The immune system in hypertension

Daniel W. Trott and David G. Harrison

Division of Clinical Pharmacology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee

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The concept that the immune system contributes to hypertension was first discovered. Several recent investigations have further defined the role of immune system, particularly the adaptive immune system, in hypertension. In this review, we summarize recent discoveries by our laboratory and others on the role of the immune system in hypertension.

Early Studies in Immunity and Hypertension

The concept that the immune system contributes to hypertension has its genesis in the 1960s, when Grollman et al. (40, 56) demonstrated that immunosuppression blunted hypertension in a model of renal infarction and that transfer of lymphocytes from rats with renal infarction induced hypertension in previously nonhypertensive animals. Later, Svenson found that hypertension was not maintained in thymectomized or athymic nude mice with renal infarction (46). In the 1980s, Ba et al. (2) found that transplanting the thymus from a Wistar-Kyoto (WKY) rat to a spontaneously hypertensive rat (SHR) resulted in a decrease in blood pressure in the SHR (2). Blood pressure was also lowered in SHR with treatment by either anti-thymocyte serum or the immunosuppressive drug cyclophosphamide (5, 12). Nerve growth in the thymus of SHR was found to be greater than that of WKY rats, suggesting a neural component of immune cell activation in hypertension (42). Rodriguez-Iturbe et al. (43) found that immunosuppression with mycophenolate mofetil blunted salt-induced hypertension after ANG II infusion. In the early 2000s, Muller and Luft (35, 36, 49) conducted a series of investigations showing that NF-κB and ROS play roles in ANG II-induced end-organ damage. More recently, advances in genetic mouse models and knowledge of immunity prepared the way for discoveries that would lead to further understanding of the importance of the immune system in hypertension.

Role of the Central Nervous System in Immune-Mediated Hypertension

In 2007, our laboratory (18) published a study demonstrating that T cells contribute to the development of hypertension. In this study, mice lacking recombinase-activating gene 1 (Rag-1−/− mice) were used as these mice cannot generate functional T cell receptors or B cell antibodies and thus lack both T and B lymphocytes. The increase in blood pressure caused by either ANG II or DOCA salt was significantly blunted in Rag-1−/− mice, suggesting that either T or B cells mediate overt hypertension. Rag-1−/− mice did not exhibit increased vascular superoxide production and endothelial dysfunction. The hypertensive response to ANG II was restored when Rag-1−/− mice received adoptive transfer of T cells but not B cells. In wild-type mice, ANG II increased circulating C6D9+, CCR5+, and CD44high T cells, markers of effector memory T cells. In addition, T cells accumulated in the perivascular adipose tissue of the aorta. The results of this study indicate that T cells play a major role in hypertension. Supporting the role of T cells in hypertension, severe combined immunodeficiency mice have also been shown to be protected against hypertension and exhibit reduced albuminuria and renal damage (10). Recently, Mattsson et al. (32) deleted the Rag1 gene in Dahl salt-sensitive rats using zinc finger nuclease technology and have shown that this attenuates blood pressure, albuminuria, and kidney damage. Thus, T cells seem to contribute to the development of various forms of hypertension in different strains of mice and in rats.
tween these tissues. Lymphoid tissues are rich in sympathetic nerves (14). Ganta et al. (17) have shown that intracerebroventricular infusion of ANG II increased sympathetic nerve activity to the spleen and increased expression of multiple cytokines in the spleen. Our laboratory has performed a series of investigations on the role of the CNS in mediating T cell activation.

The circumventricular organs (CVO) are highly vascularized and have an incomplete blood-brain barrier and can therefore be influenced by circulating hormones like ANG II. In addition, the CVO, and in particular, the subfornical organ (SFO), are important in both sending and receiving central signals that regulate cardiovascular function and electrolyte balance. Deletion of CVO extracellular (ec)SOD, using Cre-lox technology, provides a model to determine the role of central oxidative stress in hypertension. ecSOD deletion increased ROS levels in the CVO, increased heart low-frequency to high-frequency heart rate variability (indicative of increased sympathetic nervous activity), and elevated blood pressure (25). In addition, when mice with ecSOD deleted in the CVO were infused with ANG II at a dose that does not cause hypertension in normal mice (140 ng·kg⁻¹·min⁻¹), blood pressure was significantly elevated and was accompanied by aortic T cell infiltration. Interestingly, in a separate investigation, when ecSOD was specifically deleted in vascular smooth muscle, despite increases in vascular ROS, blood pressure and T cell responses were not altered compared with controls (27).

NADPH oxidases are major sources of superoxide anion production in mammalian cells. The subunit p22phox mediates trafficking of NADPH oxidase catalytic subunits to the cell membrane and is required for enzyme complex assembly and, ultimately, superoxide production. Complementing the studies that used deletion of ecSOD, our laboratory (26) also deleted p22phox in the SFO in a similar manner. Deletion of p22phox in the SFO blunted the pressor response to ANG II and decreased sympathetic outflow as assessed by heart rate variability. In addition, p22phox deletion abolished ANG II-induced aortic T cell infiltration. This study is in keeping with findings that intracerebroventricular injections of a superoxide scavenger reduces sympathetic drive, blood pressure, and renal damage in salt-induced hypertension in rats (16).

Supporting the role of the central nervous system, lesions in the anteroventral third cerebral ventricle (AV3V), a region which includes the SFO, can prevent ANG II-induced hypertension (7, 30). In addition, AV3V lesions protect against T cell activation and aortic infiltration in response to ANG II (30). This is of particular importance as it demonstrates that ANG II-induced T cell activation is not due to direct actions of ANG II on T cells but rather that central signals are required for T cell activation. Interestingly, mice infused with norepinephrine become hypertensive and exhibit T cell activation and aortic infiltration even after AV3V lesions. This supports the concept that sympathetic drive, and its attendant release of norepinephrine, likely mediates T cell activation and hypertension. In addition to the important role of the CNS, peripheral mechanisms appear to also contribute to T cell activation and vascular inflammation. Treatment with the vasodilator hydralazine to normalize blood pressure prevents T cell activation and vascular inflammation induced by ANG II infusion (30). This suggests that T cells may respond to elevations in pressure and that afferent nerves may activate central mechanisms in a feedforward manner to induce CVO oxidative stress, sympathetic outflow, further T cell activation, and overt hypertension. More recently, T cells have been shown to contribute to stress-induced hypertension (31). We exposed mice to 7 days of stress using a combination of restraint and cage switching. This stress paradigm resulted in increased blood pressure, activation of circulating T cells, and aortic T cell infiltration. Rag-1⁻/⁻ mice were protected from stress-induced hypertension, and adoptive transfer of T cells restored the hypertensive response. These findings underscore the crucial role of the CNS in orchestrating the T cell response leading to hypertension.

T Cell Subtypes, Cytokines, and Mechanisms of Activation

The above studies demonstrated that T cells contribute to the development of hypertension; however, they do not provide extensive insights into the subsets of T cells involved. CD4⁺ T cells have been generally classified as either T helper (Th)1 or Th2, depending on their activation markers and cytokine production (34). Th17 cells are a newly characterized subset of T cells; these cells produce the cytokine IL-17 and contribute to numerous autoimmune diseases, obesity, and cardiovascular disease (13, 48, 57). To investigate the role of IL-17 in hypertension, our group studied IL-17a⁻/⁻ mice. These mice exhibited a similar initial increase in blood pressure as wild-type mice in response to ANG II; however, after 7 days, blood pressure dropped in IL-17a⁻/⁻ mice (28). The ANG II-induced aortic T cell infiltration observed in wild-type mice was abolished in IL-17a⁻/⁻ mice, as were increases in vascular oxidative stress and endothelial dysfunction. Recent reports have shown that direct infusion of IL-17a mediated hypertension and endothelial dysfunction in mice (37) and that IL-17 mediated placental oxidative stress, resulting in hypertension during pregnancy in rats (11).

In addition to IL-17, other cytokines have been implicated in the pathogenesis of hypertension. Etanercept, a TNF-α antagonist, is effective in preventing hypertension (18, 50, 52). IL-6 knockout mice are also protected from ANG II-induced hypertension (6, 24, 45). Interferon (IFN)−γ is upregulated in the kidneys of hypertensive mice (10), and inhibition of IFN−γ prevents ANG II-induced end-organ damage (29). Taken together, these observations suggest that hypertension is mediated by multiple proinflammatory T cell subsets. In accordance with this concept, T regulatory (Tregs) cells, which act to restrain proinflammatory T cells, attenuate hypertension-induced end-organ damage in mice (22) and blunt hypertension in rats (53).

Classically, T cells require two signals for activation: 1) interaction of the T cell receptor with an antigen presented in the context of a major histocompatibility complex and 2) stimulation of costimulatory molecules on the T cell by ligands on the antigen-presenting cell (1). A major costimulatory molecule on T cells is CD28, which is bound by the B7 ligands CD80 and CD86 of the antigen-presenting cell. Ligation of the T cell receptor in the absence of costimulation leads to T cell apoptosis (15). The pharmacological agent CTLA4-Ig inhibits costimulation by binding to B7 ligands on antigen-presenting cells. To determine whether costimulation plays a role in hypertension, our laboratory used both pharmacological inhibition of costimulation with CTLA4-Ig and a genetic approach with B7-deficient (B7⁻/⁻) mice. CTLA4-Ig treatment blunted

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blood pressure, T cell activation, and vascular infiltration in both ANG II- and DOCA salt-induced hypertension (54). CTLA4-Ig treatment also abolished T cell production of TNF-α and IFN-γ induced by ANG II. Similar results were observed in B7−/− mice, which lack B7 ligands. These observations suggest that T cell receptor ligation and costimulation are necessary for T cell activation in hypertension.

The Innate Immune System and Hypertension

The role of the adaptive immune system in experimental hypertension has been well characterized; however, less is known about the role of the innate immune system. Recently, Abboud et al. (19) demonstrated that, in WKY rats, the cholesteronic agonist nicotine results in an anti-inflammatory response in splenic macrophages; in contrast, nicotine induced a proinflammatory response in macrophages from SHRs and enhanced Toll-like receptor-mediated cytokine release. In addition, perivascular macrophage infiltration has been observed in experimental hypertension in mice (3). Inflammatory cytokines, IL-1β, and IL-6 are elevated in SHRs compared with WKY rats; this can be reversed by treatment with an angiotensin-converting enzyme inhibitor (33). Similarly, ANG II receptor blockade prevented lipopolysaccharide-induced inflammatory responses in innate immune cells in the rat spleen (44). In humans, white blood cells from essential hypertensive patients produced more IL-1β and IL-6 when stimulated with lipopolysaccharide compared with controls (41). These observations are consistent with monocyte activation in hypertension. How the innate and adaptive immune systems interact in the development of hypertension is not well understood and is an important topic for future study.

The Immune System in Preeclampsia

Preeclampsia is characterized by the onset of hypertension during pregnancy accompanied by proteinuria. Preeclampsia is associated with the production of autoantibodies that stimulate the ANG II type 1 (AT1) receptor (55), and infusion of these antibodies can induce preeclampsia-like symptoms in pregnant mice (60). More recently, it has been shown that a specific subset of B cells produces these antibodies (21). Depletion of B cells using the anti-CD20 antibody rituximab blunts the blood pressure response in the reduced uterine perfusion pressure rat model of preeclampsia (23). Adoptive transfer of CD4+ T cells from reduced uterine perfusion pressure rats to normal pregnant rats results in increased blood pressure (39). This response is blunted by either rituximab or AT1 antagonism, suggesting an important role of cross-talk between T and B cells in preeclampsia. Supporting the role of T cells in preeclampsia, mice deficient in the cytokines IL-4 or IL-10, which skew T cells to an anti-inflammatory phenotype, develop preeclampsia-like symptoms when pregnant (8, 9). The proinflammatory cytokine IL-17 mediates placental oxidative stress and increases in blood pressure in pregnant rats (11). Together, these observations support a role for the adaptive immune system in preeclampsia where T and B cells act in a synergistic manner.

Pulmonary Hypertension and the Immune System

It has long been postulated that autoimmunity and inflammation is involved in pulmonary hypertension (38). In addition, T cells, B cells, and macrophages are present in the lungs of patients with pulmonary hypertension (51). Recently, a role of anti-inflammatory Tregs cells has been indentified in experimental pulmonary hypertension (47). In this study, in response to VEGF receptor 2 antagonism, athymic rats, which lack T cells, developed perivascular inflammation, including infiltration of B cells and macrophages in the lung and pulmonary hypertension. The authors then reconstituted different T cell subsets in these animals and found that CD4+ Tregs cells acted to blunt pulmonary vascular inflammation and the development of pulmonary hypertension. In a similar manner, Tregs cells appear to act to restrain experimental systemic hypertension in both mice and rats (3, 22, 53).

Conclusions and Future Directions

In summary, it has been known for almost 50 yr that immune cells contribute to hypertension; in the last several years, investigations from our group and others have demonstrated the importance of T cells in the development of hypertension.

Fig. 1. Working hypothesis describing the role of immune cells in hypertension. CNS, central nervous system.
with clinical "prehypertension," which results in protein modifications, possibly due to oxidative modifications. These altered proteins serve as neoantigens that no longer recognized as self, are processed and presented by dendritic cells, and promote T cell activation. In concert, antigen signals to the CNS result in increased sympathetic outflow contributing to T cell activation. Activated T cells infiltrate the kidney and vasculature and produce cytokines that promote renal Na+ and water retention and, in the vasculature, vasoconstriction and remodeling. Together, these alterations result in overt hypertension. The observation that hydratization treatment abolished the T cell response suggests that signals from the periphery operate in a feedforward manner to signal the CNS to increase central sympathetic drive and mediate overt hypertension. An important point is that the T cell response is independent of the model of experimental hypertension. T cell responses have been observed in mice in response to ANG II, DOCA salt, and norepinephrine. In rats, this response has been observed in both salt-sensitive and genetic models. Emphasizing this point is the recent study of Zhang et al. (59), where ANG II receptors were specifically deleted in mouse T cells using Cre-lox technology. These mice were not protected against ANG II-induced hypertension, and, surprisingly, kidney damage in response to ANG II was exacerbated (59). These observations indicate that the T cell response in hypertension is independent of direct actions of ANG II on T cells. We believe, based on the studies of the CNS, that central signals mediate T cell activation in a variety of hypertension models. Recent observations by Abboud et al. (19) have suggested that central signals regulate the innate immune system in hypertension as well. It should be noted that the model for the immune system in hypertension outlined here is a working hypothesis; the precise mechanisms, particularly the initiating factors in the development of hypertension, are as yet unknown. Our hypothesis will almost certainly require refinement as new information comes available.

DISCLOSURES
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