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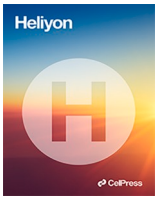

Research article

Role of plasmacytoid dendritic cells in vascular dysfunction in mice with renovascular hypertension

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ABSTRACT

Endothelial dysfunction and inflammation are clinically significant risk factors for cardiovascular diseases in hypertension. Although immune cells play a role in hypertension, the impact of plasmacytoid dendritic cells in established renovascular hypertension-induced cardiovascular complications is not fully understood. We investigated plasmacytoid dendritic cells' contribution to arterial endothelial dysfunction and inflammation in renovascular hypertension.

A two-kidney one-clip (2K1C) model for four weeks in both male and female mice was used to induce renovascular hypertension. We treated mice with or without anti-PDCA-1 antibodies for one week to deplete the plasmacytoid dendritic cells. Renovascular hypertension causes cardiac hypertrophy, lung edema, and microvascular endothelial dysfunction associated with inflammation induction in mice. Moreover, renovascular hypertension affects the profile of immune cells, including dendritic cells and macrophages, with variations between male and female mice. Interestingly, the depletion of plasmacytoid dendritic cells significantly reduces blood pressure, cardiac hypertrophy, lung edema, inflammation, and oxidative stress and improves microvascular endothelial function via the endoplasmic reticulum (ER) stress, autophagy, and mTOR-dependent mechanisms.

Plasmacytoid dendritic cells significantly contribute to the development of cardiovascular complications in renovascular hypertension by modulating immune cells, inflammation, oxidative stress, and ER stress.

1. Introduction

Hypertension is a chronic medical condition that causes vascular endothelial dysfunction, arterial calcification and fibrosis, and cardiac injury, clinically significant risk factors for cardiovascular diseases. Cardiovascular issues are considerable risk factors for myocardial infarction, coronary artery disease, and stroke in patients with established hypertension [1–3]. There are numerous antihypertensive medications available [4–6]. Nevertheless, cardiovascular complications associated with established hypertension continue to increase significantly, and only one-third of patients are able to manage hypertension with multiple therapies due to side effects. Management of hypertension alone may not be sufficient to prevent cardiovascular disorders. Therefore, developing new

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approaches to protect from cardiovascular complications in established hypertension is essential. Vascular endothelial dysfunction and cardiovascular structure pathology have long been appreciated in hypertension [7–9]. T-cells can increase arterial blood pressure through oxidative stress and sodium reabsorption in the kidney. These effects are mediated by cytokines from T cells that impact renal epithelial function and, in some cases, via direct interaction with renal tubular cells. Dendritic cells (DCs) are the most potent antigen-presenting cells, activating T-cells and playing a critical role in the immune-mediated increase in blood pressure. However, the impact of the plasmacytoid dendritic cells in these pathologies is not yet fully understood in the setting of established renovascular hypertension. Dr. Harrison's group demonstrated that the immune T-cells are preventive in hypertension-induced vascular dysfunction (angiotensin II-dependent) [10]. In 2011, we were the first to show that transferring healthy immune regulatory $CD4^{+}CD25^{+}$ cells (Treg) in mice infused with angiotensin II protected against the development of hypertension and vascular dysfunction. Dr. Schiffrin reported similar results, and other laboratories supported our findings, emphasizing that Treg cells significantly regulate cardiovascular function [11–14].

Plasmacytoid dendritic cells are a type of innate immune cells that circulate in the blood and are found in tissues [15]. These dendritic cells play a crucial role in orchestrating both innate and adaptive immune responses in hypertension and kidney disease. They also contribute significantly to T-cell responses, antiviral immunity, and autoimmune diseases [16]. Moreover, Plasmacytoid dendritic cells are also characterized by the excretion of proinflammatory cytokines including INFα/β, IL-6, IL-12, and IL-18 [17]. Besides the central role of dendritic cells in presenting antigen properties, recently, it has been reported that dendritic cells are involved in cardiovascular diseases. In 2014, Dr. Harrison's group reported that active dendritic cells contribute to hypertension induction, likely by producing reactive oxygen species and iso-ketal proteins [18]. Dieterlen et al. showed that dendritic cells are activated and produce reactive oxygen species in hypertension [19]. It has been reported that dendritic cells play a preventive role in mice lacking Fms-like tyrosine kinase 3 ligands infused by angiotensin II, stimulating the development of classical dendritic cells [20]. These studies indicate the importance of dendritic cells in hypertension-induced cardiovascular complications. However, there is no information regarding the impact of plasmacytoid dendritic cells in renovascular hypertension-induced cardiovascular complications. To determine the role of the immune dendritic cells in hypertension, it is crucial to identify the specific subpopulations of DCs responsible for promoting arterial blood pressure. Thus, in the current study, we aimed to investigate the impact of pDCs on vascular endothelial dysfunction in renovascular hypertension. This study is the first to comprehensively assess the impact of plasmacytoid dendritic cells on renovascular hypertension-induced cardiovascular dysfunction and structural remodeling. In our studies, we aimed to determine the effect of renovascular hypertension on plasmacytoid dendritic cells (pDC), conventional type 1 dendritic cell (cDC1), conventional type 2 dendritic cell (cDC2) and macrophages profiles, vascular function, and inflammation. Renovascular hypertension is a silent disease, so prevention is almost impossible. Moreover, managing patients with established renovascular hypertension is challenging because stenting the renal artery does not improve renal and cardiovascular function and structural remodeling [21]. Overall, the primary goal of the study is to elucidate that manipulating plasmacytoid dendritic cells in mice with established 2K1C renovascular hypertension could open novel avenues for therapeutic approaches to improve renovascular hypertension-induced cardiovascular complications.

2. Materials & methods

2.1. Mice

All experimental procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee at the Eastern Virginia Medical School. The male and female C57BL/6J mice, eight weeks old, were obtained from Jackson Laboratory in Bar Harbor, ME. They were kept in a temperature-controlled room (22 ± 1 °C), exposed to a 12-h dark-light cycle, and provided with a regular chow research diet and unlimited access to Reverse Osmosis water. Euthanasia was performed using an isoflurane overdose, followed by heart excision.

2.2. Renovascular hypertension induction using a two-kidney one-clip (2K1C) model

We used the method established by Wiesel and Stocker [22,23] to produce a mouse model of 2K1C renovascular hypertension. Briefly, we anesthetized mice with isoflurane (2–3% in 100 % oxygen) and injected them with buprenex^{SR}. After making a retroperitoneal incision, the right kidney was gently retracted, and a small section at the midpoint of the renal artery was separated from the renal nerve. A 0.5 mm length polytetrafluoroethylene (PTFE) tubing with an inner diameter of 0.008 in. and an outer diameter of 0.014 in., Product no. SUBL 140 from Braintree Scientific, was cut longitudinally. It was then clipped around the isolated right renal artery and secured by two 10-0 circumferential surgical sutures. In the control/sham mice, the renal artery was exposed, but no clip was implanted. Finally, the incision was closed with a 7 mm surgical reflex clip.

2.3. In-vivo treatment with anti-mPDCA-1 mAb

Male and female mice were randomly divided into three groups, each consisting of 5–6 animals: Group 1: Wild-type mice C57BL/6J served as the control group and did not receive any treatment. Group 2: Wild-type mice C57BL/6J were subjected to 2K1C to induce renovascular hypertension. Group 3: Wild-type mice C57BL/6J were subjected to 2K1C for four weeks, and then treated with anti-mPDCA-1 (Miltenyi Biotech Inc, Clone JF05-1C2.4.1, isotype: rat IgG2b, species reactivity: mouse, Lot No: 5,210,305,844) at a dose of 500 μg/mouse. The intraperitoneal injection was administered every other day for one week.

2.4. Blood pressure and heart, kidney, and lung weight measurements

Mice were initially trained and acclimated to blood pressure measurements. After the 2K1C surgery, we measured body weight and systolic blood pressure on a weekly basis. We measured systolic blood pressure (SBP) using the CODA tail-cuff blood pressure system from Kent Scientific, Torrington, USA [24]. Arterial blood pressure measurements were taken between 9 a.m. and 11 a.m. to minimize the influence of the circadian cycle. After the treatment, mice were euthanized, and blood and tissues (heart, lung, left and right kidneys, and mesenteric resistance arteries) were collected. All collected tissues were promptly placed in a cold PSS (physiological saline solution) containing NaCl 118 mM, KCl 4.7 mM, CaCl2 2.5 mM, KH2PO4 1.2 mM, MgSO4x7H2O 1.2 mM, NaHCO3 25 mM, and glucose 11 mM at pH 7.4, and processed accordingly for further studies. Blood samples were centrifuged at 14000 rpm for 5 min at 4 °C to obtain plasma, which was then immediately stored at −80 °C.

2.5. Vascular endothelial function

As previously reported, the reactivity of mesenteric resistance arteries (MRA) was assessed in all groups [24,25]. After being removed from each group, the MRAs were promptly submerged in cold PSS solution, cleared of perivascular fat and connective tissue, and then cut into 2 mm length rings. These artery rings were then placed in small dual chambers (DMT myograph; AD Instruments Ltd., Oxford, U.K.) to measure isometric tension. After a 1-h incubation, artery rings were pre-constricted with phenylephrine (PE 10^{-8} – 10^{-4} M, Sigma Aldrich P6126-50G, CAS 61-76-7). Once a steady maximal contraction was reached, cumulative dose-response curves were obtained for acetylcholine (Ach, 10^{-8} – 10^{-4} M, Sigma Aldrich, A6625-25G, Lot#BCBH3758V) and sodium nitroprusside (SNP, 10^{-8} – 10^{-4} M, Sigma Aldrich, S-0501, Lot:81K3688).

To determine the impact of autophagy, ER stress, and mTOR pathways in endothelial cell function, we isolated arteries from each group and incubated them with the following inhibitors: tauroursodeoxycholic acid (Tudca: ER Stress inhibitor) Dose: 10 mM, 30 min, Chem-Impex Int'l INC. Cat#29195, Lot# 002129–20131118; chloroquine (autophagy inhibitor, dose: 10 mM, 30 min, Alfa Aesar, Cat: J64459, Lot: Z23G009); or rapamycin (mTOR inhibitor, dose: 10 mM, 30 min, Abcam, Cat:ab120224, Lot: APN13087-1-1). After 30 min of incubation, we conducted vascular contraction to PE and relaxation to ACh and SNP on pre-contracted MRA.

2.6. Flow cytometry

Spleens were collected using aseptic techniques and processed to isolate splenocytes using a modified protocol [24]. The following procedure was used to isolate splenocytes from the spleens of C57BL/6 mice, including those subjected to 2K1C and those treated with anti-mPDCA-1 antibodies: The spleens were cut into small pieces and strained using a 40-μm cell strainer. The spleen slices were transferred to a Petri dish and incubated with 1 mg/mL of type I collagenase (Worthington) for 30 min in 1 % FBS complete RPMI media at 37 °C on a shaker. After incubation, splenic contents were collected and washed with MACS buffer at 2000 rpm at 4 °C for 5 min. Spleen fragments were gently pressed with a syringe plunger to remove clumps for clear separation and passed through a 70 μM filter in PBS. Cells were centrifuged at 2000 rpm, and RBC in the pellet was cleared in lysis buffer (8.3 g/L ammonium chloride in 0.01 M Tris-HCl buffer on ice for 5 min incubation. Cells were washed with MACS buffer at 2000 rpm for 5 min at 4 °C. The cell pellet was resuspended in 2 mL of PBS, and then the cell count was determined using a cello meter with AOPI dye. 1x 10^6 cells were stained with fluorochrome anti-markers to quantify dendritic cells and macrophages on ice for 30 min as per the manufacturer's recommendation. After incubation, the cells were washed with PBS at 2000 rpm for 5 min at 4 °C. The supernatant was decanted, and the pellet was resuspended in 200 μL of MACS buffer. Stained single-cell suspensions were analyzed using a Cytek Aurora DXP8 flow cytometer, and the data were analyzed using FlowJo software. The following antibodies were obtained from Biolegend: anti-CD45 (clone 30-F11), anti-CD163 (clone TNKUPJ), anti-CD80 (clone 16-10A1), anti-CD206 (clone C068C2), anti-CD45R (clone RA3-6B2), anti-CD19 (Clone 6D5), anti-CD23 (clone B3B4), anti-CD43 (clone S11), Siglec-H a (clone 440C), anti-CDC11C (clone N418), anti-CD11b (M1/70), anti-MHC-II (M5/114.15.2), anti-CD8a (clone 53-6.7), anti-CD103 (clone 2E7). Siglec-H a (clone 440C) was purchased from BD Bioscience, and anti-CD23 (clone B3B4) was purchased from Invitrogen.

2.7. Measurement of nitrate and nitrite

Nitric oxide's breakdown products nitrate and nitrite were measured in urine samples from all mouse groups using the nitrate/nitrite assay kit (Cayman Chemicals, # 780001, Batch 0600896) according to the manufacturer's protocol.

2.8. Cytokines/chemokines analyses using antibody array membrane

The cytokines and chemokines were analyzed using the RayBio® C-Series mouse cytokine antibody array C1 (RayBio®, #AAM–INF–1-8), following the manufacturer's protocol. The pre-coated antibody membranes were placed on the plastic tray provided in the array kit. Subsequently, 200 μl of plasma from all groups of mice was added on top of the membranes and incubated at 4 °C overnight. The next day, the plasma samples were removed, and the membranes were washed with wash buffers I and II. Following this, they were incubated with 1 ml of biotinylated antibody cocktail overnight at 4 °C. After washing, the membranes were incubated with HRP Streptavidin antibody at 4 °C overnight. Finally, we used detection buffers A and B to develop a chemiluminescent signal with the LI-COR instrument.

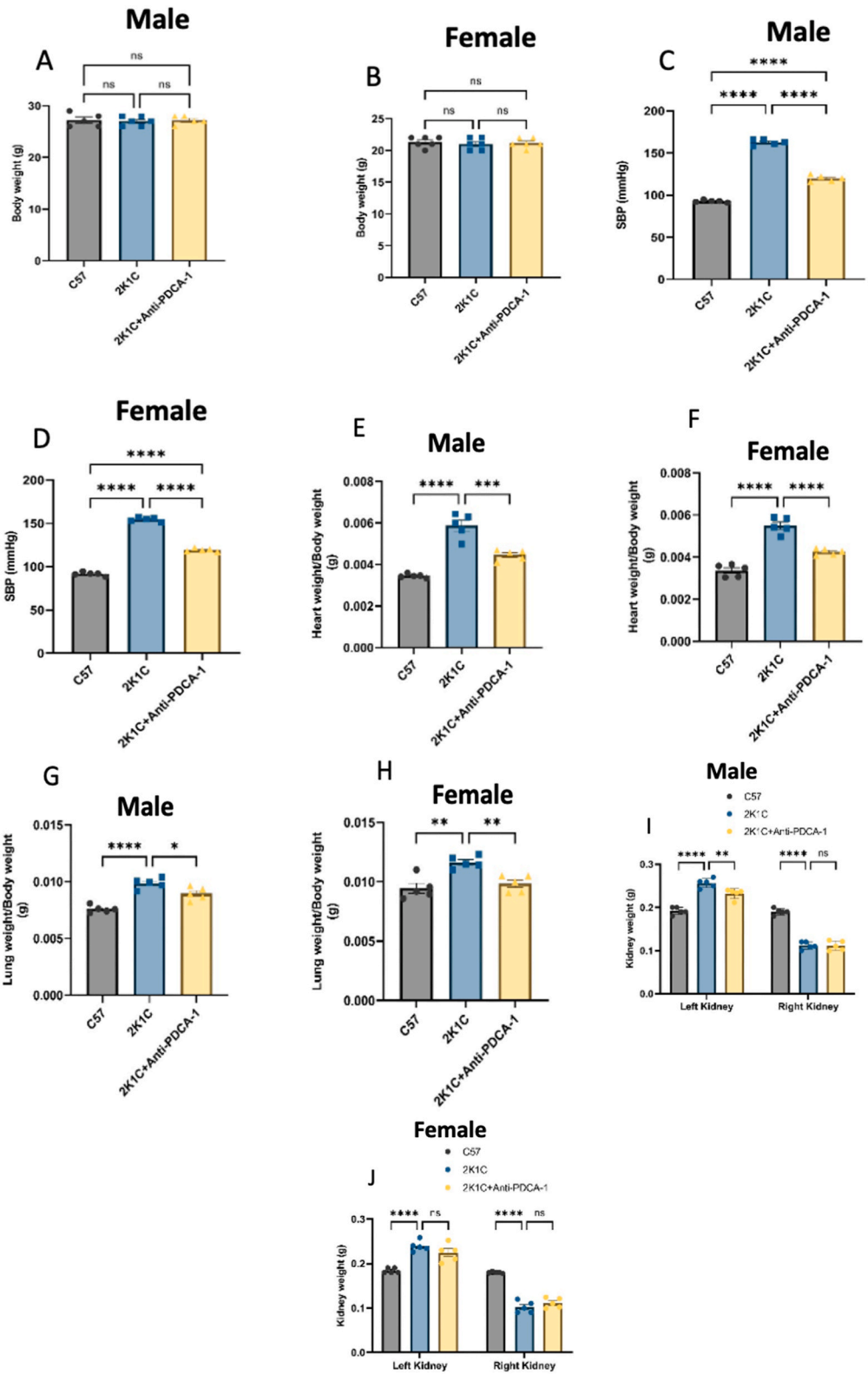

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Fig. 1. Impact of 2K1C Renovascular hypertension on various parameters in Male and Female mice (n=5–6). The study evaluates the impact of renovascular hypertension on several key physiological measures, encompassing body weight (A, B), systolic arterial blood pressure (C, D), the ratio of heart weight to body weight (E, F), the ratio of lung weight to body weight (G, H), and the weight of kidneys (I, J) within all experimental groups of male and female mice. The study utilized a meticulous assessment approach to scrutinize the detailed effects of renovascular hypertension on each parameter within the designated groups. One-way ANOVA followed by Tukey's post hoc test was applied. ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ for comparisons between C57BL/6J vs. 2K1C vs. 2K1C + anti-PDCA-1.

2.9. Quantifying angiotensin II (Ang II) levels in mouse plasma

Renovascular hypertension induced by 2K1C is characterized by elevated levels of renin and Ang II. The plasma samples from all groups of mice were used to measure the level of Ang II using the Ang II enzyme-linked Immunosorbent assay (ELISA) kit, following the manufacturer's protocol (Enzo Life Sciences, #ADI-900-204). We used optical density at 450 nm to determine the concentration of Ang II in the samples.

2.10. Western blot analysis

Western blot analysis was utilized to identify specific proteins in lysates of mouse MRA tissue, following the previously established method [24,26,27]. We harvested MRAs from all groups of mice and immediately stored them at −80 °C. Tissue lysates were prepared using an electrical homogenizer after adding ice-cooled RIPA lysis buffer (Prod# SC-24948A, Santa Cruz), sonicated for 5 s, and centrifuged for 20 min at 14,000 rpm. Protein quantification was performed according to Pierce™ BCA Protein Assay Kit (Product No. 23225). We used specific antibodies against Anti-Bip (C50B12, Cell Signaling, #3177), Anti-CHOP (Cell Signaling, #2895), iNOS (Proteintech, # 18985-1-AP), eNOS (Cell Signalling, D9A5L Rabbit mAb # 32027), Phospho-eNOS (Ser 1177, Cell Signalling (C9C3) Rabbit mAb 9570)), Anti-Cox2 ((Cayman, # 160126), Anti-NOX1 (Novus, #NBP131546), Anti-NOX2 (Proteintech, #19013-1-AP), Anti-ATF-6 (Abcam, #Ab37149), and beta-actin (Santa Cruz #SC47778). All dilutions were prepared according to the recommendation of the manufacturer. We developed the membranes using the Odyssey-imaging system LICOR, and the bands were quantified.

2.11. Statistical analysis

We presented the data as mean ± SEM. To determine significant differences, statistical calculations were achieved using GraphPad Prism 10.1.0 (GraphPad Software), which included One-way or two-way ANOVA followed by Tukey's post hoc test. Comparisons are considered non-significant when ns $p > 0.05$, and significant when *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ for C57BL/6J vs. 2K1C vs. 2K1C + anti-PDCA-1.

3. Results

3.1. Induction of 2K1C Goldblatt renovascular hypertension and its effect on various parameters in male and female mice

The body weight of male and female mice did not change across all groups (Fig. 1 A, B). 2K1C surgery increases arterial blood pressure in male and female mice. Blood pressure significantly decreases after depleting the plasmacytoid dendritic cells (Fig. 1 C, D). These results indicate the significance of plasmacytoid dendritic cells in regulating arterial blood pressure in both male and female mice. Moreover, chronic renovascular hypertension causes heart hypertrophy and lung weight, which are reduced after deleting the plasmacytoid dendritic cells (Fig. 1E- H). Furthermore, the 2K1C procedure resulted in a reduction in the size of the right kidney, while the size of the left kidney increased (Fig. 1I J); the size of both male and female mice's kidneys remained unaffected despite the depletion of the plasmacytoid dendritic cells (Fig. 1I and J).

3.2. Impact of 2K1C renovascular hypertension on immune cells profiles

The immune system significantly contributes to hypertension-induced cardiovascular complications. However, the profile and the impact of plasmacytoid dendritic cells in renovascular hypertension-induced cardiovascular complications is yet to be determined. We used flow cytometry to determine the number of dendritic cell subpopulations (plasmacytoid dendritic cells "pDC," myeloid/conventional DC1 "cDC1", and myeloid/conventional DC2 "cDC2"), proinflammatory macrophages type-I in the spleens of male and female mice from all groups. The data illustrate that renovascular hypertension increases the number of plasmacytoid dendritic cells (pDC) in both male and female mice (Fig. 2A and B). The number of cDC1 did not change between all groups of male mice (Fig. 2C). In female mice, however, the cDC1 number was lower in control and increased after 2K1C but was not affected by the depletion of pDC (Fig. 2D). The number of cDC2 was decreased in male and female mice subjected to 2K1C with and without depletion of pDC (Fig. 2E and F). Additionally, renovascular hypertension increases the proinflammatory macrophage type-I number in male and female mice (Fig. 2G and H). Interestingly, the depletion of pDC reduced macrophage type-I in mice with renovascular hypertension (Fig. 2G and H).

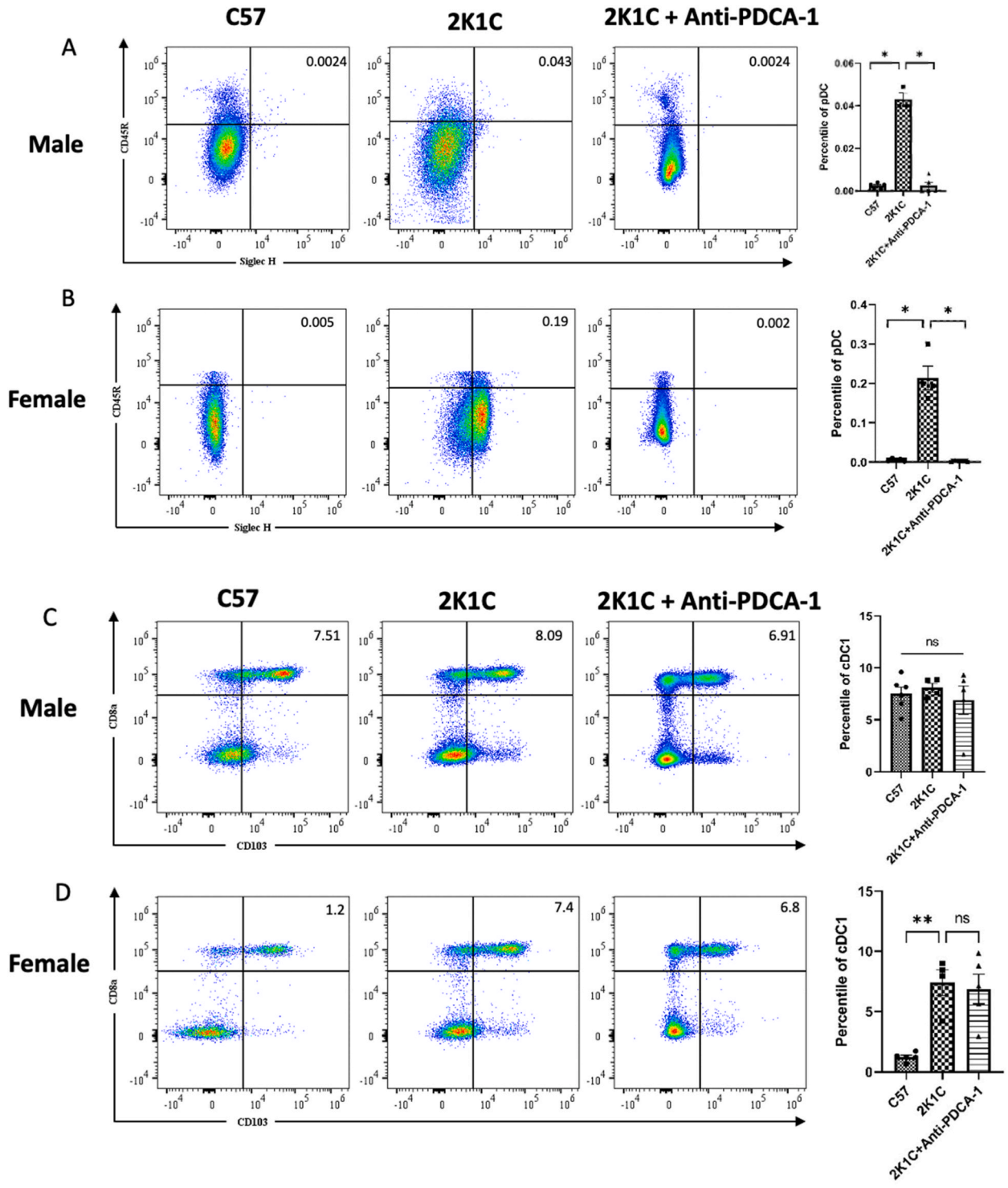

Fig. 2. Flow cytometry charts and cumulative data depicting the proportions of various dendritic cell subsets, including plasmacytoid dendritic cells (pDC), conventional type 1 dendritic cells (cDC1), and conventional type 2 dendritic cells (cDC2), alongside type-I macrophages, in the spleens of both male and female mice across all experimental groups. The flow cytometry analysis comprehensively assessed these immune cell populations, enabling a detailed comparison between treatment conditions and genders. The provided data furnish valuable insights into these immune cell subsets' intricate distribution and dynamic behavior within the specific experimental context under investigation. One-way ANOVA followed by Tukey's post hoc test was applied for **A-H.** ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$. ****$p < 0.0001$ for comparisons between C57BL/6J vs. 2K1C vs. 2K1C + anti-PDCA-1.

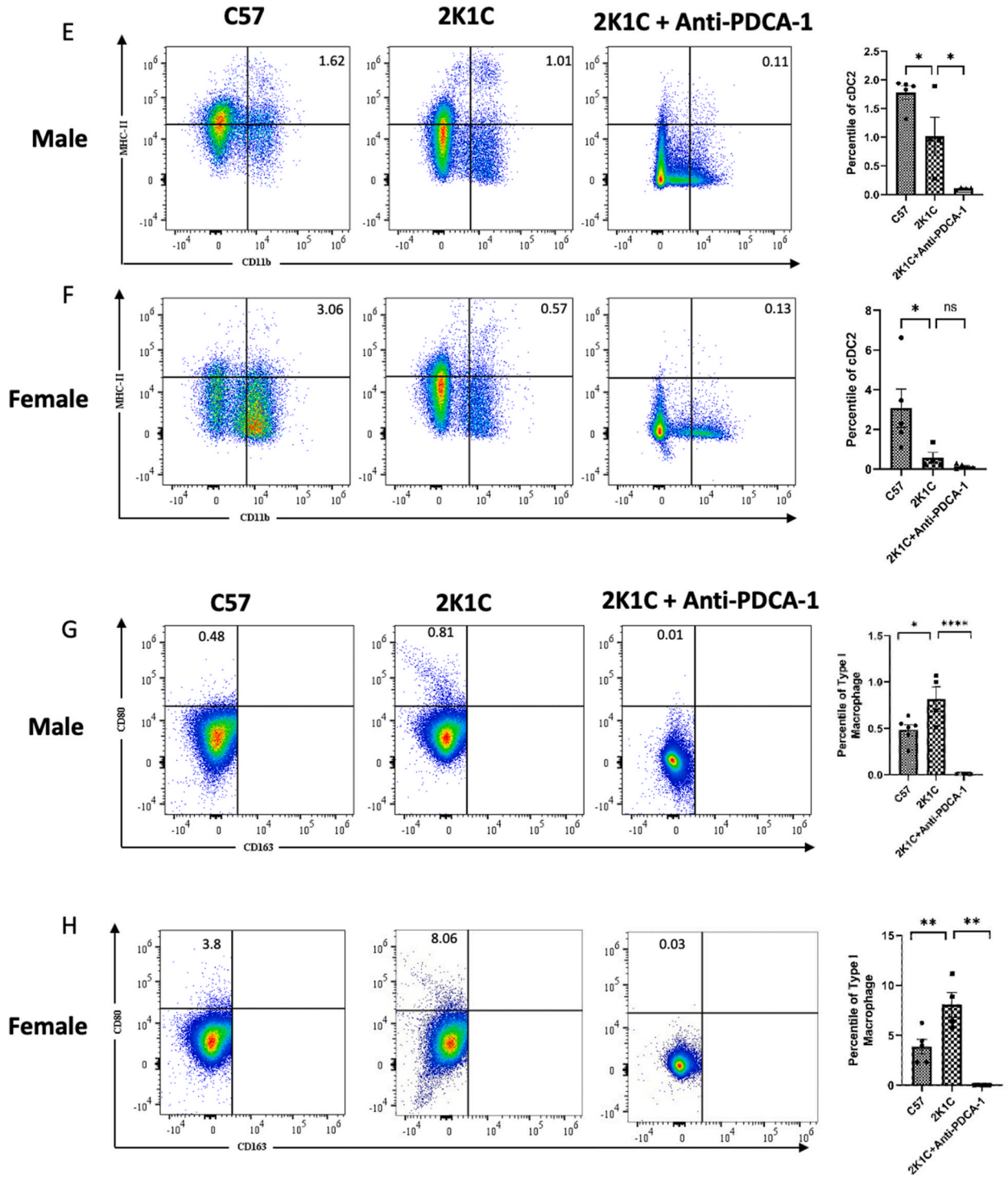

Fig. 2. (*continued*).

3.3. Impaired renal arterial microvascular function

To assess the vasoreactivity of the C57BL/6J vs. 2K1C vs. 2K1C + anti-PDCA-1 mice MRA using wire myography. The contractile response to phenylephrine and the endothelium-independent relaxation to nitric oxide donor in the mesenteric resistance artery (MRA) were similar across all groups of mice (Fig. 3A–F). However, MRA from male and female mice subjected to 2K1C showed

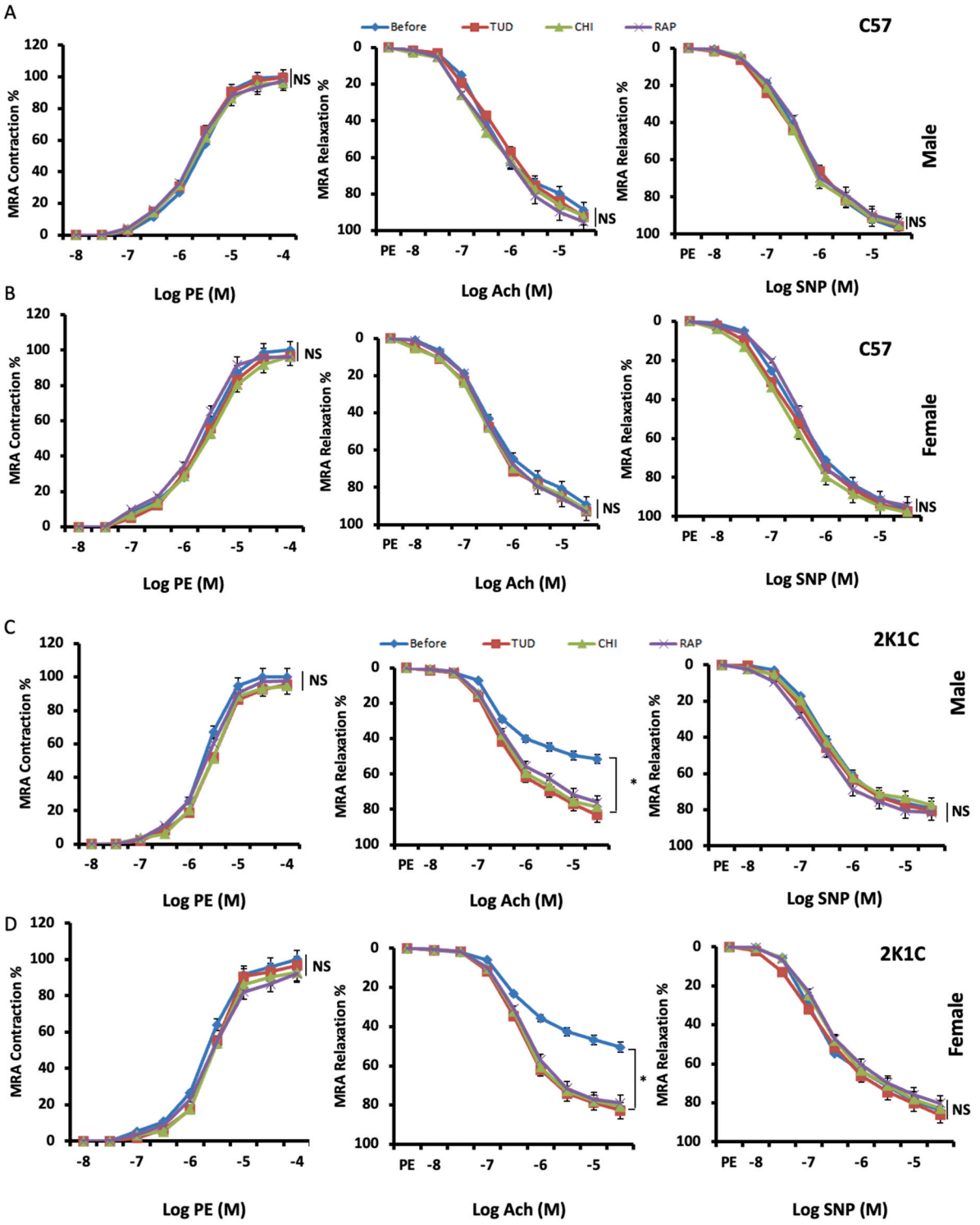

Fig. 3. Mesenteric resistance arteries (MRA) Reactivity Assessment. This figure delineates the evaluation of mesenteric resistance arteries (MRA) reactivity, specifically illustrating the contractility response to sympathetic stimulation (Phenylephrine, PE), as well as endothelium-dependent and -independent relaxation responses to acetylcholine (ACh) and sodium nitroprusside (SNP) across diverse experimental groups. Panel (A, B) represents the control group (C57BL/6J), while panel (C, D) shows C57BL/6J mice subjected to 2K1C surgery. Panel (E, F) displays C57BL/6J mice subjected to 2K1C for four weeks and treated with anti-PDCA-1 for one week. MRA reactivity was thoroughly evaluated in all groups of mice (n = 5) under different conditions, including those with and without ER stress inhibitor (Tauroursodeoxycholic acid: Tudca), autophagy inhibitor (Chloroquine: Chl), and mTOR signaling inhibitor (Rapamycin: Rap). The data presented in this figure provide valuable insights into the

vascular response in the context of the experimental manipulations and treatments, shedding light on the potential involvement of ER stress, autophagy, and mTOR signaling pathways in mediating the observed reactivity changes. ns: $p > 0.05$, *$p < 0.05$ for 2K1C vs. control and 2K1C + anti-mPDCA-1. One-way ANOVA followed by Tukey's post hoc test was applied. ns: $p > 0.05$, *$p < 0.05$ for C57BL/6J vs 2K1C vs 2K1C + anti-PDCA-1.

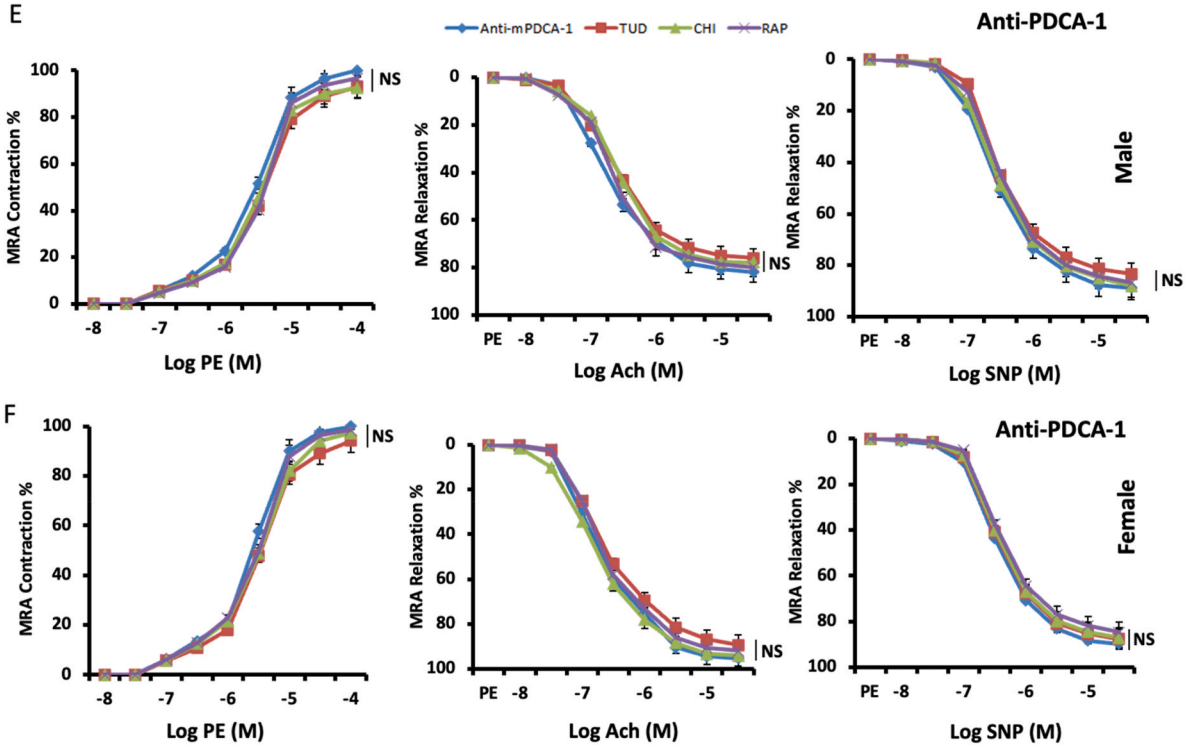

Fig. 3. (*continued*).

endothelial dysfunction, significantly improving after anti-PDCA-1 treatment (Fig. 3C–F). In terms of mechanism, MRAs were incubated with Tudca (inhibitor of ER stress), chloroquine (autophagy inhibitor), and rapamycin (mTOR inhibitor) in all groups. The contractility and relaxation of MRA from control C57BL/6 mice were identical before and after incubation with Tudca, chloroquine, or rapamycin (Fig. 3A and B). However, the incubation of MRA from hypertensive mice with Tudca, chloroquine, or rapamycin significantly improved endothelial-dependent relaxation (Fig. 2C and D).

Interestingly, the anti-PDCA-1 treatment improved MRA endothelial function in mice subjected to 2K1C (Fig. 3E and F)). Also, we did not see any effect on the relaxation of MRA isolated from mice subjected to 2K1C and treated with anti-mPDCA-1 when incubated in-vitro with Tudca, chloroquine, or rapamycin (Fig. 3E and F). These results indicate that the plasmacytoid dendritic cells are essential in regulating microvascular endothelial function, probably through ER stress, autophagy, and mTOR mechanisms in male and female mice.

3.4. Angiotensin II (Ang II) and urinary nitrate/nitrite levels in 2K1C-Induced renovascular hypertension

Ang II is a potent vasoconstrictor and an essential element of the renin-angiotensin-aldosterone system (RAAS). Nitrate (NO3-) and nitrite (NO2-) are nitrogen oxides that serve as markers of nitric oxide (NO) production. Elevated Ang II and reduced urinary nitrate/nitrite levels are also characteristic of 2K1C-induced renovascular hypertension. Plasma samples from male and female mice were analyzed for Ang II levels. The data show that Ang II levels increased in male and female mice subjected to 2K1C surgery (Fig. 4A and B). Removing the word "still" and rephrasing the sentence for clarity: Depletion of plasmacytoid dendritic cells reduced Ang II levels in 2K1C-subjected mice, although these levels remained higher than in control (Fig. 4A and B). Additionally, we measured nitrate/nitrite levels in all groups. We found that the levels of nitrate/nitrite were reduced in the urine of C57BL/6 subjected to 2K1C compared to control mice and mice subjected to 2K1C and treated with anti-mPDCA-1 (Fig. 4C and D).

3.5. ER stress and oxidative stress in 2K1C induced renovascular hypertension

Hypertension is associated with increased oxidative and ER stress and reduced eNOS phosphorylation. As shown in Figs. 1 and 3, renovascular hypertension significantly increases arterial blood pressure and causes microvascular endothelial dysfunction. Here, we showed a decrease in eNOS phosphorylation. At the same time, oxidative stress markers (NOX1, NOX2) and the ER stress markers

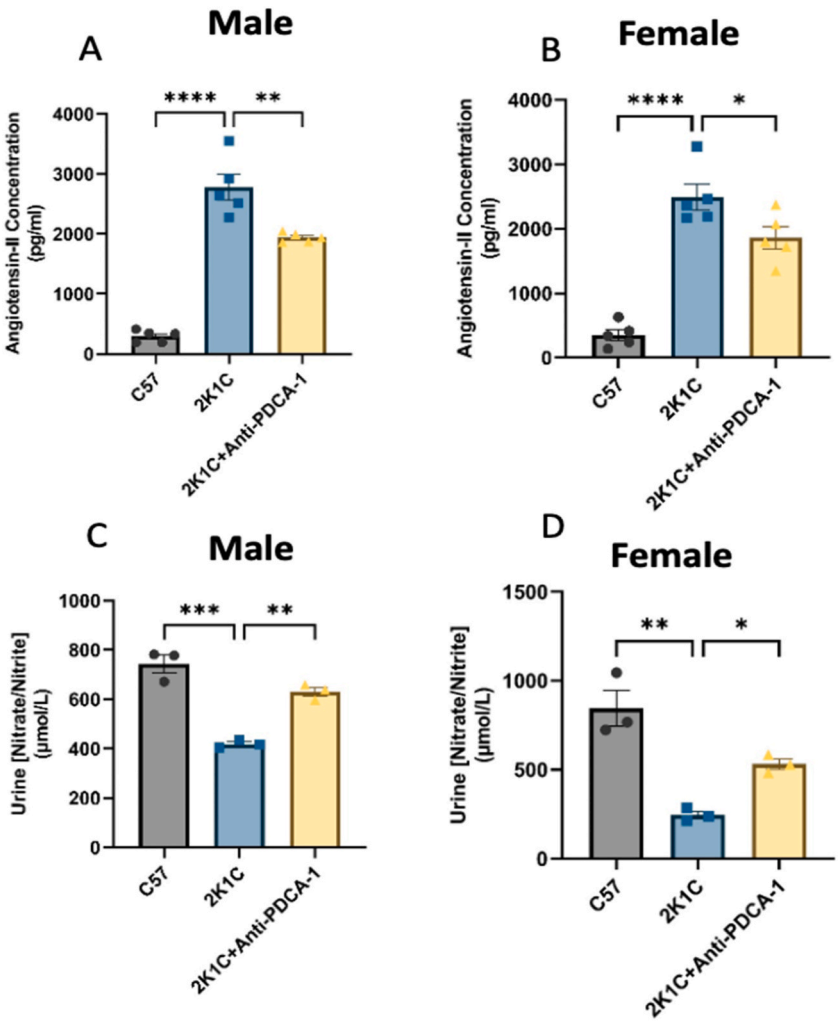

Fig. 4. Impact of Renovascular Hypertension on Plasma Angiotensin-II (Ang-II) and Urinary Nitrate/Nitrite Excretion. This figure elucidates the influence of renovascular hypertension on plasma Ang-II levels and urinary nitrate/nitrite excretion in male and female mice across the C57BL/6J, 2K1C, and 2K1C + anti-PDCA-1 groups. Panels (A, B) represent plasma Ang-II excretion expressed in pg/mL, while panels (C, D) display urinary nitrate/nitrite levels expressed in µmol/L in all male and female groups. Statistical analysis involved the application of one-way ANOVA followed by Tukey's post hoc test, which was applied for statistical analysis. Significance levels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ for comparisons between C57BL/6J vs. 2K1C vs. 2K1C + anti-PDCA-1groups.

(ATF6, BIP, and CHOP) expressions were significantly increased in MRA from both male and female mice subjected to 2K1C versus control mice and mice subjected to 2K1C and treated with anti-mPDCA-1 (Fig. 5A, B, C, D, E, F). These results suggest that plasmacytoid dendritic cells may contribute to microvascular endothelial dysfunction by affecting eNOS phosphorylation, oxidative stress, and ER stress factors. The presented data contributes to a better understanding of the cellular and molecular mechanisms involved in hypertension-related microvascular complications.

3.6. Regulation of inflammation in renovascular hypertension

It is well-known that inflammation plays a critical role in hypertension-induced cardiovascular complications. In renovascular hypertension. In both male and female mice with renovascular hypertension, we observed increased COX2 and iNOS expression in MRA compared to control mice (Fig. 6A, B & E, F). Interestingly, the expressions of COX2 and iNOS were significantly reduced in mice subjected to 2K1C and treated with anti-mPDCA-1 (Fig. 6A, B & E, F). Furthermore, we analyzed the inflammatory plasma cytokines/chemokines using an antibody array membrane kit. Data show an increase in the inflammatory factors (INF-γ, IL-1α, IL-1β, IL3, IL4, IL6, IL9, IL-12, IL13, IL17, and TNFβ) (Fig. 6C, D & G, H.) in mice subjected to 2K1C surgery compared to control mice (Fig. 6C, D & G, H). Interestingly, the depletion of plasmacytoid dendritic cells significantly reduced the inflammatory factors (INF-γ, IL-1α, IL-1β, IL3, IL4, IL6, IL9, IL-12, IL13, IL17, and TNFα) (Fig. 6C, D & G, H). These data suggest the essential role of the plasmacytoid dendritic cells in regulating inflammation in renovascular hypertension. Modifying COX2, iNOS, and plasma inflammatory factors by targeting

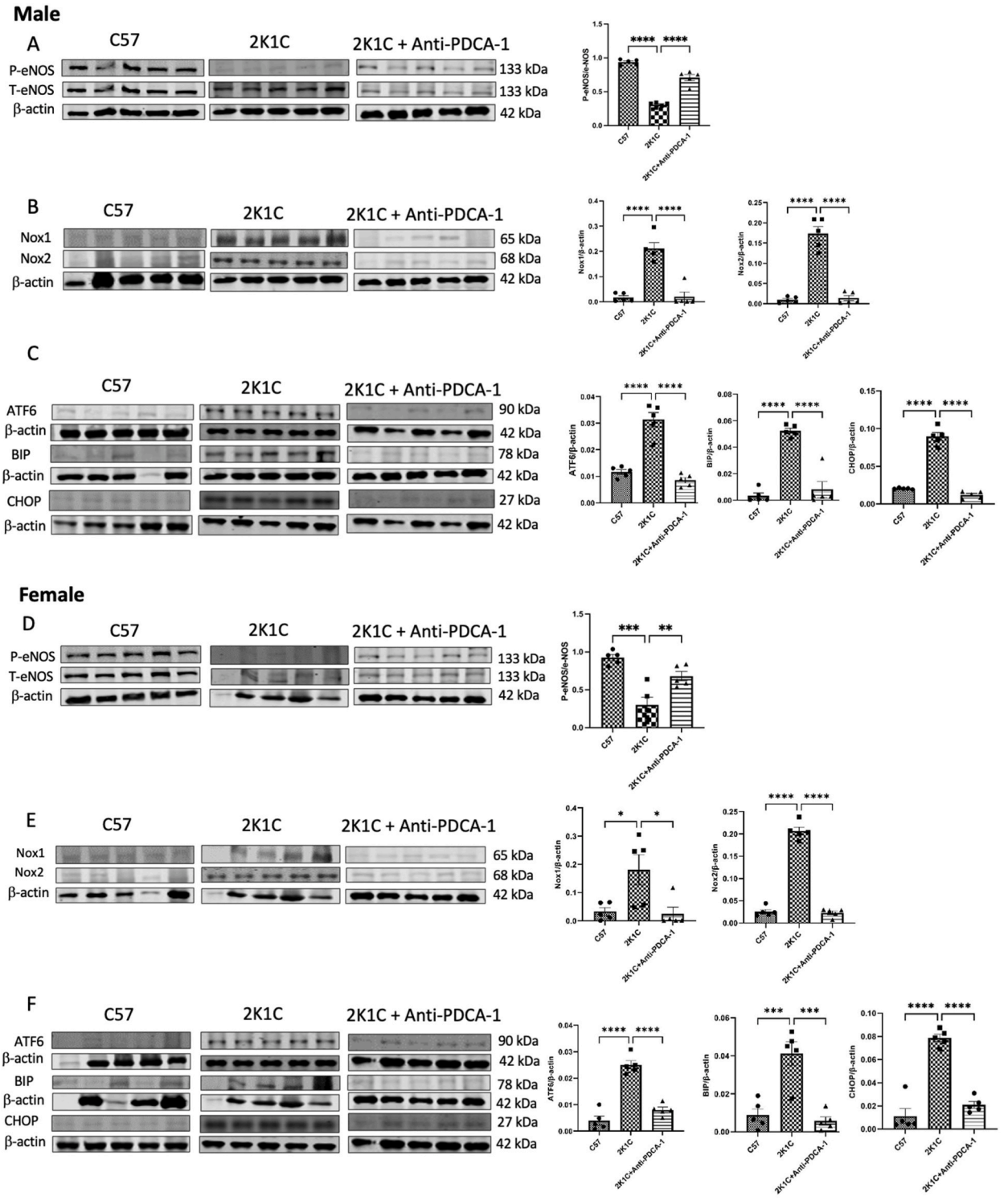

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Fig. 5. Western Blot Analysis of Vascular Function and Stress Responses: This figure presents the outcomes of Western blot analysis, offering detailed insights into the protein expression levels of pivotal molecular components implicated in vascular function and stress responses. The cumulative data presented enable meticulous comparative assessments of protein levels across distinct experimental groups, thereby facilitating the exploration of potential variations in vascular signaling pathways and stress-mediated mechanisms. This figure represents the results of Western blot analysis and Cumulative data for phosphorylated endothelial nitric oxide synthase (P-eNOS), NADPH oxidase 1 (Nox1), NADPH oxidase 2 (Nox2), along with endoplasmic reticulum stress markers Activating Transcription Factor 6 (ATF6), Binding Immunoglobulin Protein (BIP), and CCAAT/enhancer-binding protein (CHOP) in mesenteric resistance arteries (MRA) isolated from the male (A, B, C) and female mice (D, E, F) across all experimental groups (n = 5), Statistical analysis involved the application of One-way ANOVA followed by Tukey's post hoc test was applied for **A-F.** *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 for comparisons between C57BL/6J vs 2K1C vs 2K1C + anti-PDCA-1groups.

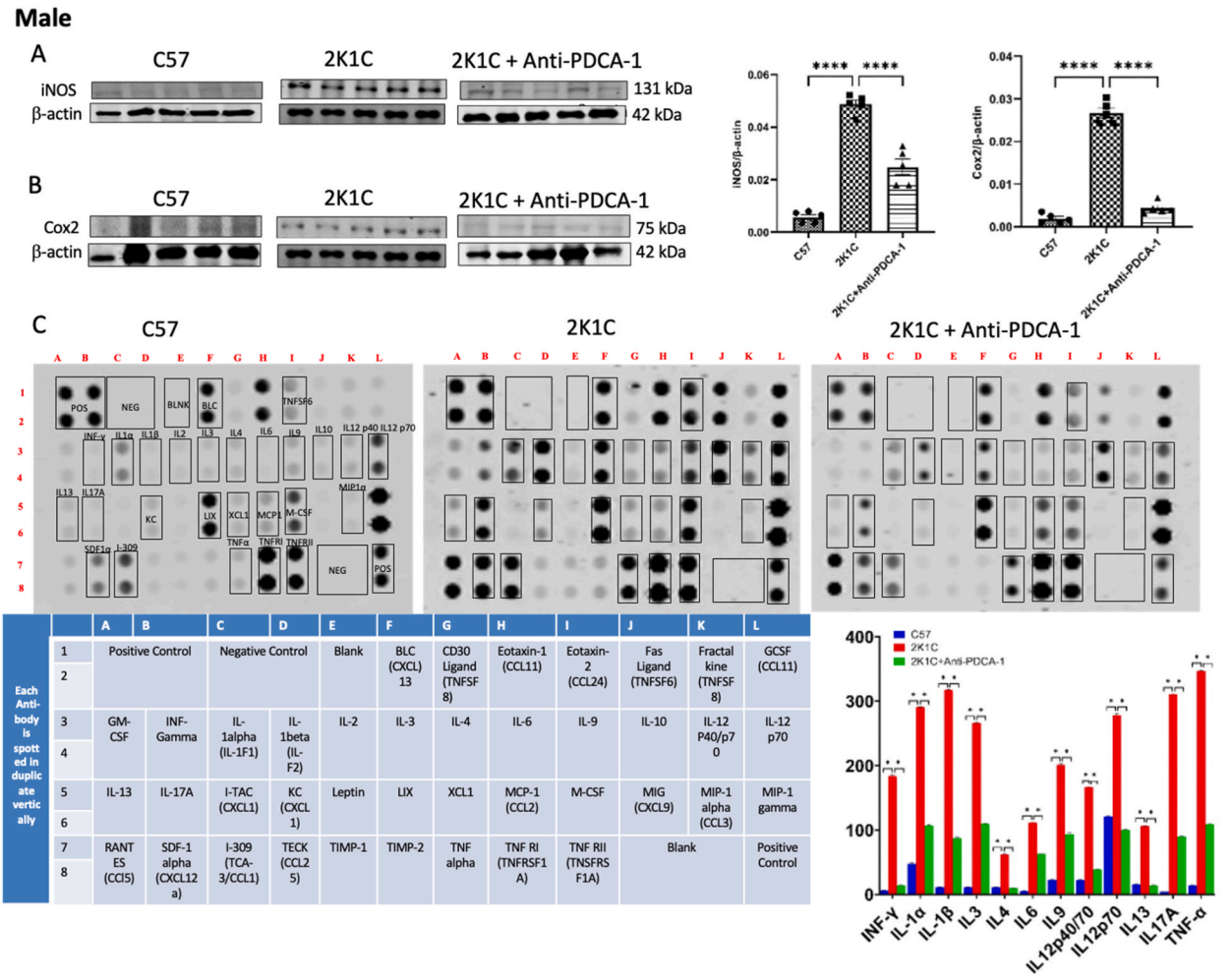

Fig. 6. Inflammatory Responses Analyzed in Mesenteric Resistance Arteries and Plasma Cytokines/Chemokines Profiling. This figure presents the results of Western blot analysis and cumulative data for the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (Cox2) in mesenteric resistance arteries (MRA). Concurrently, the inflammatory plasma cytokines/chemokines antibody array helps evaluate the inflammatory profile in the plasma of male (A, B, C, D) and female mice (E, F, G, H). Statistical analysis involved the application of One-way ANOVA followed by Tukey's post hoc test applied for A, B, E, and F. Two-way repeated measured ANOVA followed by Bonferroni post hoc test was applied for D, H. *p < 0.05, **p < 0.001. ****p < 0.0001 for comparisons between C57BL/6J vs 2K1C vs 2K1C + anti-PDCA-1 groups.

plasmacytoid dendritic cells offers therapeutic potential for mitigating hypertension-related complications.

4. Discussion

Hypertension is a chronic disease affecting billions of people worldwide and represents a significant risk for cardiovascular diseases [28,29]. Hypertension is a multifactorial disease involving the coordination of the immune, renal, brain, and cardiovascular systems.

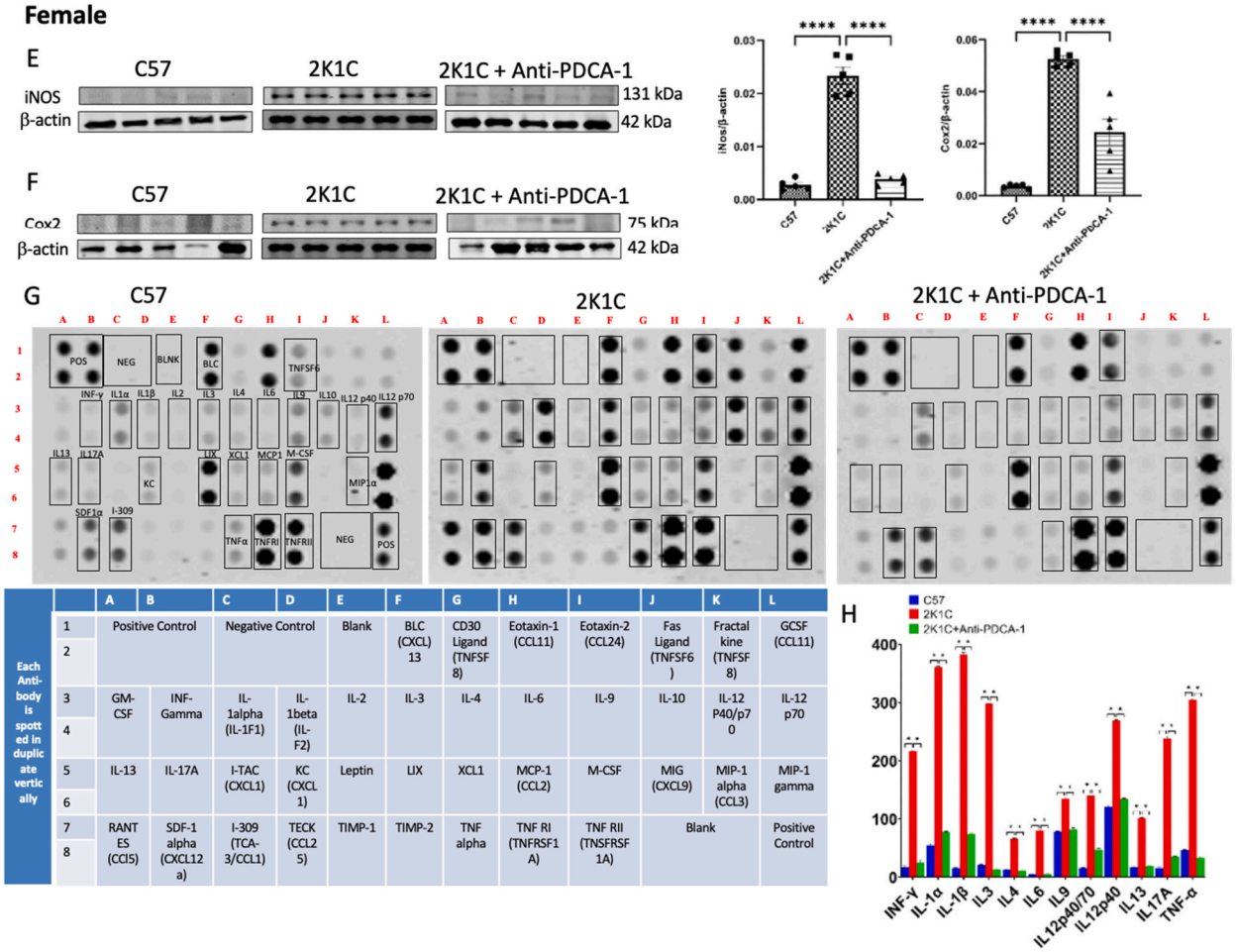

Fig. 6. (*continued*).

Specifically, renovascular hypertension is a significant disease that causes morbidity and mortality due to renal disease and cardiovascular and cerebrovascular complications [30]. Managing patients with renovascular hypertension is challenging because of the absence of an efficient therapeutical approach and the failure to improve the cardiovascular system in patients with renal artery stenting [31]. Therefore, there is an urgent need to identify treatable targets to protect the cardiovascular system and stop the progression of renovascular hypertension. In the present study, we focused on the role and mechanism of the plasmacytoid dendritic cells on renovascular hypertension-induced inflammation and cardiovascular complications. The immune system's involvement in hypertension-induced cardiovascular complications has long been appreciated. For instance, our laboratory has illustrated that the immune regulatory T (Treg) cells contribute to vascular dysfunction in mice infused with angiotensin II [26]. Dr. Schiffrin's laboratory also reported the involvement of Treg cells in vascular dysfunction induced by hypertension [32]. In 2007, Dr. Harrison's group showed that T and dendritic cells are essential in hypertension [10,18]. However, the role of the plasmacytoid dendritic cells subpopulation in renovascular hypertension-induced inflammation and cardiovascular complications has not yet been studied in both male and female mice. Our data illustrates that renovascular hypertension in both male and female mice causes vascular dysfunction, inflammation, cardiac hypertrophy, and lung weight increase.

The role of immune cells in hypertension has become a primary focus in cardiovascular research. Diverse immune cells, comprising DCs, macrophages, T cells, B cells, cytokines, and inflammatory molecules, are presently recognized as contributors to the complex mechanisms involved in hypertension. The immune system's effect on hypertension highlights the interrelationships between cardiovascular and immune pathways. Hence, the immune system exerts a crucial impact on the pathophysiology of cardiovascular complications precipitated by hypertension. Nevertheless, the characterization of plasmacytoid dendritic cells and functional involvement in renovascular hypertension-induced cardiovascular complications remains unexplored [33]. Furthermore, we used flow cytometry and we demonstrated that renovascular hypertension alters the immune cell profile (specifically dendritic cells and macrophage type-I) with variations between male and female mice. Thus, our findings show that renovascular hypertension increases the number of plasmacytoid dendritic cells, which was reduced by anti-mPDCA-1 treatment.

Dendritic cells (DCs) are crucial antigen-presenting cells that are responsible for initiating and regulating immune responses. In the

context of hypertension, DCs can significantly contribute to the development and progression of cardiovascular disease by promoting inflammation and causing dysregulation of the immune system. In hypertension, various stress signals, such as tissue damage and inflammation, activate DCs. Once activated, DCs migrate to lymph nodes, presenting antigens to T lymphocytes, specifically $CD4^+$ T helper cells. These $CD4^+$ T cells release pro-inflammatory cytokines like interleukin-17 (IL-17) and tumor necrosis factor-alpha (TNF-alpha) upon activation. These cytokines, in turn, stimulate other immune cells, including macrophages and neutrophils, leading to their infiltration into blood vessels and tissues. Activated macrophages release additional pro-inflammatory molecules. Hence, inhibiting dendritic cell activity can decrease the production and release of inflammatory cytokines and immune responses, reducing cardiovascular inflammation, lessening severe hypertension, and improving blood pressure control [19,33,34].

Interestingly, our data illustrate that the depletion of the plasmacytoid dendritic cells significantly reduced arterial blood pressure and inflammation and ameliorated vascular endothelial function in both male and female mice. Therefore, inhibiting dendritic cell activity may help preserve endothelial function, which is crucial for maintaining vascular homeostasis and reducing the risk of cardiovascular complications. These data indicate that the frequency and activity of plasmacytoid dendritic cells are essential in the cardiovascular pathology of renovascular hypertension. Previous studies showed the role of oxidative stress in vascular endothelial dysfunction in the setting of renovascular hypertension [35]. Our data show that renovascular hypertension effectively causes vascular endothelial cell dysfunction by reducing eNOS phosphorylation, induction of ER stress, and increasing autophagy, mTOR activation, and oxidative stress.

In renovascular hypertension induced by 2K1C, elevated endoplasmic reticulum (ER) stress and oxidative stress contribute to the pathophysiology of hypertension. Interestingly, our data showed that the depletion of the plasmacytoid dendritic cells significantly increased eNOS phosphorylation, reduced ER stress, autophagy, mTOR activation, and oxidative stress, and ameliorated vascular endothelium-dependent relaxation. The inhibition of inflammation by the anti-mPDCA-1 treatment could also improve vascular endothelial function in mice with renovascular hypertension. Understanding these processes may guide targeted therapeutic interventions to alleviate the impact on kidney function and overall cardiovascular health. Further studies are needed to determine whether plasmacytoid dendritic cells directly or indirectly regulate eNOS phosphorylation, ER stress, autophagy, and mTOR activation in vascular endothelial function in renovascular hypertension. A comprehensive understanding of these mechanisms offers insights into the intricate interplay between the renal and cardiovascular systems during the development of cardiac hypertrophy in renovascular hypertension. Previous reports indicate that renovascular hypertension leads to cardiac hypertrophy [36]. Consistent with these studies, we observed both heart hypertrophy and an increase in lung weight. The depletion of the plasmacytoid dendritic cells reduced cardiac hypertrophy and lung weight. A previous study found that transferring Treg cells into angiotensin II-infused hypertensive mice improved cardiac hypertrophy and reduced cardiac fibrosis despite sustained hypertension [37]. Further studies are needed to delineate whether plasmacytoid dendritic cells interact with Treg cells and macrophages type-I to regulate cardiac hypertrophy and lung edema in renovascular hypertension.

Inflammation also contributes to the pathogenesis of cardiovascular complications associated with hypertension. Renovascular hypertension, specifically, has been identified as a trigger for inflammatory responses [19,38,39]. Our study revealed significant vascular and systemic inflammation in our 2K1C mouse model of renovascular hypertension. Interestingly, the depletion of plasmacytoid dendritic cells significantly reduced inflammation. Future studies should elucidate the mechanisms by which the plasmacytoid dendritic cells are involved in renovascular hypertension-induced inflammation and determine their fate in the setting of renovascular hypertension.

5. Conclusion

Our data has shown that plasmacytoid dendritic cells play a crucial role in regulating various physiological functions, including arterial blood pressure, vascular endothelium-dependent relaxation, immune cell coordination, and inflammatory processes in renovascular hypertension. As a result, modulating these cells' number and functional activity may become a practical therapeutic approach to alleviate the cardiovascular complications caused by renovascular hypertension.

A translational perspective

Renovascular hypertension is a severe disease that causes heart attack, stroke, and vascular complications. Effectively managing patients with established renovascular hypertension caused by atherosclerosis (the most common cause) poses significant challenges due to the limited renal and cardiovascular function improvement and structural remodeling despite stenting the renal artery. In our study, we elucidated that depleting the immune plasmacytoid dendritic cells improves cardiovascular function and structure. Thus, targeting the plasmacytoid dendritic cells and their interaction with other cells or factors released by plasmacytoid dendritic cells could open new avenues for potential translational studies and therapeutic approaches to protect from renovascular hypertension-induced cardiovascular complications.

Ethics statement

EVMS IACUC Committee "IACUC #20-004"

Data Availability statement

Data have not been deposited into a publicly available repository. Data will be made available upon reasonable request.

CRediT authorship contribution statement

Balaji Srinivas: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Kiran Alluri:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Nour-Eddine Rhaleb:** Writing – review & editing, Writing – original draft. **Souad Belmadani:** Writing – review & editing, Writing – original draft, Conceptualization. **Khalid Matrougui:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31799.

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