Sex-Specific Mechanisms in Inflammation and Hypertension

Megan A. Sylvester [1] · Heddwen L. Brooks [1,2]

Abstract

Purpose of Review Despite enhanced screening and therapeutic management, hypertension remains the most prevalent chronic disease in the United States and the leading cause of heart disease, chronic kidney disease, and stroke in both men and women. It is widely accepted that hypertension is a pro-inflammatory disease and that the immune system plays a vital role in mediating hypertensive outcomes and end organ damage. Despite known discrepancies in the risk of hypertension development between men and women, preclinical models of immune-mediated hypertension were historically developed solely in male animals, leading to a lack of sex-specific clinical practice guidelines or therapeutic targets.
Recent Findings Following the NIH policy on the consideration of sex as a biological variable in 2015, significant advancements have been made into sex-specific disease mechanisms in inflammation and hypertension.
Summary This review article serves to critically evaluate recent advancements in the field of sex-specific immune-mediated hypertension.

Keywords Sex differences · Menopause · T cells · Antigen presenting cells · Cytokine · Inflammasome

Introduction

The National Health and Nutrition Examination Survey has consistently reported that early in life, women have a lower prevalence of hypertension compared with men. Importantly, after menopause, this sex disparity reverses, and postmenopausal women display a higher prevalence of hypertension than aging men [1, 2].

Sex-differences are also seen in the responsiveness of hypertensive patients to commonly prescribed therapeutics. Specifically, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are known to be less effective at reducing blood pressure in women compared with men [3]. Furthermore, the Women's Health Initiative (WHI) published in 2002 revealed that postmenopausal women taking a diuretic alone, were significantly more likely to have their blood pressure under control compared with participants taking a beta-adrenergic receptor blocker (ß-blocker), ACE inhibitor, or calcium channel blocker [4]. The WHI also reported that only one third of postmenopausal women (36.1%) had their blood pressure under control using proper antihypertensive treatment and their responsiveness to hypertensive therapeutics only worsened with increasing age [4]. These sex and age disparities are reported despite increased medication adherence in women compared with men (80.6% of women vs. 70.9% of men) [5].

Hypertension is a widespread disease not solely controlled by one organ system. The cardiovascular, central nervous, and renal systems all mediate long-term elevations in blood pressure. Moving beyond these key blood pressure mediating organs, a growing body of literature has revealed a critical role of the immune system in mediating hypertensive outcomes. Furthermore, it has been shown that the sex-differences that we see in hypertension development across the human lifespan are similarly present in immune-mediated models of hypertension. This serves as a critical review of recent work studying immune-mediated hypertension, specifically addressing sex-specific mechanisms and identifies areas where there is a paucity of published data.

✉ Heddwen L. Brooks
brooksh@email.arizona.edu

[1] Department of Physiology, College of Medicine, University of Arizona, 1656 E. Mabel St/Rm 417, P.O. Box 245218, Tucson, AZ 85724-5218, USA

[2] Sarver Heart Center, University of Arizona, Tucson, AZ, USA

Role of the Adaptive Immune System in Hypertension Development

Sex Differences in Immune-Mediated Hypertension

It has long been reported that the adaptive immune system, specifically T cells, play a critical role in regulating blood pressure responsiveness and resulting hypertensive end organ damage in male animals [6]. Early studies looking at the role of the adaptive immune system in hypertension demonstrated that male mice lacking a gene required for lymphocyte differentiation (Rag1$^{-/-}$ mice) have a dampened response to hypertensive stimuli. These studies also demonstrated that the adoptive transfer of donor male CD3$^+$ T cells was required for male Rag1$^{-/-}$ animals to produce a full hypertensive response to either Angiotensin II (Ang II) or DOCA salt [7].

Our group reported that premenopausal female animals are resistant to T cell-dependent hypertension. In a direct comparison of male and female Rag1$^{-/-}$ animals, we found that following adoptive transfer of male CD3$^+$ T cells, female Rag1$^{-/-}$ mice had a blunted hypertensive response to Ang II infusion compared to male animals that received male CD3$^+$ T cells [8••]. In this same study, we reported that female Rag1$^{-/-}$ animals were protected from the renal pro-inflammatory changes seen in hypertensive male counterparts. In males, Ang II infusion following T cell adoptive transfer, enhanced the expression of renal pro-inflammatory markers; macrophage chemoattractant protein (MCP-1), and the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), and interleukin-2 (IL-2). In contrast, no significant changes were seen in the renal expression of these pro-inflammatory markers in female Rag1$^{-/-}$ animals, indicating a protective phenotype [8••].

Other reports have further investigated how sex of the T cell itself may impact hypertensive outcomes in Rag1$^{-/-}$ studies. Interestingly, when investigators transferred female CD3$^+$ T cells into male Rag1$^{-/-}$ animals, they observed decreased hypertensive responses than when male CD3$^+$ T cells were transferred into male Rag1$^{-/-}$ animals [9]. Additionally, transfer of female CD3$^+$ T cells into male Rag1$^{-/-}$ animals resulted in decreased splenic frequencies of pro-inflammatory TNF-α and interleukin-17 (IL-17) producing cells and increases in renal anti-inflammatory interleukin-10 (IL-10) gene expression compared with when male CD3$^+$ T cells were transferred into male Rag1$^{-/-}$ animals [9]. These studies reveal that the sex of the T cell itself can impact hypertensive outcomes and calls for further investigation into sex-specific signaling mechanisms in immune-mediated hypertension.

Cytokine Signaling and Hypertension Development

Cytokines serve as major signaling molecules between the adaptive and innate immune system, as well as a multitude of other cells throughout the body. Therefore, it is not surprising that many groups have investigated cytokine signaling as a key regulator of systemic blood pressure. Clinical studies have shown increased production of pro-inflammatory cytokines in ex vivo whole blood samples from male and female patients with essential hypertension [10]. Implicated pro-inflammatory cytokines include IL-17, TNF-α, interleukin-6 (IL-6), interleukin-1 (IL-1), and interferon-γ (IFN-γ) [10–12]. These outlined pro-inflammatory cytokines have long been used as markers of renal inflammation in hypertensive research; however, recent discoveries point toward a regulatory role of cytokines in renal sodium handling, indicating local cytokine expression may be one of the key drivers of hypertensive outcomes [12, 13].

Early preclinical studies demonstrated that Ang II-mediated hypertension was attenuated in male IL-6 knockout mice and that IL-6 stimulation directly upregulates epithelial sodium channel (ENaC) expression in male cortical collecting duct cells in vitro [14, 15]. Recent work has substantiated these findings and shown that male Dahl salt-sensitive rats treated with an IL-6 neutralizing antibody, have blunted hypertensive outcomes and reduced kidney damage, implicating a major role of this cytokine in hypertensive signaling in male animals [16]. Importantly, it has also been shown that IL-6 signaling upregulates angiotensinogen expression in renal proximal tubule cells isolated from male rats in vitro [17]. Together, these studies demonstrate a direct link between IL-6 cytokine signaling and intrarenal renin-angiotensin system signaling leading to increased renal sodium retention and hypertensive outcomes specifically in male animals.

IL-17 is the hallmark signaling cytokine secreted by T-helper 17 (Th17) cells, which are known to be a highly pro-inflammatory T cell subtype. Clinical studies have revealed that male and female hypertensive patients have significantly more IL-17 producing lymphocytes in circulation compared with normotensive patients [18•]. Experimentally, injection of male mice with IL-17 has previously been shown to increase hypertensive responses to Ang II [19]. Previous reports have also shown that male IL-17 deficient mice have a blunted blood pressure response to Ang II infusion as well as reduced vascular inflammation with preserved vascular function [20]. More recently, groups have demonstrated that male IL-17 knockout animals have reduced activation of the sodium-chloride cotransport (NCC) and ENaC in the distal nephron following Ang II infusion. Additionally, this same study used human male proximal tubule (HK-2) cells to show IL-17 signaling directly increased sodium hydrogen exchanger 3 (NHE3) expression in vitro [21].

IFN-γ is secreted by CD4$^+$ T helper type 1 (Th1) cells as well as CD8$^+$ cytotoxic T cells in pro-inflammatory states. Similar to IL-17, it has been shown that numbers of CD4$^+$ and CD8$^+$ IFN-γ producing cells are elevated in the peripheral blood of male and female hypertensive patients compared

with normotensive patients [18•]. Recent experimental work has shown that male IFN-$\gamma^{-/-}$ mice have a blunted hypertensive response to Ang II infusion [22]. This study also revealed that expression and activation of the sodium potassium chloride cotransporter (NKCC2), NHE3 cotransporter, and NCC cotransporter were all blunted in the male IFN-$\gamma^{-/-}$ mice following Ang II infusion suggesting a direct role of IFNγ in regulating renal sodium handling and therefore blood pressure regulation in male animals.

It is important to note that to our knowledge, no significant work has been done to understand the role of IL-6, IL-17, or IFN-γ signaling in the female animal or how these signaling molecules could be impacting sex-differences in immune-mediated hypertension across the female lifespan and into menopause.

Protective Role of T Regulatory Cells in Immune-Mediated Hypertension

T regulatory cells (Tregs) are a major CD4$^+$ T cell subpopulation known to act as anti-inflammatory T cells during times of inflammation and have been reported to have sex-specific expression patterns in models of immune-mediated hypertension. Studies have reported that adoptive transfer of additional Tregs into Ang II-infused male mice blunts the hypertensive response [23]. However, until recently very little work had been done to specifically understand how Tregs are implicated in sex-specific hypertensive outcomes.

It has been reported that female spontaneously hypertensive rats (SHR) have enhanced renal Treg infiltration compared with males; in contrast, male SHR had enhanced renal infiltration of pro-inflammatory Th17 cells compared with females [24]. Building upon these reports, it has been shown that treating female SHR with an antibody to transforming growth factor-ß (TGF-ß), a cytokine known to be crucial to the maturation of Tregs, does not increase systemic blood pressure despite significantly reducing the circulating Treg population as expected [25••]. Interestingly, investigators did not see a change in renal Treg populations following anti-TGF-ß administration [25••]. These results suggest that systemic and renal Treg populations may function through different signaling mechanisms during a hypertensive stimulus and may play distinct roles in hypertensive outcomes.

Similarly, sex-specific T regulatory cell differences have been observed in a high fat diet-induced hypertensive Dahl Salt Sensitive (DSS) rat model. Male and female DSS rats placed on a high fat diet for 4 weeks have similar hypertensive outcomes compared to standard chow-fed control groups [26]. The findings of this study contradict previous reports that female Wistar and Fischer rats are resistant to high fat diet-induced hypertension, compared with males suggesting a strain-specific sex-difference in hypertensive response when using DSS rats [27, 28]. Male DSS rats fed a high fat diet had increased pro-inflammatory IL-17$^+$ T cells in their thoracic aortic tissue compared with female DSS rats, while females had increased anti-inflammatory FoxP3$^+$ Tregs compared with males suggesting sex-specific compensatory mechanisms [26].

To further study the role of Tregs in female protection from hypertension, our group selectively depleted Tregs in premenopausal female mice, and eliminated the premenopausal protection from Ang II-induced hypertension [29••]. In this study, we injected premenopausal female mice with the anti-CD25 antibody, PC-61, to selectively deplete Tregs. Anti-CD25 induced a 30% depletion in splenic and renal Treg populations, following which premenopausal females responded to Ang II infusion with a significant elevation in systolic blood pressure compared with Ang II-infused premenopausal females [29••]. Furthermore, while not all systemic Tregs were depleted following PC-61 injections, we found that the remaining Tregs in the premenopausal females were inactive, as represented by a lack of CD25 surface expression [29••]. These results, taken together with previous findings within the field, suggest that not only are Tregs implicated in sex-specific hypertensive outcomes, but that activation status likely plays a large role in mediating premenopausal protection from hypertension. All in all, more work is needed to fully understand the protective role of Tregs in sex-specific immune-mediated hypertension.

Models of T Cell Activation in Hypertension

One of the greatest challenges in understanding immune-mediated hypertension is addressing the mechanism by which T cells are activated by hypertensive stimuli rather than pathogenic antigens as traditionally described in the immunology field.

A large body of work has been published addressing this very concern. It has previously been shown that salt signaling can directly influence T lymphocyte activity and maturation in males. Specifically, the T cell serum/glucocorticoid regulated kinase 1 (SGK1) has been linked to increased maturation of male pro-inflammatory Th17 cells by altering the expression of the key activation marker interleukin-23 receptor (IL-23R) [30]. This same salt-sensing kinase has been implicated in driving hypertensive outcomes in male mice. Male mice lacking T cell SGK1 displayed a blunted hypertensive response to Ang II infusion, as well as decreased renal and vascular inflammation [31•]. Similarly, another group has shown that when SGK1 is pharmacologically inhibited in vivo, male mice have decreased Ang II-induced renal and cardiac dysfunction [32]. This group demonstrated that pharmacological inhibition of SGK1 in males increased Th17 and decreased Treg infiltration in both kidney and cardiac tissue, indicating a significant shift in the pro/anti-inflammatory balance in these Ang II-infused male animals. To our knowledge, no work has been

done to identify the role of T lymphocyte SGK1 in mediating hypertensive outcomes in female animals.

Another recent discovery is the role of T cell mineralocorticoid receptor (MR) in immune-mediated hypertension. MR antagonists have long been used as anti-hypertensive therapeutics due to their ability to alter salt and water retention in the kidney; however until recently, it was not known if or how the MR expressed directly within the T cell regulates blood pressure. It has now been shown that T cell MR knockout animals do not develop severe hypertension or the expected end organ damage following Ang II-infusion [33]. This study suggests that removal of the MR from CD8$^+$ T cells is protective against Ang II hypertension due to downregulation of IFN-γ production in these animals, implicating a direct link between mineralocorticoid signaling and T cell pro-inflammatory cytokine production. Similar to SGK1 signaling, the role of MR signaling in hypertensive outcomes has not been sufficiently studied in female animals to identify any sex-specific disease mechanisms.

Impact of Menopause on Immune-Mediated Hypertension

It is important to note that female protection from immune-mediated hypertension is lost following menopause. Clinically, postmenopausal women have increased expression of the pro-inflammatory markers MCP-1, TNF-α, IFN-γ, and IL-6 in their serum as well as significant changes in the proportion of pro- and anti-inflammatory immune cell populations [34, 35].

It is hypothesized that this shift in inflammatory environment following menopause is due to the sudden lack of estrogen signaling on immune cells. Interestingly, it has long been known that immune cells express estrogen receptors [36]. The most abundantly expressed estrogen receptor on T cells is estrogen receptor-α (ER-α) [37]. It has been reported that ER-α signaling on the T cell itself inhibits pro-inflammatory Th17 and type 1 T helper (Th1) cell expansion [38]. Furthermore, one previous report demonstrated that premenopausal female ER-$\alpha^{-/-}$ animals have increased hypertensive outcomes compared with wildtype premenopausal females, suggesting a protective role of ER-α signaling in blood pressure regulation [39]. Additional work is still needed to specifically understand how estrogen signaling on immune cells may be altered during menopause and what specific estrogen receptors may be implicated in this loss of protection.

Using the 4-vinylcyclohexene diepoxide (VCD) ovarian failure mouse model of menopause, our group has shown that female mice lose their protection from hypertension following complete follicular depletion [40••]. In this study, we reported that not only are VCD-treated postmenopausal female animals no longer protected from Ang II-mediated hypertension following follicular depletion, but that estrogen supplementation during the perimenopause transition attenuates hypertensive outcomes observed in postmenopausal females [40••].

We previously demonstrated that females (premenopausal) were protected against T cell-mediated Ang II-induced hypertension. Using the VCD model of menopause in Rag1$^{-/-}$ mice we examined if females would lose this protection, following progression in menopause. VCD treated Rag1$^{-/-}$ animals were infused with Ang II with or without the adoptive transfer of male CD3$^+$ T cells. Menopausal Rag1$^{-/-}$ animals responded to Ang II infusion with a significant increase in SBP [29••] similar in response to that seen in male mice [7,8••]. Additionally, we reported enhanced renal pro-inflammatory gene expression (IL-2, TNF-α, and MCP-1), enhanced renal macrophage infiltration, and decreased pro-inflammatory renal M2 macrophages in postmenopausal Rag1$^{-/-}$ females following T cell transfer and Ang II infusion [29••]. Taken together, these results demonstrate an enhanced pro-inflammatory environment following menopause which likely contributes to increased susceptibility to hypertensive outcomes and end organ damage.

Role of Antigen Presenting Cells in Hypertension Development

Preclinical research on immune-mediated hypertension has traditionally focused on the adaptive immune system as previously discussed. In the majority of previous studies, changes in innate immune cells such as macrophages and dendritic cells were solely used as markers of end organ damage, rather than as key mediators of hypertensive development. Over the past few years, a growing body of work has revealed that the innate immune system, specifically antigen presenting cells, may play more of a regulatory role in immune-mediated hypertension than previously thought.

In order to better understand the interaction between the innate and adaptive immune system, a recent report highlighted the role of the macrophage and dendritic cell surface marker CD70. CD70 is known to drive formation of memory T cells in times of chronic inflammation. This report revealed that male CD70$^{-/-}$ mice treated with L-NAME/high salt or Ang II were unable to expand their renal memory T cell population and thus had decreased secretion of renal pro-inflammatory cytokines compared with control male mice [41]. Additionally, the male CD70$^{-/-}$ mice were protected from hypertension when challenged with L-NAME/high salt or Ang II indicating a significant role of this innate immune marker in the generation of end organ damage and hypertensive outcomes specifically in males.

Another recent report links salt signaling directly with dendric cell activation in males. This study proposed that in a high salt environment, sodium enters dendritic cell via amiloride-sensitive channels which triggers an intracellular

cascade to activate the dendritic cell. An in vitro model was used to show that male dendritic cells cultured in high salt medium had elevated NADPH oxidase activity compared with control conditions and that this altered activity could be blocked by amiloride [42•]. This study also demonstrated that high-salt activated male dendritic cells significantly increased susceptibility to hypertension when adoptively transferred into naïve male mice. For this experiment, male dendritic cells were first cultured in control media, normal salt media, or high salt media before being adoptively transferred into male recipient mice. Results revealed that male animals given high-salt activated dendritic cells had an increased sensitivity to a low pressor dose of Ang II compared with animals transferred dendritic cells from control or normal salt culture conditions [42•].

While these studies indicate a significant role of the innate immune system in regulating hypertensive outcomes, these models have only been used to study immune-mediated hypertension in males. Therefore, future studies are needed to clarify the role of the innate immune system activation in female animals, as well as how menopause may alter these immune mechanisms and result in varying hypertensive outcomes across the female lifespan.

Intracellular Inflammatory Signaling Pathways and Hypertension

Sex Differences in the COX-2 Signaling Pathway

Apart from studying adaptive and innate immune cell types, many groups have started to highlight sex differences in general inflammatory pathways including cyclooxygenase-2 (COX-2) signaling. Renal COX-2 signaling is known to be induced by inflammation and drives prostaglandin release within the kidney which drives salt and water excretion and vasodilation within the kidney leading to a reduction in systemic blood pressure [43, 44].

It has previously been reported that female SHR have higher renal COX-2 expression and urinary prostaglandin E2 (PGE2) levels compared with male SHR [45]. These sex differences in COX-2 expression and PGE2 excretion, combined with the known blunting of hypertensive outcomes in female SHR, suggest a sex-specific protective role of COX-2 signaling in female animals. Recently, a follow-up study was published looking at sex differences in blood pressure response using the COX-2 inhibitor celecoxib in the SHR model of hypertension. This study reported that although expression of COX-2 is elevated in female SHR compared with male, inhibition of COX-2 did not eliminate female protection from hypertension [46•]. The authors concluded from this study that COX-2 does not contribute to sex differences in hypertensive outcomes in the SHR model; however, further

investigation is still needed to fully understand how the reported sex-specific discrepancies in renal COX-2 and prostaglandin expression may alter the renal immune profile during hypertension development.

Emerging Role of the Inflammasome Pathway

A growing body of work has highlighted the NLRP3 inflammasome pathway, which stimulates release of the pro-inflammatory cytokines IL-1ß and IL-18, as a potential therapeutic target in immune mediated hypertension. The NLRP3 inflammasome pathway has been implicated in a multitude of chronic inflammatory diseases including Type 1 diabetes, Alzheimer's disease, as well as autoimmune conditions [47–49]. Therefore, it is not surprising that recent research into immune-mediated hypertension has found that when the NLRP3 inflammasome pathway is genetically knocked out or pharmacologically inhibited, hypertensive outcomes as well as renal inflammation are attenuated in male mice [50, 51•]. Targeted silencing of the NLRP3 gene has also been shown to improve vascular remodeling and hypertensive outcomes in male spontaneously hypertensive rats (SHR) [52].

Interestingly, clinical genotyping studies have suggested a sex-specific role of NLRP3 polymorphisms in the risk of developing a myocardial infarction. Their results suggested that women containing a specific polymorphism of the NLRP3 gene were at a lower risk of developing a myocardial infarction compared with men who expressed the exact same polymorphism [53]. Further clinical studies have found that placental tissue from women with preeclampsia, a spontaneous form of hypertension developed during pregnancy, express significantly higher levels of NLRP3 inflammasomes and associated signaling molecules [54, 55]. Animal models of preeclampsia have confirmed that NLRP3 deficiency in female mice is protective against Ang II-induced hypertension during pregnancy [56].

To our knowledge, no preclinical studies have been performed to evaluate the potential role of the NLRP3 inflammasome in non-pregnant female animal models of hypertension, nor has the effect of menopause on inflammasome signaling been sufficiently explored.

Conclusion and Perspectives

In this review, we have highlighted many advancements in understanding immune-mediated hypertension over the last few years. While a significant body of referenced work has focused specifically on sex-specific disease mechanisms, there is still much progress to me made. Namely, preclinical investigation into the role of specific cytokine signaling pathways, antigen presenting cells, and the inflammasome pathway have still only been performed in male models of

immune-mediated hypertension. Future research in the field should prioritize closing this gap in sex-specific knowledge in order to fully understand disease mechanisms in both men and women equally.

Additionally, very little work has been done to understand the impact of menopause on immune-mediated hypertension, or why postmenopausal women have a higher propensity toward hypertension development compared with age-matched men. The life-expectancy of a woman in the United States is now reported to be 81 years old according to the Centers for Disease Control, meaning that the average American woman will spend nearly 40% of their life in menopause. With the average female lifespan only expected to increase over time, there is a significant need for more preclinical research specifically investigating the mechanisms of post-menopausal immune-mediated hypertension.

In conclusion, as our medical system moves more toward personalized medical treatments, it is increasingly important for preclinical research to continue building an understanding of sex-specific mechanisms in immune-mediated hypertension and to highlight how these mechanisms may change across the male and female lifespan.

Compliance with Ethical Standards

Conflict of Interest   The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent   This article does not contain any studies with human or animal subjects performed by any of the authors.

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