCCCGTTCAG |
| IL-1β | CTTCCCCAGGGCATGTTAAG | ACCCTGAGCGACCTGTCTTG |
| IL-6 | GCTACCAAACTGGATATAATCAGGA | CCAGGTAGCTATGGTACTCCAGAA |
| TNF-α | ATGGCCTCCCTCTCATCAGT | CTTGGTGGTTTGCTACGACG |
| ICAM-1 | GTGATGCTCAGGTATCCATCCA | CACAGTTCTCAAAGCACAGCG |
| MCP-1 | CTGGACCCATGCATGCTT | CTGGCTCTCTCTCTTTCTTC |
| NOX1 | CCCATCCAGTCTCCAAACATGAC | ACCAAAGCTACAGTGGCAATCAC |
| NOX2 | GGGAACTGGGCTGTGAATGA | CAATTGTGTGGATGGCGGTG |
| $p22^{Phox}$ | CTCCTCTTCACCCTCACTCG | GTGGACTCCCATTGAGCCTA |
| IL-17A | TTCATCTGTGTCTCTGATGCT | TTGACCTTCACATTCTGGAG |
| IL-22 | CATGCAGGAGGTGGTGCCTT | CAGACGCAAGCATTTCTCAG |
| IL-23 | ACACTAGCCTGGAACGCACA | GAGGCATCTGTTGGGTCTCC |
| TGF-β1 | CTCCCGTGGCTTCTAGTGC | GCCTTAGTTTGGACAGGATCTG |
| IFN-γ | GGAACTGGCAAAAGGATGGTGAC | GCTGGACCTGTGGGTTGTTGAC |
| IL-2 | CCTGAGCAGGATGGAGAATTACA | TCCAGAACATGCCGCAGAG |
| IL-4 | ACAGGAGAAGGGACGCCAT | GAAGCCCTACAGACGAGCTCA |
| IL-5 | GGGCTTCCTGCTCCTATCTA | CAGTCATGGCACAGTCTGAT |
| IL-12 | CAACATCAAGAGCAGTAGCAG | TACTCCCAGCTGACCTCCAC |
| IL-13 | CCTGGCTCTTGCTTGCCTT | GGTCTTGTGTGATGTTGCTCA |
| IL-10 | AGCCGGGAAGACAATAACTG | CATTTCCGATAAGGCTTGG |

**Western Blot Analysis**

Mice (n=6 per group) were anesthetized with 1.5% isoflurane after Ang Ⅱ infusion for 14 days. Vessel tissues were lysed with RIPA lysis buffer (Solarbio, Beijing, China) and protein was separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinylidene difluoride (PVDF) membranes. The primary antibodies used were anti-CD1d (abcam, ab119846, 1:1000), anti-RORγ (abcam, 78007,1:1000), anti-pSTAT3 (CST, 9145S,1:1000), anti-STAT3 (CST, 8768S,1:1000) and anti-GAPDH (CST, 5174S,1:1000), followed by incubation with secondary antibodies (CST,7074S,7076S,1:3000) for 1 hour at room temperature. The chemiluminescent system was used to develop the signals of all blots which analyzed with FluorChem FC3, Santa, Clara, CA USA).

**Enzyme-Linked Immunosorbent Assay**

Mice (n=6 per group) were anesthetized with 1.5% isoflurane after Ang Ⅱ infusion for 14 days. The vessel tissues were cut and homogenized using ultrasonic processor in PBS buffer, and the protein concentration was determined using a BCA Protein Assay Kit (from ThermoFisher, Waltham, MA). IL-6 and IL-17A from the aorta lysate were detected using ELISA kit (Arigo, ARG80199, ARG80161).

**Bone Marrow Chimeric Mice**

Chimeric mice were generated as previously described[48]. In brief, bone marrow cells were harvested from C57BL/6J WT mice (n=5-6) and CD1dko mice (n=5-6) by flushing the femurs and tibiae with RPMI-1640 medium. The recipient mice were then subjected to irradiation (8.5 Gy) and received $1\times10^7$ bone marrow cells from either C57BL/6 or CD1dko mice. These mice were housed in clean, individually ventilated cages and were provided with acidified (containing 5% Levofloxacin and 0.5% fluconazol) and sterilized food. After 4 weeks of transplantation, the chimeric mice were infused with Ang Ⅱ for 2 weeks before being used for experimentation.

**Flow Cytometry**

Mice (n=3-6 per group) were anesthetized with 1.5% isoflurane after receiving Ang Ⅱ infusion for 14 days. Aortic vessels were extracted and immune cells were analyzed using flow cytometry as previously described[24]. Single-cell suspensions were stained with CD45 Percp, CD3 FITC, CD11b FITC, F4/80 BV421, CD4 BV510, CD8 APC, and IL-17A PE, along with isotype-matched negative controls (from BD, Franklin Lakes, NJ). Events were chosen on a live gate and acquired on a Fortessa flow cytometer (BD) and analyzed. The expression of surface molecules was collected and analyzed by a Beckman Coulter Epics XL flow cytometer (from Beckman Coulter, Miami, FL).

**Vascular Relaxation Studies**

Mice (n=4-6 per group) were anesthetized with 1.5% isoflurane after Ang Ⅱ infusion for 14 days. Intact aortas were gently isolated and placed in cold Krebs Henseleit buffer (37°C, pH 7.2-7.4, containing 120 mM NaCl, 5.5 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2·6H2O, 1.2 mM Na2HPO4, 20 mM NaHCO3, 0.03 mM EDTA-Na2, 10 mM Glucose bubbled with Carbogen gas containing 95% O2 and 5% CO2). The thoracic aorta was cut into 4mm segments and connected with force transducers (Power Laboratory, AD Instruments, Bella Vista, Australia) in four chambers. Changes in isometric tension were recorded using a PowerLab recording system (AD Instruments). The initial tension was set to 0.75g. To test the aorta's relaxation ability, the aorta was stimulated to contract with $1\times10^{-6}$M Noradrenalin (NE), and then a concentration gradient acetylcholine (ACh) and sodium nitroprusside (SNP) was applied to relax the aorta. Relaxation was expressed as a percentage of the precontraction tension by phenylephrine.

**Array analysis for cytokines**

Mice (n=3-6 per group) were anesthetized with 1.5% isoflurane after Ang II infusion for 14 days. The aortic vessels were extracted and the concentrations of cytokines were analyzed using the RayBio Mouse Cytokine Array kit (QAM-TH17-1, RayBiotech) following the manufacturer's instructions. The kit was used to determine the level of 18 cytokines, including Interferon gamma (IFN-γ), IL-1β (IL-1 F2), IL-10, IL-12 p70, IL-13, IL-17A, IL-17F,

IL-2, IL-21, IL-22, IL-23 p19, IL-28A, IL-4, IL-5, IL-6, macrophage inflammatory protein 3(MIP-3 α), TGFβ1 and TNF-α.

**Human Study and Flow Cytometry Analysis**

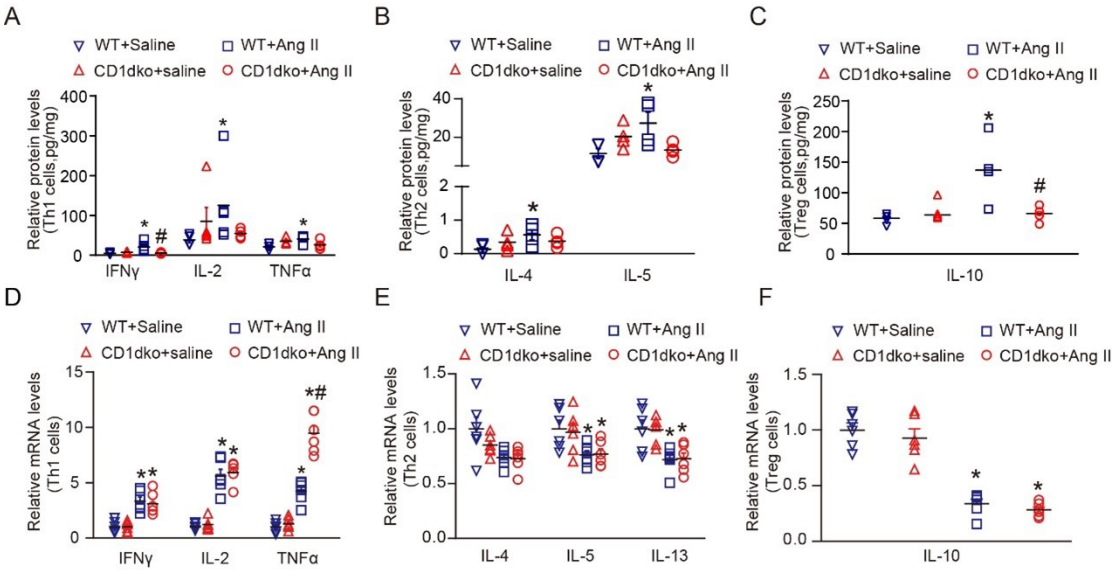
To analyze the changes in the number of NKT cells in patients with hypertension, 87 normotensive individuals and 57 essential hypertensive patients (with systolic blood pressure ≥ 140mmHg and diastolic blood pressure ≥ 90mmHg) were recruited for the study. The baseline information of normotensive individuals is present in Table S1. Blood samples were collected from all participants via antecubital venipuncture using a 21-gauge needle in the recumbent position, with K2EDTA used as anticoagulant. RBC lysis buffer (BD) was used to remove erythrocytes and then the blood was incubated with CD45 Percp-cy5.5, CD3 FITC, CD56 BV421 and TCRγδ APC. The events were acquired using a FACS Canto II$^{TM}$ flow cytometer (BD) and analyzed based on a live gate.

**Table S1. Baseline Information of Normotensive Control Subjects and Hypertensive Patients**

| Parameter | Normotensive Controls(n=87) | Hypertension(n=57) | *P* value |
|---|---|---|---|
| Age(years) | 62.5±0.5 | 60.3±13.4 | 0.236 |
| Male, n (%) | 40(46) | 31(54.4) | 0.324 |
| LVEF (%) | 59.4±1.9 | 57.0±6.9 | 0.015 |
| SBP (mmHg) | 127.4±13.4 | 161.1±24.0 | <0.001 |
| DBP (mmHg) | 78.8±11.5 | 89.4±18.0 | <0.001 |
| Heart rate(bpm) | 68.0±11.3 | 77.5±12.1 | <0.001 |
| Total cholesterol(mmol/L) | 5.6±1.0 | 4.8±1.1 | <0.001 |
| LDL cholesterol(mmol/L) | 3.1±0.7 | 2.7±0.7 | 0.001 |
| HDL cholesterol(mmol/L) | 1.4±0.3 | 1.1±0.3 | <0.001 |
| Triglycerides(mmol/L) | 1.8±1.3 | 1.9±1.8 | 0.668 |
| Creatinemia(umol/L) | 69.3±42.4 | 71.5±20.8 | 0.0714 |
| Fasting blood glucose(mmol/L) | 5.4±1.1 | 6.2±2.8 | 0.039 |
| White blood cell count(10^9/L) | 5.8±1.5 | 6.8±1.9 | 0.001 |

Hypertension patients versus normotensive control. LVEF indicates left ventricular ejection fraction; SBP indicates systolic blood pressure; DBP indicates diastolic blood pressure. LDL and HDL indicates low or high density lipoprotein. All the parameters are shown as mean ± SEM except Male, n (%).

**Figure S1. CD1d deficiency does not exacerbates Ang Ⅱ infusion induced Th1/Th2/Tregs cytokines mRNA and protein level.**




**A)** Protein chip measurement of Th1 cytokines including IFNγ, IL-2 and TNFα protein level in the aorta tissues(n=5-6). **B)** Protein chip measurement of Th2 cytokines including IL-4 and IL-5 protein level in the aorta tissues(n=3-6). **C)** Protein chip measurement of Treg cytokine including IL-10 protein level in the aorta tissues(n=3-6). **D)** qPCR analysis of Th1 cytokines including IFNγ, IL-2 and TNFα mRNA level in the aorta tissues(n=5-6). **E)** qPCR analysis of Th2 cytokines including IL-4, IL-5 and IL-13 mRNA level in the aorta tissues(n=6). **F)** qPCR analysis of Treg cytokine including IL-10 mRNA level in the aorta tissues(n=5-6). Data are expressed as mean±SEM. *$p$< 0.05 compared to WT+Saline; $^{\#}p$< 0.05 compared to WT+Ang Ⅱ.