

# The immunology of hypertension

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Although systemic hypertension affects a large proportion of the population, its etiology remains poorly defined. Emerging evidence supports the concept that immune cells become activated and enter target organs, including the vasculature and the kidney, in this disease. Mediators released by these cells, including reactive oxygen species, metalloproteinases, cytokines, and antibodies promote dysfunction of the target organs and cause damage. In vessels, these factors enhance constriction, remodeling, and rarefaction. In the kidney, these mediators increase expression and activation of sodium transporters, and cause interstitial fibrosis and glomerular injury. Factors common to hypertension, including oxidative stress, increased interstitial sodium, cytokine production, and inflammasome activation promote immune activation in hypertension. Recent data suggest that isolevuglandin-modified self-proteins in antigen-presenting cells are immunogenic, promoting cytokine production by the cells in which they are formed and T cell activation. Efforts to prevent and reverse immune activation may prove beneficial in preventing the long-term sequelae of hypertension and its related cardiovascular diseases.

## Introduction

In 2010, hypertension was ranked as the leading risk factor for global burden of disease, and it affects individuals in both economically developed and developing nations alike (Bromfield and Muntner, 2013). Recent guidelines have defined hypertension as a sustained systolic blood pressure greater than 130 mm Hg, making almost half of the adult population hypertensive (Whelton et al., 2017). End-organ damage to the kidneys, heart, brain, and vasculature is an important manifestation of this disease. As such, those who suffer from hypertension are more likely to develop atherosclerosis, stroke, myocardial infarction, heart failure, chronic kidney disease, and dementia (Lionakis et al., 2012; WHO, 2013). Although occasional cases of hypertension are a result of identifiable causes, such as renal artery stenosis, pheochromocytoma, excessive adrenal aldosterone production, or monogenetic causes, more than 90% of cases do not have an identifiable etiology and are classified as “essential.” Essential hypertension often coexists with obesity, disorders of lipid metabolism, aging, and insulin resistance, and thus is often considered as part of a complex metabolic phenotype that has myriad manifestations (Carretero and Oparil, 2000).

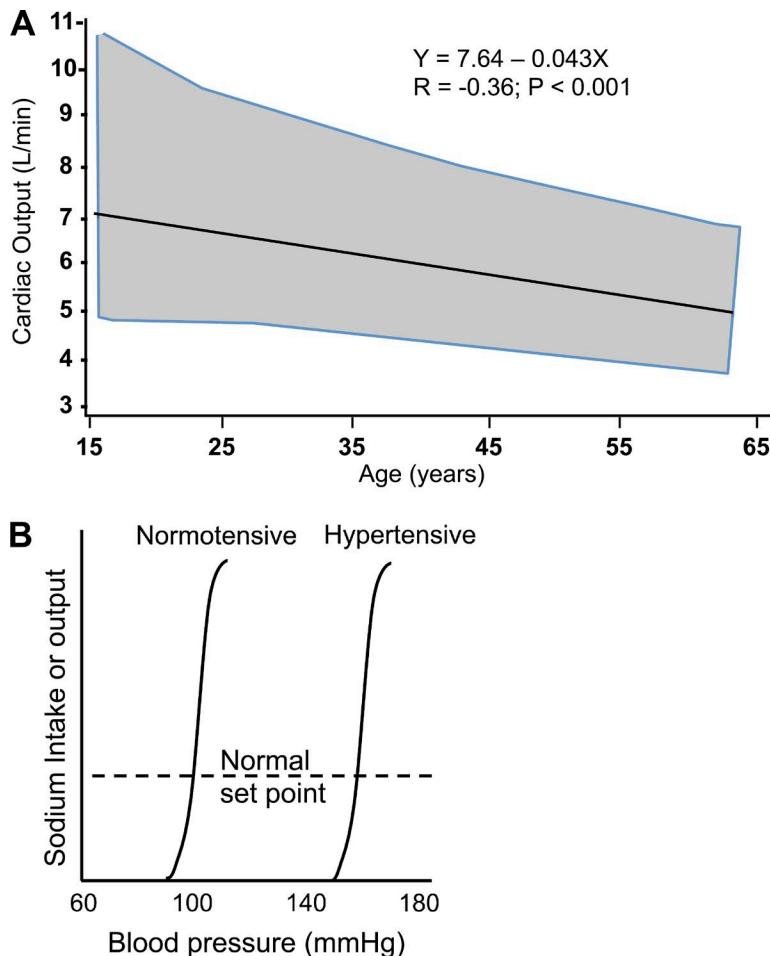
## A brief primer of hypertension

Perturbations of the vasculature, central nervous system, and kidneys have all been implicated in essential hypertension, and likely all contribute to elevations of blood pressure. Yet the precise manner in which these interact, and the factors that recruit these systems, remain a focus of continued investigation. Blood pressure is the product of cardiac output and systemic vascular resistance. Thus, either cardiac output or systemic vascular resistance must be elevated in chronic

hypertension. Interestingly, it appears these play different roles depending on age and likely duration of hypertension. Fagard and Staessen (1991) measured cardiac output at rest and during exercise in 110 hypertensive individuals ranging in age from 16 to 64 yr and found that cardiac output is elevated in younger individuals (age <25 yr) with hypertension but was within the normal range in older patients (Fig. 1 A). Although this might reflect differences in the etiology of hypertension in younger versus older individuals, this pattern is compatible with the concept that blood volume, and thus cardiac output, is elevated early in hypertension and that there are vascular adaptations that occur later in the disease. These vascular events likely increase systemic vascular resistance and concomitantly normalize cardiac output.

Why would blood volume be increased in hypertension? There is substantial support for the concept that renal retention of sodium and water must occur to sustain hypertension. Simply stated, in the setting of normal kidneys, an increase in blood volume and elevation of blood pressure leads to a brisk diuresis and ultimately the normalization of blood pressure. Guyton (1987) defined the relationship between blood pressure with sodium and water excretion as the “pressure-natriuresis” curve, and proposed that there must be a rightward shift in this relationship for hypertension to be sustained. This is schematically illustrated in Fig. 1 B. Any insult or change to the kidney that alters this ability to excrete sodium and water will result in a “natriuretic handicap” wherein the mean arterial pressure over which salt and water is excreted will increase to account for a decrease in the capacity of kidney function. This is often not reflected by overt changes in renal function, but by enhanced reabsorption of

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**Figure 1. Changes in cardiac output and renal function in hypertension.** (A) Relationship between cardiac output (liters/min) and age (yr) in 110 male individuals diagnosed with essential hypertension. Measurements were taken while subjects were at rest in a seated position, from Fagard and Staessen (1991). (B) Guytonian pressure-natriuresis curve relationship. Normotensive individuals undergo a brisk diuresis, excretion of sodium and water, in response to elevations in blood volume and blood pressure, allowing for maintenance of a normal mean arterial pressure. In order for sustainment of hypertension, this curve must be shifted. The shifting of the curve results from changes that occur within the kidney that decrease its capacity to excrete sodium and water. Thus, the mean arterial pressure over which sodium and water are excreted increases to compensate for these deficits in kidney function.

sodium and water along the nephron regulated through differential activity of the various proximal and distal transporters. Sustained hypertension is associated with vascular rarefaction within the kidney (described in the next paragraph) and fibrotic replacement of renal parenchymal tissue, and ultimately defects in glomerular function. These perturbations in renal function underlie the importance of sodium restriction and use of diuretics in the treatment of hypertension, which act in part to normalize the pressure natriuresis curve.

If blood volume is increased in younger patients with hypertension, why does it normalize with time, and why is there a concomitant increase in systemic vascular resistance? Guyton proposed that the increase in cardiac output would lead to “systemic autoregulation,” or widespread vasoconstriction, which further raises blood pressure. This promotes pressure natriuresis and return of blood volume (and cardiac output) to near normal levels. This should occur rather rapidly; however, as shown in Fig. 1 A, there is a gradual decrease in cardiac output over the years. It is now clear that hypertension is associated with other events that increase systemic vascular resistance that evolve slowly. These include enhanced vasoconstriction as a result of local production of potent mediators such as endothelin-1, prostaglandin H<sub>2</sub>, angiotensin

II (Ang II), and increased production of ROS, such as superoxide. Related to this is loss of endothelium-derived nitric oxide (NO), commonly measured as a reduction in endothelium-dependent vasodilatation in isolated vascular preparations or in flow-mediated vasodilatation in humans. This is uniformly present in both human and experimental hypertension, and is in part an oxidative event. There is also remodeling of vessels in hypertension, initially described by Folkow (1982), in which the vascular lumen is narrowed as the media thickens. Finally, there is frank loss of capillaries and resistance vessels, known as vascular rarefaction, in hypertension (Bobik, 2005). All of these events ultimately reduce the cross-sectional area of the vasculature and predispose to increased systemic vascular resistance and hypertension.

There is also strong evidence that the central nervous system plays a critical role in hypertension. Increased sympathetic outflow is a uniform finding in essential hypertension, and contributes to vasoconstriction, vascular remodeling, and vascular smooth muscle cell proliferation (Mancia et al., 1999). Furthermore, activation of adrenergic receptors increases renin release and promotes sodium retention by tubular epithelial cells (Nishi et al., 2015). Centers in the forebrain, the hypothalamus, and the brain stem have all been

implicated in hypertension and have been proposed to alter the balance between sympathetic and parasympathetic outflow. Indeed, renal nerve ablation and carotid sinus stimulation have been used to treat hypertension in humans, albeit with conflicting outcomes. Adrenergic receptor-blocking agents are commonly used as treatments for hypertension, although not considered first-line therapies. Most studies have focused on the role of increased sympathetic outflow; however, parasympathetic withdrawal is also an early event in hypertension (Goit and Ansari, 2016). Interestingly, conditions commonly associated with hypertension, including obesity, sleep disturbances, and chronic stress, are all associated with increased sympathetic outflow.

Emerging evidence over the past decade suggests that these alterations of the vasculature, kidney, and sympathetic nervous system stem, at least in part, from an underlying inflammatory condition during which innate and adaptive immune cells become activated, invade target tissues, and promote end-organ damage through production of various cytokines and chemokines. This review aims to provide a synopsis of the initial discoveries through more recent advances that have been made toward the understanding of the contribution of both the innate and adaptive immune system to blood pressure elevation and end-organ damage.

### Immune system in hypertension

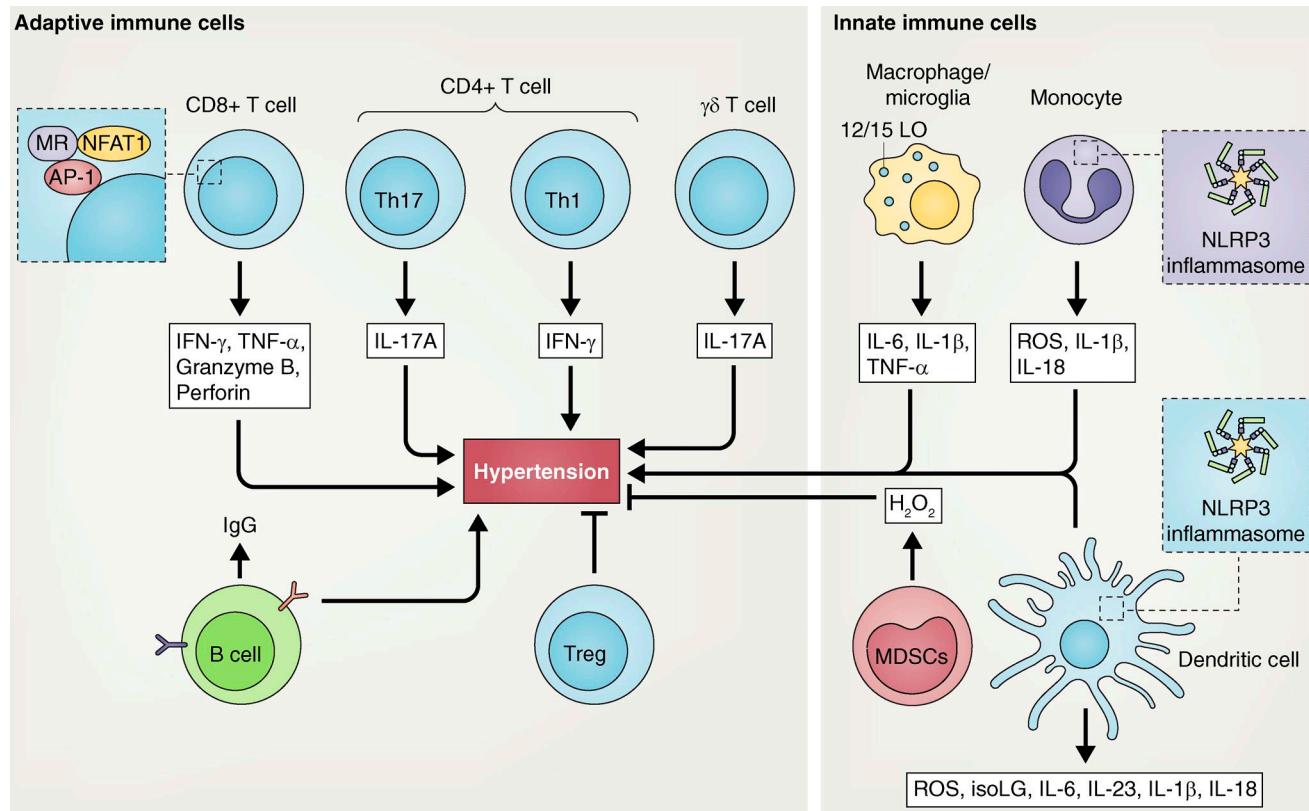
The idea that the immune system and inflammation plays a role in hypertension is an old concept. In the 1960s, Okuda and Grollman (1967) showed that the transfer of lymphocytes from rats with unilateral renal infarction caused hypertension in recipient rats. Additionally, White and Grollman (1964) demonstrated that immunosuppression lowers blood pressure in rats that had partial renal infarction. Olsen (1972) described an inflammatory reaction occurring inside the vasculature of humans with different causes of hypertension; specifically, he noted a periadventitial accumulation of T cells and monocytic cells. In the 1970s, Svendsen (1976) discovered that mice that were thymectomized or athymic nude mice do not maintain hypertension after renal infarction. In the 1980s, Ba et al. (1982) discovered that transplant of a thymus from a Wistar-Kyoto rat reduced blood pressure in a recipient spontaneously hypertensive rat (SHR). They also noted that transplanting a compatible thymus into neonatal SHRs resulted in significant suppression of blood pressure if full immunological restoration was achieved. These studies set the stage for recent advances regarding the role of the immune system in hypertension.

Virtually every cell type involved in innate and adaptive immunity has been implicated in hypertension (Fig. 2). T cells have been observed in the kidneys of hypertensive rodents and humans for many years, and drugs like abatacept and mycophenolate mofetil have been shown to lower blood pressure in experimental models (Rodríguez-Iturbe et al., 2001; Vinh et al., 2010). In 2007, our group showed that mice lacking the recombination-activating gene 1 (RAG1<sup>-/-</sup>

mice), which lack both T and B cells, develop blunted hypertension and have preserved vascular endothelial function when infused with Ang II or after challenge with deoxycorticosterone acetate (DOCA) and salt. Adoptive transfer of T cells restored the hypertensive response and vascular dysfunction observed in WT mice. Subsequently, Mattson et al. (2013) deleted the RAG1 gene in Dahl salt-sensitive rats and showed that this blunted the blood pressure increase that occurs upon high-salt feeding. A striking finding was that the RAG1<sup>-/-</sup> rats seem protected against glomerular damage, albuminuria, and renal damage. In a following study, this group deleted the  $\zeta$  chain (CD247) from CD3<sup>+</sup> in Dahl salt-sensitive rats and found a very similar phenotype to that observed in the rats lacking RAG1 (Rudemiller et al., 2014). In keeping with these findings in RAG1-deficient animals, Crowley et al. (2010) showed that SCID mice, which are deficient in lymphocytes, develop blunted hypertension and cardiac hypertrophy during Ang II infusion.

There has been substantial interest in the subtypes of T cells involved in hypertension, and what they are doing to contribute to this disease. We found that mice lacking CD8<sup>+</sup> T cells were protected from hypertension, whereas mice lacking CD4<sup>+</sup> T cells or MHC class II were not. In this study, deep sequencing revealed an increase in V  $\beta$  chain clonality of CD8<sup>+</sup> T cells in the kidney, but not in blood vessels or the spleen of hypertensive mice. Likewise, there was no clonal skewing of CD4<sup>+</sup> T cells in any organ. We noticed that CD8<sup>-/-</sup> mice also displayed less vascular rarefaction and remodeling in the kidney as compared with WT or CD4<sup>-/-</sup> mice. An interesting study by Youn et al. (2013) compared circulating T cell phenotypes in newly diagnosed hypertensive patients to age- and sex-matched controls. They found that the number of circulating “immunosenescent” proinflammatory CD8<sup>+</sup> T cells is increased in humans with hypertension. These cells produce increased amounts of IFN- $\gamma$ , TNF- $\alpha$ , and the cytotoxic molecules granzyme B and perforin compared with CD8<sup>+</sup> T cells from normal subjects.

As further support for a role of CD8<sup>+</sup> T cells in hypertension, Sun et al. (2017b) recently showed that these cells express the mineralocorticoid receptor (MR), and that this T cell receptor plays a major role in systemic hypertension. The MR, which is a nuclear protein, was found to complex with nuclear factor of activated T cells 1 (NFAT1) and with activator protein 1 (AP1) to promote IFN- $\gamma$  production by CD8<sup>+</sup> T cells and that specific deletion of the MR receptor in T cells caused a dramatic decrease in blood pressure elevation and renal and vascular damage caused by Ang II. In contrast, overexpression of the MR in T cells exacerbated hypertension. Eplerenone, a commonly used MR receptor antagonist, prevented IFN- $\gamma$  production by CD8<sup>+</sup> T cells in hypertension. Traditionally, research has focused on the role of the MR on epithelial cells of the distal kidney nephron in promoting sodium and volume retention; however, there is growing evidence that the MR has prohypertensive effects in other cells (McCurley et al., 2012; Jia et al., 2016). This



**Figure 2. Innate and adaptive immune cells that have been shown to play a critical role in hypertension.** Adaptive immune cells: CD8<sup>+</sup> T cells, CD4<sup>+</sup> cells (Th1, Th17, and T reg cells), T cells, and B cells produce factors that promote or inhibit hypertension. Innate immune cells: Macrophages, microglia, monocytes, DCs, and MDSCs also produce cytokines and ROS, which promote or inhibit hypertension. The NLRP3 inflammasome in monocytes and DCs plays a key role in hypertension.  $\gamma\delta$  T cells function on the border of innate and adaptive immunity.

study showed that the T cell MR has a previously undefined role in hypertension.

There is also evidence that CD4<sup>+</sup> T cells are activated in hypertension and likely play an important role. We and others have shown that IL-17A, produced in large part by CD4<sup>+</sup> T cells, plays a critical role in hypertension (Madhur et al., 2010; Nguyen et al., 2013). IL-17A has been shown to raise blood pressure when infused into normal mice, and to induce phosphorylation of the endothelial NO synthase (eNOS) on threonine 495, an inhibitory site, leading to impaired endothelium-dependent vasodilatation (Nguyen et al., 2013). In humanized mice, we found that long-term infusion of Ang II caused a striking increase of human CD4<sup>+</sup> T cells in lymph nodes and accumulation in the kidneys and aorta. Likewise, we found that the production of IL-17A is markedly increased in the circulating CD4<sup>+</sup> T cells of humans (Itani et al., 2016).

Approximately 10% of CD4<sup>+</sup> T cells are T regulatory (T reg) cells, and these have been shown to modulate hypertension. Barhoumi et al. (2011) found that adoptive transfer of T reg cells into WT mice reduced the hypertension, endothelial dysfunction, and immune cell infiltration caused by Ang II. Matrougui et al. (2011) saw a reduction in T reg cells in Ang II-infused mice. These authors also noted improved coronary

arteriolar endothelial function in mice that received adoptive transfer of T reg cells. Mian et al. (2016) demonstrated that microvascular injury, measured by examining microvascular remodeling and stiffness, was exaggerated in Rag1<sup>-/-</sup> mice given T cells from Scurfy mice, which lack T reg cells, compared with mice given WT T cells in response to Ang II. Scurfy mice are deficient in T reg cells because of their mutated forkhead box p3 (Foxp3) gene. These data add further evidence showing that functional T reg cells are crucial for controlling the hypertensive response in mice to Ang II.

Although the majority of T cell receptors are composed of  $\alpha/\beta$  chains, a small percentage possess  $\gamma/\delta$  receptors, and recent evidence suggests that these also contribute to hypertension. Caillon et al. (2017) recently showed that  $\gamma/\delta$  T cells are important in hypertension, and that mice lacking these cells exhibit a markedly blunted rise in blood pressure and preserved endothelial function in response to Ang II infusion.  $\gamma/\delta$  T cell-deficient mice also exhibited fewer activated CD4<sup>+</sup> T cells expressing the marker CD69 in the spleen and mesenteric arteries, suggesting that  $\gamma/\delta$  T cells might have an initiating role in hypertension. Likewise, antibody clearance of  $\gamma/\delta$  T cells reduced the hypertensive response to Ang II. The precise mechanisms by which these cells promote

hypertension remain to be defined, but these cells are also major sources of IL-17A, which has prohypertensive actions in both the kidney and vasculature (Saleh et al., 2016).

There is also evidence that B cells and the antibodies that they produce contribute to hypertension. This has been extensively studied in preeclampsia, a devastating illness affecting between 2 and 8% of all pregnancies, associated with hypertension, proteinuria, fetal growth retardation, and placental ischemia. Antibodies that are agonistic to the angiotensin type 1 receptor (AT<sub>1</sub>R) have been observed in both humans with preeclampsia and in experimental models of this illness (Dechend et al., 2000; LaMarca et al., 2011). There is evidence that T<sub>H</sub>1 CD4<sup>+</sup> cells are required for production of such autoantibodies (Wallace et al., 2011), and that T reg cells markedly inhibit their presence in experimental preeclampsia (Cornelius et al., 2015). Similar AT<sub>1</sub>R antibodies occur in humans with secondary malignant hypertension (Fu et al., 2000) and with renal allograft rejection (Dragun et al., 2005).

Chan et al. (2015) recently showed an increase in the number of activated B cells and of plasma cells in the spleens of mice made hypertensive by Ang II infusion. Additionally, they found that circulating IgG is markedly increased and accumulates in the aortic adventitia in hypertension. In B cell-activating factor receptor-deficient mice (BAFF-R<sup>-/-</sup> mice), which lack mature B cells, the increase in circulating IgG was eliminated and the increase in systolic blood pressure was attenuated in response to Ang II. Furthermore, Chan et al. (2015) showed that depletion of B cells using a CD20 antibody attenuates the blood pressure increase caused by Ang II infusion. These data demonstrate the importance of B cells in the development of hypertension and warrant further studies into the role of B cells and the antibodies they produce in hypertension. The role of antibodies that might activate prohypertensive receptors such as the AT<sub>1</sub>R is particularly interesting.

In addition to cells of the adaptive immune system, there is ample evidence that innate immune cells play a role. The earliest evidence of this was derived from studies of osteoprotic mice (Op/Op) mice that are deficient in the macrophage colony stimulating factor and thus have reduced macrophages. De Ciucis et al. (2005) showed that these animals exhibited markedly blunted hypertension in response to chronic Ang II infusion, and had preserved endothelium-dependent vasodilatation and vascular morphology as compared with WT littermates. Wenzel et al. (2011) used diphtheria toxin to deplete monocytes in mice expressing the diphtheria toxin receptor on myeloid cells and showed that this completely prevented the hypertension and attenuated the endothelial dysfunction caused by chronic Ang II infusion. The hypertensive response was fully restored by adoptive transfer of WT monocytes into these mice. Kriska et al. (2012) showed that mice lacking 12/15 lipoxygenase (12/15 LO), produced by macrophages and other cells, are markedly resistant to hypertension caused by DOCA-salt or the NO synthase inhibitor nitro L-arginine methyl ester (L-NAME). Adoptive transfer

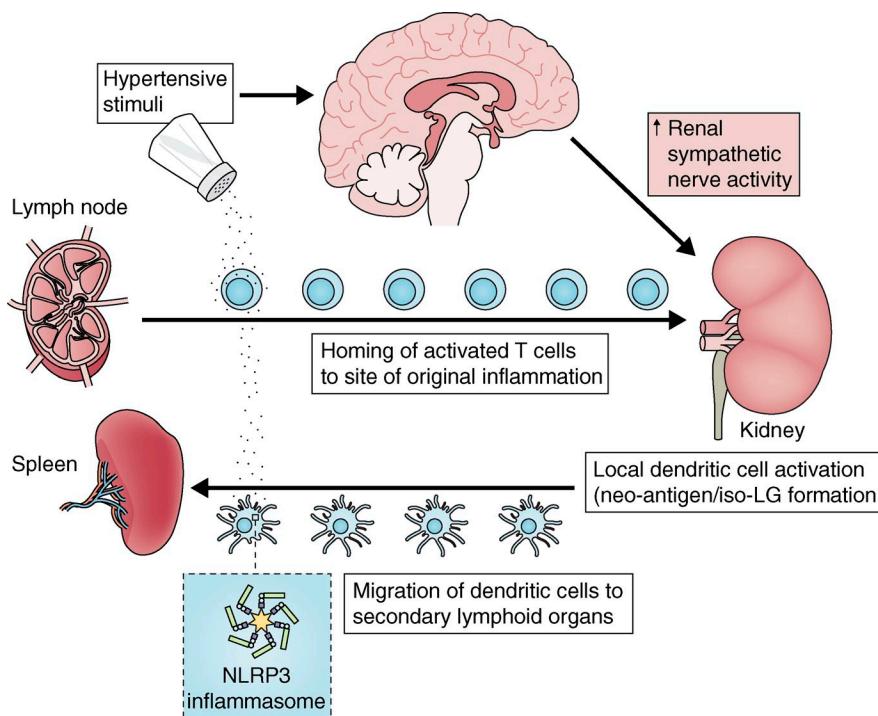
of WT macrophages to these animals restored their hypertensive response to L-NAME, and clearing of macrophages using clodronate prevented L-NAME-induced hypertension. The precise role of 12/15 LO in hypertension was not defined in this study; however, the importance of this enzyme in macrophages was clear.

Kirabo et al. (2014) identified a previously unknown role of dendritic cells (DCs), and particularly monocyte-derived DCs, in the genesis of hypertension. DCs of hypertensive mice produce increased amounts of ROS, ultimately leading to the production of isolevuglandin protein adducts (isoLG). These modified proteins seem to act as neoantigens to promote T cell, and in particular CD8<sup>+</sup> T cell, proliferation and cytokine production. The increase in isoLG adducted proteins corresponded with an increase in surface expression of CD86 and increased production of IL-6, IL-1 $\beta$ , and IL-23. Moreover, adoptive transfer of DCs from hypertensive mice resulted in a striking increase in blood pressure in the recipient mice in response to a generally suppressor dose of Ang II. Subsequent studies have shown that catecholamines stimulate isoLG adduct formation in these cells, and that the kidney is a major site for DC activation and isoLG formation (Xiao et al., 2015).

Another innate immune cell that seems to have a role in hypertension is the myeloid-derived suppressor cell (MDSC). These are a heterogeneous population of immature myeloid cells first described to suppress immune responses to cancer. Shah et al. (2015) showed that these cells increased in the circulation during prolonged Ang II infusion or after L-NAME/high-salt-induced hypertension. They further showed that hypertension increased the ability of MDSCs to suppress T cell activation, and this was at least in part because of hydrogen peroxide production by these cells. Inhibition of MDSCs raised blood pressure, and adoptive transfer of these cells lowered blood pressure in Ang II-infused mice.

### Mechanisms of immune activation in hypertension

The precise reasons immune cells are activated in hypertension remain poorly defined. As discussed in the brief primer on hypertension above, sympathetic outflow is commonly increased in hypertension, and there is substantial evidence that this plays a role in activation of both myeloid cells and T cells. Sympathetic nerves innervate the spleen and lymph nodes (Felten et al., 1984; Bellinger et al., 1992; Rosas-Ballina et al., 2011; Lori et al., 2017), and most immune cells possess adrenergic receptors. In particular, T and B cells almost exclusively express the  $\beta$ 2 subtype (Sanders, 2012). As an example, Ganta et al. (2005) showed that administration of Ang II in the lateral cerebral ventricle increased mRNA expression of proinflammatory splenic cytokines such as IL-1 $\beta$  and IL-6, and splenic sympathetic denervation abrogated these responses. We produced lesions of the forebrain in mice that prevent sympathetic outflow and showed that these prevented immune cell activation in Ang II-induced hypertension (Marvar et al., 2010; Lob et al., 2013). In contrast, we enhanced sympathetic outflow



**Figure 3. Working hypothesis for activation of renal DCs by sympathetic activation in hypertension.** Hypertension enhances sympathetic outflow to the kidney, where DCs become activated, accumulate isoLG-adducts, and transmigrate to secondary lymphoid organs. T cells activated by DCs return to the kidney to promote renal dysfunction and damage. From Xiao et al. (2015).

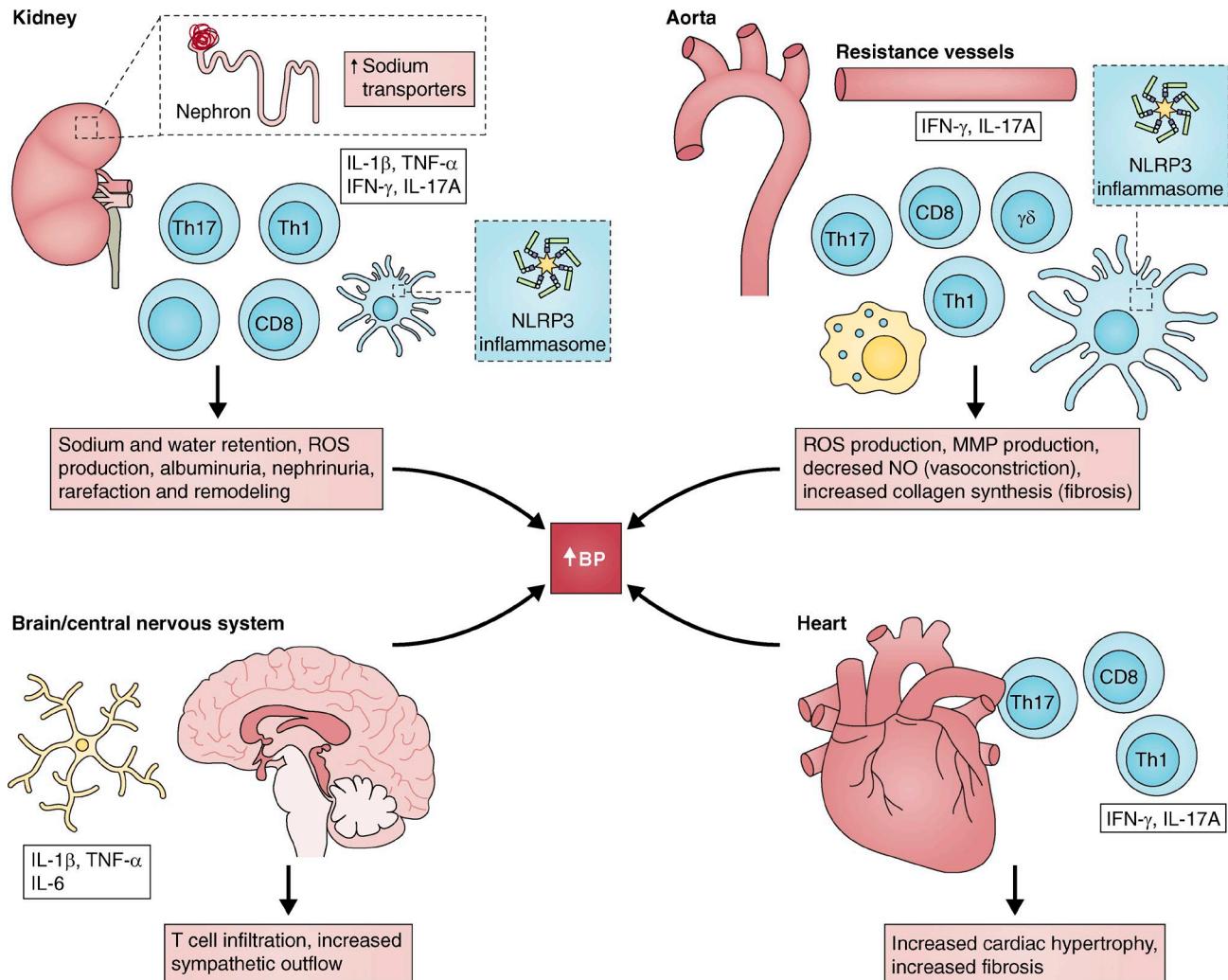
by deleting an antioxidant enzyme in this region and showed that this enhanced T cell activation and vascular infiltration. We also showed that renal denervation has a dramatic effect on the renal infiltration of T cells and the production of cytokines by myeloid (CD11b<sup>+</sup>/CD11c<sup>+</sup>) DCs in both the kidney and the spleen, suggesting that the kidney is a major site of immune activation by sympathetic nerves in this model of Ang II-induced hypertension (Xiao et al., 2015). We found that renal denervation prevents formation of isoLG adducts in DCs of the kidney and the appearance of DCs with these adducts in the spleen. These data are compatible with the working hypothesis shown in Fig. 3, in which DCs in the kidney accumulate isoLG adducts and are activated by neuronally released norepinephrine to produce cytokines like IL-6, IL-1 $\beta$ , and IL-23. These cells likely transmigrate to secondary lymphoid organs, where they activate T cells to return to the kidney and vasculature.

In addition to catecholamines, another stimulus common to the hypertensive milieu is altered mechanical forces experienced by the vascular wall. In particular, hypertension increases the cyclical stretch of larger vessels, and as the proximal vasculature stiffens, enhances delivery of the pulse wave to the distal vasculature. The resultant increased stretch increases endothelial production and release of IL-6, IL-8, ROS, endothelin, and other proinflammatory mediators (Jufri et al., 2015). Likewise, increased stretch increases endothelial expression of the vascular cell adhesion molecule 1 (VCAM1), the intracellular adhesion molecule 1 (ICAM), and CD40. Although not extensively studied, it is likely that factors such as these can enhance activation of adjacent monocytes, macrophages, and DCs.

Another stimulus for immune activation in hypertension is increased sodium. In contrast to classic teaching, it has become apparent that interstitial concentrations of sodium can exceed the blood plasma concentrations by as much as 40 mmol/liter in the interstitium of hypertensive animals and humans (Machnik et al., 2009; Kopp et al., 2012). Such concentrations of sodium have been shown to stimulate IL-17A production by T cells via activation of the salt-sensing kinase serum and glucocorticoid-regulated kinase 1 (SGK1; Kleinewietfeld et al., 2013; Wu et al., 2013). We have recently shown that deletion of T cell SGK1 blunts hypertension and reduces end-organ damage in response to either Ang II or DOCA-salt (Norlander et al., 2017), supporting a role of this enzyme in the T cell in hypertension. Very recently, we have shown that similar concentrations of sodium can promote DCs to form ROS and isolevuglandin-protein adducts (Barbaro et al., 2017). We established that sodium enters DCs via an amiloride-sensitive channel and leads to activation of the NADPH oxidase. DCs affected in this fashion stimulate T cells to proliferate and to produce IL-17A, and when adoptively transferred to naive mice, worsens the hypertensive response to low-dose Ang II infusion. Thus, salt stimulates T<sub>H</sub>17 cell formation not only directly but also indirectly via actions on APCs.

#### Organ involvement

In the next paragraphs, we will discuss recent understanding of the contribution of the immune system in specific organs in hypertension. These organs are not only targets of hyper-



**Figure 4. Inflammation in the kidney, aorta, brain, and heart promote hypertension and end-organ damage.** Kidney: CD4<sup>+</sup> Th1 and Th17 cells as well as γδ T cells and DCs infiltrate the kidney and produce cytokines such as IL-1β, TNF-α, IFN-γ, and IL-17A. Mediators from these cells up-regulate sodium transporter expression and activity, resulting in sodium and water retention; damage kidney tissue, resulting in albuminuria and nephritis; alter/ damage kidney vasculature, resulting in rarefaction and remodeling; and increase ROS production within the kidney. Blood vessels: CD4<sup>+</sup> Th1 and Th17 cells, CD8<sup>+</sup> T cells, γδ T cells, macrophages, and DCs infiltrate the blood vessels and produce IFN-γ and IL-17A. As a result, there is increased ROS production, increased matrix metalloproteinase production, and decreased NO bioavailability, which leads to vasoconstriction and increased collagen synthesis, resulting in stiffening of the vessels within the vasculature. Brain/CNS: Microglia, which are resident in the brain, become activated and produce IL-1β, TNF-α, and IL-6. These cytokines increase sympathetic outflow and promote the infiltration of T cells. Heart: CD4<sup>+</sup> Th1 and Th17 cells as well as CD8<sup>+</sup> T cells infiltrate and produce IFN-γ and IL-17A. This increases cardiac hypertrophy and fibrosis within the heart.

tension, but once they become dysfunctional, they promote further elevations of blood pressure (Fig. 3).

**Kidney.** The kidney is not only a major determinant of blood pressure but also a key target of inflammatory end-organ damage associated with hypertension. Inflammatory cells and their products contribute to blood pressure elevation at least in part by increasing renal sodium transport. Ultimately, uncontrolled inflammation results in renal fibrosis, oxidative stress, glomerular injury, and chronic kidney disease (Fig. 4).

Inflammatory cytokines secreted by innate and adaptive immune cells, as well as renal epithelial cells, modulate the ex-

pression and activity of sodium transporters at various points along the nephron, leading to defective pressure natriuresis, sodium and water retention, and hypertension. Cytokines can regulate renal sodium transporters directly or indirectly (i.e., through modulation of the intrarenal renin angiotensin system or NO production). For a detailed review on this topic, see Norlander and Madhur (2017).

Infiltrating APCs, including monocytes and DCs, contain multiple important sensors of the innate immune system; one of these sensors is the inflammasome, activated by pathogen- and danger-associated molecular patterns (PAMPs and DAMPs) that lead to the maturation of IL-1β and IL-18

from inactive proforms. Two recent studies have demonstrated the importance of the inflammasome to hypertension. Wang et al. (2014) showed that hypertension is blunted in inflammasome-deficient  $ASC^{-/-}$  and  $NLRP3^{-/-}$  mice using a model of renin-dependent renovascular hypertension. Krishnan et al. (2016) demonstrated that apoptosis- $ASC^{-/-}$  mice are protected from renal inflammation, fibrosis, and elevated blood pressure induced by DOCA-salt hypertension. These authors also demonstrated that a specific  $NLRP3$  inhibitor reverses DOCA-salt-induced hypertension, indicating a causal role for the inflammasome and its key cytokines, IL-1 $\beta$  and IL-18, in the development of hypertension. In keeping with this concept, Zhang et al. (2016) recently showed that mice lacking IL-1 receptor 1 ( $IL-1R1^{-/-}$ ) develop blunted hypertension when administered Ang II, and this is associated with reduced activation of sodium potassium chloride cotransporter 2(NKCC2) and diminished sodium retention during the onset of hypertension. These effects were mimicked by treatment with the IL-1R1 receptor antagonist Anakinra. The authors linked IL-1R1 signaling to reduced NO production and enhanced formation of ROS in the kidney, factors known to modulate NKCC2 activity.

We recently investigated the effect of inflammation on hypertension and end-organ dysfunction using a mouse model of LNK deficiency. Multiple genome-wide association studies identified a polymorphism in the gene *Sh2b3* (which encodes the lymphocyte adaptor protein LNK) to be associated with several autoimmune and cardiovascular disorders, including hypertension, in humans. Expressed in all hematopoietic cells and some somatic cells, LNK functions as a “brake” to cell proliferation and cytokine signaling. We found that  $LNK^{-/-}$  mice have elevated levels of inflammatory cells in their kidneys and vessels at baseline and that these are further increased by Ang II infusion.  $LNK^{-/-}$  mice develop exaggerated hypertension, and their kidneys show evidence of increased superoxide production, albuminuria, and nephritis (markers of glomerular injury) and increased sodium and water retention after Ang II infusion compared with WT mice. Interestingly, we found a specific increase in IFN- $\gamma$ -producing CD4 $^+$  (Th1) cells and CD8 $^+$  (Tc1) cells and demonstrated that loss of IFN- $\gamma$  protects against hypertension (Saleh et al., 2015). Thus, the effects of LNK deficiency on hypertension and renal dysfunction may in part be a result of increased IFN- $\gamma$  signaling. However, the effect of IFN- $\gamma$  on blood pressure has been mixed. Similar to our results, Garcia et al. (2012), found that IFN- $\gamma$  deficiency protected against hypertension in a mouse model of uninephrectomy combined with aldosterone infusion and salt feeding, whereas Zhang et al. (2014) and Markó et al. (2012) found no effect of loss of IFN- $\gamma$  or the IFN- $\gamma$  receptor, respectively, on blood pressure using hypertension models involving much higher doses of Ang II with or without uninephrectomy. Nevertheless, Markó et al. (2012) did report decreased renal inflammation and tubulointerstitial damage in IFN- $\gamma$  receptor-deficient mice compared with WT mice after Ang II infusion.

Another cytokine that seems to play a role in hypertension is TNF- $\alpha$ . Venegas-Pont et al. (2010) showed that TNF- $\alpha$  contributes to the development of hypertension observed in a genetic mouse model of systemic lupus erythematosus (SLE). Treatment of female SLE (NZBWF1) mice with etanercept, a TNF- $\alpha$  antagonist, or vehicle for 4 wk reduced mean arterial pressure, albuminuria, monocyte/macrophage infiltration, and renal cortex NADPH activity. Mathis et al. (2014) found that depletion of B cells with anti-CD20 antibodies reduced glomerular and tubular injury and prevented the development of hypertension. These effects were associated with a marked reduction in renal cortical TNF- $\alpha$  expression. The precise link between B cells and TNF- $\alpha$  expression was not defined; however, B cell depletion was accompanied by a reduction in renal T cells, which are potential sources of this cytokine.

**Blood vessels.** In hypertension, innate and adaptive immune cells accumulate in blood vessels, particularly in the perivascular fat and adventitia, where they communicate with the vessel wall through the production of various factors including ROS, cytokines, and matrix metalloproteinases. Mikolajczyk et al. (2016) demonstrated that this is effect is, in part, caused by the chemokine RANTES. Many of the cells, particularly T cells that infiltrate the perivascular adipose tissue, bear the RANTES receptor CCR5. The authors showed that vascular accumulation of IFN- $\gamma$ -producing T cells and endothelial function are preserved in RANTES $^{-/-}$  mice infused with Ang II.

Although immune cells are potent sources of ROS, cytokines from these cells likely diffuse to the adjacent vascular cells and stimulate ROS formation by vascular smooth muscle and endothelial cells. Excess ROS reduces the bioavailability of NO, leading to impaired vasodilation. Inflammatory cytokines can also lead to reduced NO production, increased collagen synthesis, and further recruitment of inflammatory cells (McMaster et al., 2015).

We and others have identified IL-17A, produced by CD4 $^+$  (Th17) cells and  $\gamma\delta$  T cells, to play a major role in the vascular dysfunction associated with hypertension (Saleh et al., 2016). IL-17A $^{-/-}$  mice develop blunted hypertension, abrogated vascular inflammation and vascular oxidative stress, and preserved vascular reactivity in response to Ang II infusion (Madhur et al., 2010). Nguyen et al. (2013) demonstrated that IL-17A phosphorylates the eNOS at an inhibitory site, leading to increased superoxide production and reduced NO production. Likewise, we have shown that treatment of mice with the TNF- $\alpha$  antagonist etanercept mitigates the increase in vascular ROS production and enhances endothelium-dependent vasodilatation in Ang II-induced hypertension (Guzik et al., 2007).

Another manifestation of vascular inflammation in hypertension is vascular fibrosis. Increased stiffness of the proximal vessels leads to enhanced pulse wave propagation to the peripheral tissues and enhanced end-organ damage. We found that both RAG1 $^{-/-}$  mice and IL-17A $^{-/-}$  mice

are protected against aortic stiffening (Wu et al., 2014). The role of IL-17A in fibrosis is controversial. We found IL-17A directly stimulates production of collagens I, III, and V by aortic fibroblasts, whereas others have found an inhibitory role (Sun et al., 2017a).

Finally, an important consequence of hypertension is vascular rarefaction, or frank loss of the small resistance vessels and capillaries. We found that mice lacking CD8<sup>+</sup> T cells develop less vascular rarefaction in the kidney than WT mice during Ang II-induced hypertension, and that this is associated with preserved capacity to secrete a sodium load.

**Brain.** The brain is both an initiator and an end-organ target of hypertension. Sympathetic outflow is uniformly increased in hypertension via coordinated actions of the forebrain centers, the hypothalamus and the brain stem centers. Shi et al. (2010) demonstrated that microglial cells, resident macrophage cells of the brain, become activated and contribute to hypertension during Ang II infusion. These cells produce inflammatory cytokines including IL-1 $\beta$ , IL-6, and TNF- $\alpha$  locally. These investigators showed that intracerebroventricular injections of the antibiotic minocycline, which inhibits microglial cells, attenuates the hypertensive response to Ang II. Shen et al. (2015) subsequently selectively depleted microglia through intracerebroventricular injection of diphtheria toxin into CD11b-diphtheria toxin receptor mice and discovered that neuroinflammation in response to Ang II and L-NAME were blunted. These studies link activated microglia to the development of neurogenic hypertension.

The forebrain circumventricular organs, including the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis, are the highest known centers to modulate sympathetic outflow in hypertension. The SFO in particular lacks a well-formed blood-brain barrier and senses blood-borne signals such as salt and Ang II. Pollow et al. (2014) observed CD3<sup>+</sup> cells infiltrating around this organ in male but not female mice after Ang II-induced hypertension. Our own group has manipulated sympathetic outflow from this region and has shown that increases in sympathetic outflow enhance peripheral T cell activation (Lob et al., 2010), whereas blockade of sympathetic outflow has the opposite effect (Marvar et al., 2010; Lob et al., 2013).

Katsuki et al. (2015) found that numbers of T reg cells decrease during the development of hypertension in the stroke-prone SHR (SHRSP) and that this is prevented by splenic denervation in prehypertensive animals. This intervention was found to delay the onset of hypertension, implicating CNS control over T reg cell number and function in hypertension. Viel et al. (2010) found dysfunctional T reg cells in the Dahl SS rat. Compared with results in Brown Norway rats, the suppressive capacity of T reg cells was impaired. Collectively, these studies highlight the benefits of functional T reg cells for the prevention of hypertension and the importance of sympathetic outflow to the spleen for activation of T reg cells.

Santisteban et al. (2015) linked bone marrow cells to the development of neuroinflammation during hypertension. The authors demonstrated that reconstitution of SHRs with bone marrow from normotensive Wistar Kyoto rats resulted in a marked decrease in mean arterial pressure, whereas Wistar Kyoto rats reconstituted with bone marrow from SHRs developed a hypertensive phenotype complete with increased inflammation. Additionally, these authors went on to show that the antiinflammatory antibiotic minocycline lowers blood pressure in SHRs and in normal rats infused with Ang II. Minocycline also prevented extravasation of bone marrow cells into the paraventricular nucleus of the hypothalamus. These studies provided the first evidence linking bone marrow cells to neuroinflammation.

Although unrelated to hypertension, a recent study by Tawakol et al. (2017) illustrated a correlation between brain amygdalar activity and activity of the bone marrow, both quantified by <sup>18</sup>F-FDG uptake. Similarly, amygdalar activity also correlated with increased carotid uptake of <sup>18</sup>F-FDG, indicative of arterial inflammation. Amazingly, over a 5-yr follow up of 293 subjects, cardiovascular events were much higher in those with high compared with those with low amygdalar activity. In a subgroup of patients, perceived stress was also associated with arterial inflammation and increased amygdalar activity. This study further emphasizes an evolving understanding of how brain centers activate bone marrow cells, ultimately leading to end-organ disease.

**Heart.** The heart is also an important target of activated immune cells during hypertension. A hallmark of hypertension is cardiac hypertrophy and fibrosis. Markó et al. (2012) showed that cardiac hypertrophy, fibrosis, and inflammation caused by Ang II were reduced in IFN- $\gamma$ R-deficient mice, although blood pressure was not changed. Amador et al. (2014) found activation of Th17 cells in both the heart and kidney of rats through measurements of IL-17 and ROR $\gamma$ t after induction of DOCA-salt hypertension. Moreover, the authors found that treating DOCA-salt-challenged rats with an anti-IL-17 antibody reduced blood pressure and reduced expression of many proinflammatory and profibrotic mediators in both the heart and the kidney. These studies confirm the importance of IL-17 signaling to the development of hypertension. Kvakan et al. (2009) show that adoptive transfer of T reg cells into Ang II-infused mice resulted in less cardiac hypertrophy and fibrosis of the heart despite no change in blood pressure. Additionally, the authors show reduced T cell infiltration into the heart, displaying a critical role for T reg cells in suppression of the inflammatory response that results from Ang II infusion.

**Gastrointestinal system and the microbiome.** Although the gut is not traditionally thought to be a target of end-organ damage, emerging evidence suggests that hypertension is associated with alterations of the gut microbiome. Several experimental models display a switch in the ratio of intestinal Bacteroidetes to Firmicutes bacterial species and less bacterial

diversity (Marques et al., 2017). Yang et al. (2015) demonstrated such an imbalance in spontaneously hypertensive rats, Ang II–infused rats, and humans with hypertension. A consequence of this dysbiosis is a reduction in short chain fatty acids such as butyrate and acetate, which have antiinflammatory properties. Related to this, Karbach et al. (2016) showed that germ-free mice were protected against the hypertensive effects of Ang II infusion and had less renal and arterial leukocyte infiltration than conventionally raised mice. This was associated with blunted aortic expression of mRNA for the monocyte chemoattractant peptide 1 and reduced endothelial leukocyte rolling. Likewise, cardiac infiltration of myelomonocytes was decreased in germ-free mice. Recently, Wilck et al. (2017) showed that feeding a high-salt diet to mice depletes intestinal *Lactobacillus murinus* and that this promotes T<sub>H</sub>17 cell formation and aggravates both encephalitis and hypertension. Concomitant treatment with *L. murinus* blunted hypertension in these animals. In humans, a 14-d challenge with a high-salt diet increased blood pressure and circulating IL-17A<sup>+</sup>/TNF- $\alpha$ <sup>+</sup>/CD4<sup>+</sup> T cells while reducing fecal *Lactobacillus* species. This is an exciting new direction of research that likely has enormous implications for understanding how diet and the gut can affect inflammation in distant organs and ultimately blood pressure.

### Conclusion and future directions

Our understanding of immune activation and inflammation in hypertension is evolving, and numerous laboratories are now devoting substantial effort to this topic. Knowledge of how immune cells are activated and exactly what they do in hypertension remains insufficient. The precise role of myeloid cells, T cells, B cells, and their respective subtypes requires further study. It is unclear exactly where these cells are activated, i.e., in the kidney, the vasculature, the gut, the bone marrow, or secondary lymphoid organs. There appears to be cross talk between these sites by mechanisms that are poorly understood. Interactions with the nervous system and its various mediators require additional investigation. Of particular note, it is now clear that local inflammation can elicit afferent signals and an “immune reflex” (Oke and Tracey, 2009); however, the role of this reflex in hypertension needs to be defined. The nature of potential antigens generated in hypertension is not clear. Although some evidence suggests that oxidatively modified proteins might serve this role, the specific proteins or peptides that are altered in this fashion remain to be defined. As evident in the discussion above, the immune system is highly interdependent, and the manners by which these various cells and mediators interact in hypertension remain to be elucidated.

A major goal of hypertension treatment is prevention of end-organ damage. Increasingly it has become apparent that a substantial portion of the vascular, renal, cardiac, and brain damage and dysfunction that accompanies hypertension is mediated by inflammation within these target organs. The data discussed in this review implicate the innate and adaptive

immune responses as critical to the development of hypertension and its untoward consequences. Each cell type discussed is a potential target for future therapies to complement currently used antihypertensive agents. Although immune modulation might not be commonly used for hypertension, such treatment might be warranted in difficult-to-treat populations or in patients with aggressive end-organ damage. Moreover, targeted therapies such as preventing formation of isolevuglandin adducts, blockade of specific adrenergic receptors, or MR blockade might have previously unappreciated antiinflammatory effects that are worthy of further study.

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