Published in final edited form as:

Transl Res. 2018 January ; 191: 45–63. doi:10.1016/j.trsl.2017.10.011.

Macrophages Under Pressure: The Role of Macrophage Polarization in Hypertension

Sailesh C. Harwani, MD, PhD^{1,2,3}

¹Department of Internal Medicine, 200 Hawkins Dr., Iowa City, IA, US, 52242

²Center for Immunology and Immune Based Diseases, 2193 MERF, Iowa City, IA, US, 52242

³Abboud Cardiovascular Research Center, 2269 CBRB, Iowa City, IA, US 52242

Abstract

Hypertension is a multi-factorial disease, involving the nervous, renal, and cardiovascular systems. Macrophages are the most abundant and ubiquitous immune cells, placing them in a unique position to serve as key mediators between these components. The polarization of macrophages confers vast phenotypic and functional plasticity, allowing them to act as pro-inflammatory, homeostatic, and anti-inflammatory agents. Key differences between the M1 and M2 phenotypes, the two subsets at the extremes of this polarization spectrum, place macrophages at a juncture to mediate many mechanisms involved in the pathogenesis of hypertension. Neuronal and non-neuronal regulation of the immune system, i.e. the “neuro-immuno” axis, plays an integral role in the polarization of macrophages. In hypertension, the “neuro-immuno” axis results in synchronization of macrophage mobilization from immune cell reservoirs and their chemotaxis, via increased expression of chemoattractants, to end-organs critical in the development of hypertension. This complicated system is largely coordinated by the dichotomous actions of the autonomic neuronal and non-neuronal activation of cholinergic, adrenergic, and neurohormonal receptors on macrophages, leading to their ability to “switch” between phenotypes at sites of active inflammation. Data from experimental models and human studies are in concordance with each other and support a central role for macrophage polarization in the pathogenesis of hypertension.

Introduction

Mononuclear phagocytic immune cells serve as a cohesive cellular component in explaining the immune system’s role in the pathophysiology of hypertension. Essential or primary hypertension is a complex multi-factorial disease that involves the nervous, renal, and

Corresponding Author: Sailesh C. Harwani, MD, PhD, University of Iowa, Carver College of Medicine, Department of Internal Medicine, Division of Cardiovascular Diseases, 501 Newton Rd., 616B MRC, Iowa City, IA, 52242, Phone: 319-335-6982, Fax: 319-335-6969, sailesh-harwani@uiowa.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of interest: The authors have read the journal’s policy on disclosure of potential conflicts of interest and have none to declare.

cardiovascular systems. Macrophages are the most abundant and most widespread, yet most primitive, immune cells in tissues, including the organ systems involved in hypertension (1). Thus, it is no surprise that to function in diverse environments, macrophages possess an extraordinary degree of plasticity. This plasticity is manifested in a sophisticated framework of functional and phenotypic differentiation, i.e. polarization. At its extremes, this polarization is characterized by the M1 and M2 phenotypes. Although this is an oversimplification of macrophage polarization, identifying key differentiating characteristics between these two macrophage populations can be utilized to begin understanding the complex role of macrophages in the pathophysiology of hypertension. Building a “macrophage-centric model” will help explain the interrelatedness of the neural, renal, and vascular components of hypertension. The most important contribution of this review will be to introduce the “neuro-immuno” axis as an important force in macrophage polarization. Based on the available data, this review will synthesize an interdisciplinary model of macrophage polarization, where the nervous system plays an integral role in synchronizing mobilization of macrophages from immune reservoirs and their chemotaxis to tissues/organ systems involved in the development of hypertension. Finally, we will also review the evidence for the role of inflammation, with a focus on macrophages, in clinical essential hypertension.

Key Aspects of Macrophage Polarization

Macrophages not only play an integral role in the innate immune response, but also carry out homeostatic functions. To carry out these diverse duties, macrophages harbor the ability to polarize and morph into subsets, demonstrating diverse phenotypic and functional plasticity. Although macrophage polarization is deeply intriguing and profound, a complete review of macrophage polarization is beyond the scope of this review. Here we will briefly review macrophage polarization, focusing on important characteristics pertinent to our discussion of hypertension.

M1 vs M2

Following their original discovery as phagocytic cells over 100 years ago by Elie Metchnikoff, the plasticity of macrophages began to become more apparent (2). In an oversimplified model, macrophage polarization can be thought of as consisting of two subsets: M1 and M2. The concept of macrophage polarization was initially borne from the observations that macrophages exposed to interferon-gamma (IFN- γ) expressed a different gene-expression profile than those exposed to interleukin-4 (IL-4) (3, 4). IFN- γ induces the differentiation of M1-macrophages, which are inflammatory and referred to as “classically activated” macrophages; where IL-4 induces the anti-inflammatory M2 macrophages, which are categorized as “alternatively activated” (5).

At the outset, there are a couple main differences between M1 and M2 polarized macrophages that position macrophages to play central roles in hypertension. First, it is important to note that the nomenclature of “M1” and “M2” was derived from the effects that these two macrophage populations have on the differentiation of T-lymphocytes. Specifically, M1 macrophages are known to activate and guide Th1 T-lymphocytes and M2

macrophages are associated with induction of Th2 T-lymphocyte responses (6). Based on the T-lymphocyte associations of macrophage subsets, there is indirect evidence for the role of macrophage polarization in hypertension. In a small study of 45 hypertensive patients, Ji et al demonstrated a significant **increase** in circulating Th1 and Th17 T-lymphocytes, in contrast to a dramatic decrease in Th2 T-lymphocytes (7). In a RAG KO murine angiotensin II (Ang II) dependent model of hypertension, Guzik et al definitively demonstrated dependence of Ang II hypertension on the presence of T-lymphocytes (8). Similarly, IL-17 has been shown to be integral in the promotion of Ang II mediated hypertension (9). Other studies have also confirmed the critical role of inflammatory T-lymphocytes as essential in the pathophysiology of hypertension (10–13). Second, L-arginine's metabolic fate, which differentiates the M1 and M2 phenotypes at the molecular level, has potentially important implications for hypertension. M1 macrophages metabolize L-arginine to nitric oxide (NO) via inducible nitric oxide synthase (iNOS or NOS2); where M2 macrophages metabolize L-arginine to ornithine (which promotes tissue growth, repair, and fibrosis) via activation of arginase-1 (14). Discovered in the 1980's, NO has long been known to play an important role in cardiovascular disease, including hypertension (15). However, NO has a complicated relationship with cardiovascular disease. Not only can NO be synthesized by different tissues and enzymes (neuronal NOS or NOS1, inducible NOS or NOS2, endothelial NOS or NOS3), but its effects are concentration-dependent (16). Abnormalities in NO and endothelial vasodilation have been shown to be abnormal in hypertensive patients (17). Correlating with clinical studies, experimental models of hypertension have also been shown to have abnormalities in NO synthesis and bioavailability (18–20). A major mechanism in the development of essential hypertension appears to be the decreased bioavailability of NO (17) or interference in its regulation of natriuresis (21). Reactive oxygen species (ROS), a product of M1 macrophages, are known to scavenge NO, thereby implicating it as a possible mechanism in limiting NO bioavailability (17). Interestingly, the role of ROS in tilting the balance of polarization towards the M1 or M2 phenotype is less clear. ROS are known to play an important role in skewing the polarization of macrophages towards the inflammatory M1-phenotype as well as recruiting M2-macrophages (22). Despite the fact that ROS are known to be increased in patients with hypertension and experimental models of hypertension, their causative role in essential hypertension has not been shown and remains uncertain (23, 24). Nonetheless, ROS do likely play a key role in the pathophysiology of hypertension, including end-organ damage (25). Finally, macrophages are involved with the process of sodium balance, a cellular activity that has been shown to be related to lymphangiogenesis and blood pressure regulation, discussed further below (26). Thus, at the outset, based on key physiological aspects of macrophage polarization, it appears that there is strong evidence for the role of macrophage polarization as a key mechanism in the development of hypertension. Adding to the complexity is the limited knowledge about macrophage polarization itself.

Macrophage Polarization – Still Developing

Although the dichotomous polarizations of M1 and M2 phenotypes appear straight forward and concrete, it is much more complicated, with new phenotypes and polarizing stimuli (beyond IFN- γ and IL-4) being continuously discovered. For example, similar to IL-4, interleukin-21 (IL-21) has been shown to influence macrophage polarization towards the

M2-phenotype (27, 28), through which it may play an important role in the pathogenesis of hypertension and end-organ damage. Similarly, aldosterone has been shown to promote the balance of macrophage polarization in the direction of the M1 inflammatory phenotype (29). Consequently, the picture of a 'simple phagocytic macrophage' has morphed into an image of an immune cell that is at the center of a sphere with the potential to follow innumerable radiant pathways of phenotypic and functional diversity. Within just 4 years of their initial description in 2000 (6), the M1–M2 paradigm had already morphed into further subclassification of the M2 macrophage populations into M2a, M2b, M2c, and M2d (5, 30). The complexity of the various macrophage subsets, both known and yet to be discovered, is perceived as being potentially so vast that it has been equated to a color-wheel (31), where slight variations may exist on even known macrophage populations, leading to new phenotypes. Although useful, a color-wheel does not necessarily indicate relatedness between the colors. Here we introduce the phylogenetic tree as a conceptual framework for understanding macrophage polarization, where variations in subsets possess some degree of relatedness to each other (Figure 1). This is exemplified by the various subsets within the M2-phenotype and the, more recently identified, Tumor Associated Macrophages (TAM) (32, 33). Additionally, recent identification of macrophages that express CD169 and the T-cell receptor (TCR), not yet clearly falling into either the M1 or M2 "camps" further complicate matters (34). CD169-positive cells are implicated in patients with renal disease and have been shown to play a role in inducing proteinuria and renal damage (35–37), potentially playing a role in the development of hypertension.

Identification of macrophage subsets is based on expression of specific extra and intracellular proteins. Traditionally, M1 macrophages are identified by the presence of increased levels of CD68, major histocompatibility complex-II (MHC-II), CD80, CD86, CC chemokine receptor 2 (CCR2, receptor for monocyte chemoattractant protein-1, MCP-1), Ly6, and CD62L on their cell surface. On the other hand, M2 macrophages are phenotyped by increased cell-surface expression of CD163 (scavenger receptor), CX3C chemokine 1 (CX3CR1, the receptor for fractalkine), CD206 (mannose receptor), YM1 and YM2 (members of chitinase and chitinase-like molecules), vascular endothelial growth factor (VEGF), and acidic mammalian chitinase (AMCase) (30–32). In addition, the M1 and M2 macrophages have individual cytokine and chemokine secretion profiles. The "classically activated" M1 phenotype is marked by secretion of pro-inflammatory cytokines that include tumor necrosis factor- α (TNF- α), interleukin 1 β (IL-1 β), interleukin 6 (IL-6), interleukin 12 (IL-12), interleukin-18 (IL-18), and interleukin 23 (IL-23). This cytokine profile specifically and primarily directs the development of the Th1 T-lymphocyte, but is also involved in development of Th17 T-lymphocytes (IL-6 and IL-23), which are involved in secretion of interleukin 17 (IL-17) and implicated in autoimmune diseases. As noted earlier, both of these T-lymphocyte subsets are implicated in the development of hypertension (38). Conversely, the cytokine profile of M2 macrophages, which includes tumor growth factor-beta (TGF- β), induces differentiation and activation of regulatory T-lymphocytes (Tregs). M2 macrophages also secrete interleukin-10 (IL-10), a potent anti-inflammatory cytokine (31). In experimental models of hypertension, not only are Tregs decreased in number, but adoptive transfer of Tregs results in a decrease in blood pressure. These studies demonstrate that the anti-inflammatory actions of these cells may be

protective against the development of hypertension and end-organ damage (39–41). As referenced above, the concept of macrophage polarization is relatively new and still evolving with more cellular markers and cytokine profiles remaining to be discovered or, in some cases, rediscovered and reclassified. Thus, the ‘Tree of Macrophage Polarization’ is only a sapling and has much growth to accomplish. This is exemplified by studies in the Spontaneously Hypertensive Rat (SHR), a genetic model of essential hypertension.

Macrophage Polarization in the Spontaneously Hypertensive Rat

CD161a: A New Marker of M1 Macrophages

CD161a, a member of the lectin-like receptor subfamily B member 1 (NKR-P1a), was initially identified as a marker of natural killer (NK) cells (42). However, CD161a is also expressed on antigen presenting cells such as monocytes and macrophages (43). During the late 1990’s, CD161a was noted to be a robust cellular marker of an inflammatory macrophage phenotype involved in acute kidney allograft rejection in rats (44). Lectin-Like-Transcript-1 (LLT1) has been identified as a ligand for CD161a and this interaction may play a key role in immunomodulatory functions of Th17 cells (45). Our group was the first to identify an abnormal predominance of CD161a-positive immune cells in young (3–5 weeks old) SHR **prior** to the elevation of blood pressure (46), suggesting that this immune cell population may play a causative role in the development of hypertension. Later, we showed that this immune cell population differentiated into CD68-positive M1-macrophages following activation of nicotinic cholinergic receptors (nAChR) (Figure 2a) and that monocyte chemoattractant protein (MCP-1) and LLT1 were over-expressed in the kidney of the SHR, suggesting a mechanism for homing of these cells (47). Cholinergic stimulation also led to the infiltration of these CD161a+/CD68+ M1 macrophages into the renal medulla and *subsequent* development of hypertension in young pre-hypertensive SHR (47), implicating renal inflammation by these cells as a causative mechanism in the development of hypertension (a process we refer to as “cholinergic-mediated hypertension”) (47). In support of these findings, treatment with a novel therapeutic agent inhibited the induction of iNOS and M1 macrophage activation, via increased expression of peroxisome proliferator-activated receptor-gamma (PPAR- γ), and led to normalization of blood pressure in the SHR (48). This is in line with the known effects of PPAR- γ to polarize macrophages towards the M2-phenotype (49). Normalizing blood pressure via the inhibition of M1 macrophage activation is a key factor in demonstrating the role of macrophage polarization in hypertension (*loss* of macrophage ‘inflammatory potential’). To strengthen the causative link between macrophage polarization and hypertension, based on Hill’s criteria, it is also important to show that induction or activation of the anti-inflammatory M2 macrophage retards or normalizes elevations in blood pressure (*gain* of macrophage ‘Anti-inflammatory potential’).

The M1/M2 Ratio

It appears that the ratio of M1/M2 macrophage plays an integral role in the pathophysiology of hypertension. Ndisang et al. have documented over a 50% decrease in the M2 macrophage markers CD163 and CD206 by Western blot of hepatic homogenates in the SHR and demonstrate that induction of the M2-macrophage population, thereby decreasing

the M1/M2 ratio, results in normalization of blood pressure in adult SHR with established hypertension (Figure 3) (50). Using flow cytometry, we characterized the CD163 positive M2 macrophage population as CD4^{Lo} in the pre-hypertensive SHR and also find an over 50% reduction, compared to the age-matched control (Wistar Kyoto, WKY) following the induction of 'cholinergic-mediated hypertension' (Figure 2b). In addition, Gomolak et al. demonstrated a dose-response effect between angiotensin-II (Ang II) infusion, the induction of systolic hypertension, plasma IL-6 levels, vascular infiltration with CD68-positive M1 macrophages, and endothelial dysfunction (51). Beginning at a 'low-intermediate' dose (200ng/kg/min) of Ang II, there was a subacute rise in systolic pressure with a trend towards increased vascular M1-macrophage infiltration, and endothelial dysfunction. These changes were more pronounced at a 'high-intermediate' (400ng/kg/min) and "high" (1000ng/kg/min) dose of Ang II (51). Utilizing a murine model with inducible diphtheria toxin receptor, Wenzel et al. elegantly established that Ang II-induced hypertension is dependent on the presence of CD11b+/F4-80+ M1-macrophages (52). Additionally, deficiency of m-CSF (monocyte colony stimulating factor) results in selective monocytopenia and resistance to Ang II dependent vascular injury, oxidative stress, and hypertension (53–56). Although, the increased M1/M2 ratio appears to be a critical component in the development and maintenance of hypertension, the native causative factor/s responsible for this imbalance in hypertension have not been clearly identified. One known factor in the induction of the M1-phenotype is activation of innate immune receptors (aka 'pathogen recognition receptors', PRR).

Toll-like Receptors and Macrophage Polarization

Toll-like receptors (TLR) are innate immune receptors that detect "pathogen-associated molecular patterns" (PAMPs) and "danger associated molecular patterns" (DAMPs). Macrophages are known to express a wide range of TLR (57). Activation of TLR skews the polarization of macrophages to the M1 phenotype (58), potentially, playing an important role in essential hypertension by altering the M1/M2 ratio. To explain the pro-inflammatory milieu and polarization of macrophages towards the M1 phenotype in hypertension, some have postulated the presence of "neoantigens" that trigger an inflammatory innate immune response (59). To date, however, the identity of these "neoantigens" remains elusive. Nevertheless, it is known that extraneous factors can regulate TLR expression and activation, skewing macrophage polarization towards the M1 phenotype. To this effect, we demonstrated that activation of nAChR not only induces the expansion of a CD161a+/CD68+ M1 macrophage population, but also modulates the innate immune response, leading to an exaggeration of the pro-inflammatory cytokine secretion in response to TLR stimuli from young pre-hypertensive SHR derived immune cells. This was in stark contrast to the observed anti-inflammatory cholinergic effect following activation of TLR in age-matched WKY-derived immune cells (60). Interestingly, the immune response to TLR activation of immune cells derived from the young SHR and WKY were similar in the absence of pre-exposure to cholinergic stimuli (46). These findings confirm the presence of a previously described "cholinergic anti-inflammatory" effect (61, 62) and demonstrate the presence of an paradoxically abnormal "cholinergic mediated pro-inflammatory effect" in the pre-hypertensive SHR. This abnormal pro-inflammatory regulation of macrophage polarization

by cholinergic receptors represents a novel key mechanism in macrophage polarization and the development of hypertension. More importantly, it establishes precedence for the regulation of immune responses, including macrophage polarization, by engagement of cholinergic, adrenergic, and neurohormonal receptors (e.g. angiotensin type 1 receptor, AT1R and the mineralocorticoid receptor) directly on immune cells.

The “Neuro-Immuno” Axis in Hypertension: a new force in Macrophage Polarization

An important force in the polarization of macrophages is the interaction between the nervous and immune system, i.e. the “neuro-immuno” axis. Specifically, the “neuro-immuno” axis refers to the bidirectional communication between the nervous and immune system. In the forward direction, central nervous system (CNS) activity directly regulates immune cell function and differentiation through efferent nerve fibers; while, in the reverse direction, immune cells/cytokines directly impact the activation state of the CNS via afferent neural pathways. To complicate matters, neural regulation of immune cells can occur via neuronal (direct) and non-neuronal (indirect) mechanisms. Neuronal regulation refers to the direct effects of the CNS nerve fibers on immune cells; where non-neuronal regulation refers to the effects of neurotransmitters and neurohormonal agents secreted from non-neuronal cells and acting on the immune cells in an autocrine or paracrine manner.

CNS to Immune

Neuronal Regulation—The autonomic nervous system has long been known to directly innervate immune cell compartments (63), where nerve fibers make close contact with immune cells at the “neuro-immune synapse” (64). Studies have demonstrated evidence for autonomic neuronal regulation of immune responses (61, 65), leading to a streamlined model of the “neuro-immuno” axis, where the parasympathetic nervous system (PNS) mediates anti-inflammatory and the sympathetic nervous system (SNS) mediates inflammatory immune responses (Figure 4a). Borovikova et al. had initially demonstrated that stimulation of the vagus nerve (PNS) exerts an inhibitory influence on endotoxin induced M1 macrophage secretion of pro-inflammatory cytokines (61), referred to as the “cholinergic anti-inflammatory reflex”. The anti-inflammatory effect of the vagus nerve was found to be mediated by the $\alpha 7$ -nicotinic acetylcholine receptor ($\alpha 7$ -nAChR) (62). Vagal nerve stimulation also induces the activation of the Jak2-Stat3 transcription factors, which are known to polarize macrophages to the M2 phenotype (66, 67). In contrast, induction of splenic sympathetic nerve activity, in response to infusion of Ang II into the CNS, has been shown to lead to an increase in mRNA of M1 macrophage pro-inflammatory cytokines in the spleen (68). Carnevale et al, has also demonstrated that sympathetic innervation of the spleen is required for the development of hypertension in response to infusion of Ang II, definitively demonstrating the integral role of Ang II’s “neuro-immuno” effects in the development of Ang II-dependent hypertension. Splenic sympathetic innervation appears to mediate communication between CD68-positive M1 macrophages and T-lymphocytes, leading to egression of the T-lymphocytes, end-organ damage, and the development of hypertension (69, 70). More recently, in patients, increased renal sympathetic nerve activity was shown to be involved in macrophage activation (65).

Conversely, renal denervation results in a significant decrease of F4/80-positive inflammatory M1 macrophages into the renal medulla and increase in the expression of kruppel-like factor-4 (klf4) (71), a factor that induces polarization of macrophages to the M2 phenotype (72). Hence, based on these studies one may conclude that direct activity of the PNS favors the M2 phenotype and SNS favors the M1 phenotype (Figure 4b). Leaving the overview of the neuronal “neuro-immuno” axis, it appears the direct neuronal effects of the PNS and SNS on the immune system are discrete and straightforward. Unfortunately, the lines between the effects of activating adrenergic (sympathetic) and cholinergic (parasympathetic) receptors directly on immune cells in an autocrine or paracrine fashion, i.e. non-neuronal regulation, are less crisp.

Non-Neuronal Regulation—It is well known that macrophages express cholinergic and adrenergic receptors (63). Although direct neuronal regulation of immune cells via the SNS mediates inflammatory immune responses consistent with activation of M1 macrophages, activation of adrenergic receptors on macrophages is slightly more complicated. Activation of alpha-1 and alpha-2 adrenergic receptors seems to induce polarization towards the inflammatory M1 phenotype (73); whereas activation of beta-2 adrenergic receptors mediates polarization towards the anti-inflammatory M2 phenotype (74). Less is known about the effects of cholinergic receptors on macrophages, with the exception of the well-investigated alpha7-nAChR ($\alpha 7$ -nAChR), which has been shown repeatedly to exert anti-inflammatory effects, skewing the innate immune response towards the M2 phenotype (75). Activation of the $\alpha 7$ -nAChR has not been demonstrated to decrease blood pressure or prevent/delay the onset of hypertension, but it has been demonstrated to inhibit end-organ damage in experimental models of hypertension (76). However, although the $\alpha 7$ -nAChR exerts anti-inflammatory effects, cholinergic receptors are a diverse group of receptors. Following the template of the adrenergic receptors, it is clear that while some nAChR exert anti-inflammatory effects ($\alpha 7$ -nAChR), others harbor the ability to elicit pro-inflammatory immune responses (77, 78). To this effect, our laboratory first demonstrated that activation of nAChR ex vivo and in vivo leads to the selective induction of M1 macrophages in the young pre-hypertensive SHR, but not in the age-matched WKY (47), corresponding with the premature development of hypertension. Investigating the effects of cholinergic receptors on innate immune function, much less on macrophage polarization, is a new frontier. As it stands, the current database of knowledge regarding nAChR is based on studies of nerve cells and muscle tissue, which serves as the bedrock of our understanding of nAChR; hence the categories of neuronal nAChR and muscle-type nAChR (79). Studies investigating the expression of nAChR in immune cells have primarily been limited to the $\alpha 4$, $\alpha 7$, and $\beta 2$ -nAChR (80). Thus, there is a large gap in our knowledge of the repertoire of cholinergic receptors in immune cells. Currently, our laboratory is actively working to characterize nAChR expression in immune cells, in an attempt to identify the nAChR/s involved in mediating the pro-inflammatory M1 differentiation of the pro-hypertensive CD161a-positive macrophages.

Immune to CNS

Generally, the nervous system is considered an immune-privileged site, separated from the circulating immune cells and cytokines/chemokines by the blood-brain barrier (BBB). In

thinking about the interfaces of the CNS with the peripheral immune cells as cytokines, it is important to identify 3 main interfaces: the blood-brain barrier (BBB), choroid plexus (CP), and the circumventricular organs (CVO) (Figure 5). These interfaces are presented in order of increasing “leakiness” and contact with the systemic circulation. The BBB is the most impenetrable to circulating cytokines, neurohormonal factors, and immune cells; where the CVO are penetrated by fenestrated capillaries and are considered to have a high level of permeability, allowing direct contact with systemic factors and cells (81). The CP is a barrier between the systemic circulation and the brain that lines the ventricular compartment in vertebrates and is composed of a combination of endothelial and epithelial cells; although other immune cells are also known to be present, including macrophages. The primary function of the CP is to generate cerebrospinal fluid for the CNS, which it does through a close interaction with fenestrated capillaries blood vessels (82). The CVO is comprised of the median eminence, neurohypophysis, organum vasculosum lamina terminalis (OVLT), the subfornical organs (SFO), and area postrema (AP). In addition, there are areas within the CNS (the forebrain and hindbrain) that are in very close proximity to the systemic circulating factors and immune cells. Important structures in these areas are the paraventricular nucleus (PVN), rostroventrolateral medulla (RVLM), anteroventral third ventricle (AV3V), and nucleus tractus solitarius (NTS) (Figure 5). A comprehensive review of neurobiology is beyond the scope of this review, but a simplified overview is presented. In essence, the NTS and PVN are areas for ‘input’ from the CVO and afferent nerve fibers. Neuronal projections from these areas then communicate with other areas of the CNS, such as the RVLM, which is the primary center for sympathetic outflow from the CNS. Key to implicating these centers in the development of immune-mediated hypertension was a study by Shi et al. revealing the pro-hypertensive effects of IL-1 β delivered directly to the PVN or into the ventricular space of rats (83).

With the basic view presented above, one can begin to understand the communication of the immune system with the CNS. Pro-inflammatory cytokines, that are generated in the periphery as a result of immune responses to antigens (self or foreign), such as IL-1 β , IL-6, and TNF- α , are pivotal in this communication (84, 85). These cytokines can communicate directly with the CVO or induce alterations in the adherens and tight junctions of the BBB and induce increased permeability to cytokines to gain access to the CNS (86, 87). Cytokines at local sites of inflammation also have the ability to trigger afferent nociceptive (somatic) and vagal fibers in the periphery, thereby communicating with the NTS and PVN via neuronal pathways (84), potentially activating increasing sympathetic outflow from the CNS and leading to hypertension. Thus, inflammatory M1 macrophages at localized sites of inflammation in the periphery can signal to the CNS to raise blood pressure transiently through inflammatory cytokines (which would be protective in acute illness); however, in the chronic inflammatory state, this signal may trigger an abnormal pro-inflammatory immune response within the CNS, leading to essential hypertension.

CNS Macrophages

Although there are a variety of immune cells within the CNS, we will focus on those cells relevant to the discussion of macrophage polarization, only briefly mentioning other cells as needed. It is most useful to think of immune cells as 1) outside the CNS, 2) at the interfaces

of the CNS with the periphery, and 3) within the CNS. The role of macrophages and immune cells outside the CNS has been discussed elsewhere in this review. Here we will focus on how macrophages and their actions at the interfaces and within the CNS can regulate hypertension. The BBB is a complicated structure primarily composed of processes of astrocytes with close proximity to penetrating blood vessels in the brain parenchyma. Astrocytes are the most numerous non-neuronal cells in the CNS and play an important structural role in the maintenance of the BBB, but have also been found to have immune functions (marked by the secretion of IL-6) (88). Perivascular macrophages (PVM) are an important mononuclear phagocytic cell that also surround the blood vessel and are just outside the BBB, able to communicate with astrocytes (89). TGF- β , a hallmark of the M2-phenotype, is secreted by multiple cells at the BBB in an attempt to maintain its integrity (89). Thus, it is not surprising that PVM primarily express CD163, a M2-phenotype marker (90). PVM surround the penetrating blood vessels in the brain parenchyma and are primarily replenished by blood-derived macrophages (91). Despite their native, anti-inflammatory state, the PVM can be polarized towards an M1 phenotype by known pro-M1 stimuli, such as endotoxin (systemic or local). Once activated, PVM signal to neighboring immune cells, endothelial cells, and parenchymal microglia through pro-inflammatory cytokines such as IL-1 β (92). Activation of PVM leads to induction of ROS and is implicated in damaging neurocognitive and vascular effects of hypertension (93). Consistent with this finding, Liu et al found an approximately 50–100% increase in the number of PVM present in adult hypertensive SHR and stroke prone SHR, compared to the age-matched WKY (94). Similarly, macrophages are also present in the stroma of the CP and their activation to the M1-phenotype in hypertension has also been implicated in cognitive dysfunction (95). Macrophages in the stroma of the choroid plexus (CP-macrophages) can transmigrate across the choroid plexus and line the ventricular boundary of the CNS (96). CP-macrophages are also derived from systemic blood-derived macrophages, but their roles in hypertension development/maintenance or hypertensive cerebral dysfunction have not been studied in-depth. Nonetheless, due to their relationship with blood derived macrophages they can polarize to M1 or M2 macrophages and secrete cytokines directly into the CSF, inducing M1 polarization in parenchymal microglia (discussed below). Summarizing the role of macrophages at the BBB and CP, it appears activation to the M1-phenotype primarily leads to transduction of hypertensive damage to the CNS, leading to neurocognitive dysfunction. However, microglia play a much more important role in the development of neuroinflammation and hypertension.

Neuroinflammation is known to play a significant role in the development of essential hypertension (97). The concept of neurogenic hypertension has traditionally revolved around the increased sympathetic outflow of the CNS to the periphery, leading to increased vascular resistance and end-organ damage (98–100). Microglia are resident macrophages within the brain parenchyma. Microglia are derived from the embryonic yolk sac prior to the ninth embryonic day in rodents (101), proliferating and maintaining homeostasis within the CNS. In contrast to PVM and CP-macrophages, microglia have little to no dependence on systemic circulating monocytic/macrophage precursors (102). It has become evident in the past several years that microglial activation, i.e. neuroinflammation, leads to the secretion of inflammatory cytokines that are sympathoexcitatory, linking immune activation to increased

sympathetic outflow from the CNS, a key mechanism in pathogenesis of essential hypertension (83, 103–107). Similar to blood-derived macrophages, microglia can polarize towards an M1 or M2 phenotype (58, 108). Although microglia can be influenced to polarize towards an M1 phenotype in response to cytokine signals transduced through immune cells at the interfaces of the CNS (PVM, CP-macrophages, and T-lymphocytes), other factors also directly activate the microglia. At this point, the importance of the renin-angiotensin-aldosterone system (RAAS) becomes a central and critical component of neuroinflammation and neurogenic hypertension.

Ang II is a key component of RAAS that results in elevated blood pressure via vasoconstriction, increased sodium reabsorption, as well as sympathomimetic effects on the CNS. The mechanism for the CNS effects of Ang II has been an area of active investigation. Recently, Ang II was shown to activate microglia, polarizing them towards an M1-phenotype, inducing the development of hypertension (83). Minocycline, an anti-inflammatory antibiotic, was shown to abrogate hypertension in this model by reversing the polarization of the microglial towards an M2-phenotype as marked by an increase in IL-10, and decrease in the pro-inflammatory M1 cytokines (IL-1 β , IL-6, and TNF- α) (83). In 2015, Shen et al., utilized a CD11b-DTR transgenic mouse model to selectively deplete microglia in the motor cortex and PVN, resulting in resolution of hypertension in an Ang II dependent model, confirming the role of M1-polarized microglia in neurogenic hypertension (106). This is consistent with earlier findings of an anti-inflammatory effect of angiotensin II type I receptor (AT1R) antagonists in microglia exposed to endotoxin (109). Vascular inflammation in the brainstem has been proposed as a potential explanation in the SHR for the development of hypertension, possibly through decreased oxygen delivery to the CNS (110). Interestingly, cerebral ischemia leads to upregulation of microglial expression of Ang II, AT1R, and AT2R (angiotensin type 2 receptor) in microglia, establishing a role for the autocrine and paracrine inflammatory action of Ang II in the CNS (111). Of note, Ang II's induction of inflammatory effects in microglia has been shown to be mediated by the presence of functional TLR4, demonstrating cross talk between the AT1R and TLR pathways (112). Similarly, we had shown that Ang II primed immune cells from young pre-hypertensive SHR in vitro and in vivo to exert an exaggerated pro-inflammatory immune response to TLR activation (46). Taken together, it is clear that Ang II and innate immune receptors work together to shift the balance of microglial activation towards an M1 phenotype, leading to the development and propagation (in a feedforward manner) of essential hypertension. Increased microglial activation and polarization may also result from various inflammatory cytokine input from the CNS interfaces as well. Moreover, polarization towards the M1 phenotype of macrophages, including microglia, within the CNS likely plays a key role in the neurocognitive dysfunction associated with hypertension (93, 94, 113).

RAAS, Inflammation & Macrophage Polarization

At face value, the RAAS system also plays dual contradictory roles in hypertension with regards to inflammation. Systemic Ang II infusion is known to induce the expression of MCP-1 in vascular smooth muscle cells and the kidney (114–116). As a consequence, it is no surprise that, both, the vascular and renal compartments are marked by increased

infiltration of inflammatory M1 macrophages with Ang II infusion, along with the corresponding inflammatory cytokine milieu (117–120). From this, it logically follows that MCP-1 deficiency would be protective against vascular inflammation and infiltration of macrophages (117, 118). Interestingly, recent studies have revealed a dynamic component of macrophage biology in the context of renal and vascular inflammation. Specifically, although inflammatory M1-phenotype macrophages initially infiltrate these structures, these cells can ‘switch’ to the M2-phenotype. Confirming the Ang II induced infiltration of the aorta with M1-phenotype Ly6C^{hi} macrophages, Moore et al. showed that at 7–14 days, these cells began to express the M2 phenotype, marked by increased CD206 and arginase-I (121). Further they showed that antagonism of MCP-1, prevented the macrophage infiltration and subsequent fibrosis associated with Ang II infusion, the latter of which is most likely mediated by the M2 macrophages (121). Similarly, Aki et al. demonstrated the ability of systemic AT1R blockade with olmesartan to induce a switch from an M1 macrophage profile to an M2 phenotype, along with an increase in IL-4, IL-13, and IL-10 (an M2 anti-inflammatory cytokine profile) (122). From these studies, it is important to note that M1 macrophages infiltrate vessel walls and renal interstitium in response to elevated levels of **systemic** Ang II, which crosses the BBB (123). Moreover, the plasticity of macrophages in their phenotypic and functional diversity is dynamic, with the fluent ability to transition between phenotypes. Despite this, there appears to be a disconnect with studies looking at the direct effects of AT1R engagement on the surface of immune cells.

Immune cells are known to express components of RAAS, including AT1R (124, 125). It appears that the direct engagement of the AT1R on immune cells mediates the polarization of macrophages in the direction of the anti-inflammatory M2-phenotype (126, 127). Kato et al had investigated the effects of selective deletion of AT1R in mice expressing human renin and angiotensinogen genes (chronic RAAS activation) and found that there was a preserved hypertensive response, increased renal fibrosis, and increased severity of atherosclerosis (128). Similarly, Crowley et al demonstrated that Ang II infusion in bone marrow chimeric mice with deletion of the AT1R in hematopoietic cells have elevated blood pressures, albuminuria, as well as increased T-lymphocyte and macrophage infiltration into the kidneys (129). In line with these basic science studies, Merino et al demonstrated that the use of losartan, an angiotensin receptor blocker (ARB), in *patients* led to a significant decrease in the M2 macrophage (CD16-positive) population (130). However, studies demonstrate that renal denervation decreases the expression of MCP-1 and protects against renal fibrosis in mice (131), decreased infiltration of F4/80 macrophages in chronic Ang II infusion (71), and decreased inflammatory macrophages [Harwani unpublished data].

The “neuro-immuno” axis likely explains these seemingly discordant findings. Increased sympathetic drive leads to the increased mobilization of mononuclear phagocytic cells from immune cell reservoirs (70) and simultaneously increases the expression of chemoattractant signals, e.g. MCP-1, in the end-organs, such as the kidney. Simultaneous sympathetic-mediated macrophage mobilization and tissue chemoattractant expression results in an increased influx of M1 Inflammatory macrophages into endo organ tissues. Within these tissues engagement of innate immune receptors, e.g. TLR, with “neoantigens”, activates the M1 macrophages. As the inflammatory cascade ensues, these macrophages encounter

activation of AT1R on their cell surface through local Ang II in the tissue (they may even secrete Ang II, and undergo activation in an autocrine/paracrine fashion), which then triggers a gradual transformation to the M2 phenotype, with its consequences of fibrosis etc... (Figure 6).

Relationship between Salt and Macrophages Polarization

Polarization of macrophages is also an important component in sodium balance, which has been an important mechanism in the development of hypertension clinically and in experimental models for decades. Osmotic forces due to the presence of sodium are believed to play an important role in the pathogenesis of essential hypertension. By tightly regulating total body sodium, the body attempts to maintain a steady-state to counter the pro-hypertensive effect of sodium. There is no doubt that renal mechanisms play an important role in this process and the renal infiltration of macrophages and inflammation are important in the development of hypertension, but it is also clear that macrophages play an important role in the regulation of dermal interstitial sodium. Dermal interstitial sodium stores were shown to correlate with the induction of hypertension, which was countered by macrophage induced lymphangiogenesis, mediated by the expression of VEGF-C. The expression of VEGF-C was dependent on the expression of tonicity-enhancer binding protein (TONEBP/NFAT5) (132, 133). Increased dietary sodium has also been shown to be a key factor in the M1 macrophage dependent development of Th17 T-lymphocyte mediated autoimmune disease (134, 135). Hence, it is no surprise that TLR induction of iNOS, a hallmark of M1 macrophages, is dependent on expression of TONEBP (136), since NO has the potential to interfere with natriuresis (21). Likewise, TONEBP inhibits IL-10 mediated polarization towards the M2-phenotype (137), supporting the pro-M1 effects of sodium and its regulatory mechanisms.

Evidence Suggesting a Role for Macrophage Polarization in Clinical Essential Hypertension

Multiple organ systems have been implicated in the development of hypertension and pharmacological therapies target these various systems. Despite this, approximately 12% of treated patients remain hypertensive and are categorized as being “treatment resistant” or “refractory to medical therapy” (138). Of those that have reached adequate control of their blood pressure, many of them are on multiple agents. This would seem to suggest that although patients are responding to agents targeting multiple organ systems and pathways, our current therapies are missing an essential core mechanism in the pathophysiology of hypertension: the immune system.

Human data is less abundant than experimental models, but the data that is available supports a role for inflammation and macrophage polarization in essential hypertension, as well as cardiovascular disease. Inflammation has been demonstrated in patients with hypertension by the presence of increased circulating levels of inflammatory cytokines and immune cells in various organ systems (renal, vascular, and neural) that are implicated in the development and maintenance of essential hypertension.

The majority of studies have correlated the presence of hypertension with elevated levels of circulating inflammatory markers, cytokines, and antibodies. Increased levels of IgG immunoglobulins were noted in patients with hypertension (139–141). Hypertensive patients are known to have an increased level of IL-6, IL-1 β , IL-1 α , IL-18, IL-2, IL-8, TNF- α , IFN- γ , C-reactive protein (CRP), and MCP-1. In contrast, anti-inflammatory cytokines such as IL-10 have been noted to be decreased in patients with hypertension (142). Studies have also noted the presence of agonistic autoantibodies to the angiotensin type I receptor (AT1R) as well as the presence of host derived antigens that activate innate immune responses (143–145). In 2007, Sesso et al. attempted to demonstrate a temporal relationship between the presence of inflammatory cytokines and the risk of developing essential hypertension in a nested case-control study of 400 women, showing that IL-6 and CRP were elevated prior to the development of the hypertension (146). The results showed a strong association between the elevated presence of CRP and the development of hypertension, but not IL-6. Similarly, elevated levels of inflammatory cytokines (CRP, IL-6, and soluble ICAM-1) were associated with the development of hypertension in an analysis of men in the Physician Health Study in another prospective study (147). Although the presence of cytokines have been an important initial step in establishing inflammation as a possible causative factor in the development of hypertension, the presence and lack of immune cells have been helpful in shedding light on the mechanisms.

Both the innate and adaptive immune cells have been implicated in the development of hypertension. The immunological paradigm is that immune responses begin with activation of the innate immune system, which then goes on to activate the adaptive immune arm through presentation of antigens and secretion of pro-inflammatory cytokines. In the 1950's investigators demonstrated a correlation between renal lymphocyte infiltration and scarring in hypertensive patients undergoing sympathectomy (148, 149). Another study demonstrated that severity of hypertension was independent of race and closely associated with the presence of CD68-positive M1 inflammatory macrophages in the kidney (150). Vascular inflammation has been suggested to lead to vascular "stiffness" based on the correlation of increased CRP, TNF- α , IL-6, and adiponectin in blood vessels with increased pulse-wave velocity, a measurement of vascular stiffness that corresponds with hypertension (151–153). These studies are supported by evidence that peripheral blood monocytes (PBMCs) isolated from hypertensive patients are preactivated based on the increased secretion of IL-1 β when cultured with Ang II and lipopolysaccharide (LPS), M1 polarizing stimuli (154). Interestingly, clinical inhibition of IL-1 β in patients over 4 years was shown to decrease cardiovascular events in patients independent of their cholesterol levels, providing evidence for the role of the innate immune response in cardiovascular disease (155). In hypertensive patients, CD8-positive, but not CD4-positive T-lymphocytes are increased in the circulation of (156), serving as indirect evidence for a Th1 mediated immune response (which is mediated by M1 macrophages). Overall, the inflammatory state in patient studies early on supports a role for the M1/Th1 mediated pro-inflammatory immune response. However, since that time, we have more recent studies that have looked at the differentiation of macrophages by subsets in hypertensive patients.

In humans, macrophages subsets are grouped by the expression of CD14 and CD16 based on a nomenclature system approved by the Nomenclature Committee of the International Union

of Immunologic Societies. This system breaks the macrophage populations into three subsets: CD14⁺⁺/CD16⁻⁻⁻ ("Classical Monocytes/Macrophages"), CD14⁺/CD16⁺ or CD14⁺⁺/CD16⁺ ("Intermediate Monocytes/Macrophages"), and CD14^{dim} or ⁺/CD16⁺⁺ ("Non-classical Monocytes/Macrophages") (157). In essence, one can simplify these into two primary groups of macrophages: CD14-positive and CD16-positive, which can be taken to be correlates of the M1 and M2 phenotypes, respectively. Interestingly, similar to the effects seen in the SHR, activation of PPAR- γ decreased the expression of CD14, **inhibiting** the polarization of macrophages towards the M1 phenotype in diabetic patients (158). This CD14-positive vs CD16-positive nomenclature has been helpful in identifying polarization of macrophages in clinical hypertension and serving as a marker for morbidity and mortality in hypertensive patients with cardiovascular disease. A family of molecules referred to as angiopoietin and their receptor Tie-2 have been implicated in patients with hypertension (159) and are involved in the vascular 'stiffness', remodeling, and angiogenesis of blood vessels (160). Marketou et al. demonstrated that CD14⁺ M1 phenotype macrophages were present in the blood vessels of patients with hypertension and express angiopoietin and their receptor, Tie-2 (161). Re-invoking the M1/M2 ratio, the presence of circulating CD14-positive M1 macrophages was found to positively correlate with the incidence of cardiovascular events; where the presence of CD16-positive M2 macrophages negatively correlated with the development of cardiovascular events, suggesting a protective mechanism of a decreased M1/M2 ratio (162). Directly implicating CD14-positive human macrophages in the development of clinical hypertension is their robust expression of angiotensin converting enzyme (ACE) (163), demonstrating their potential involvement in hypertension through participation in the RAAS, which may lead to feedforward activation of monocytes and "switching" between the M1 and M2 phenotypes. This theory is consistent with the finding that monocytes isolated from hypertensive patients are preactivated based on their increased secretion of IL-1 β when cultured with Ang II and LPS (154). Supporting the theory of the "Neuro-Immuno" axis as a force in macrophage polarization and participation in RAAS and the development of hypertension, the presence of CD14-positive macrophages in the kidney directly correlated with the severity of renal damage in patients with chronic kidney disease (130). In addition to their activation state, the ability of macrophages from patients with elevated blood pressure to mobilize was increased and correlated with systemic levels of TNF- α (164).

Translational Significance

The translational significance of the role of macrophage polarization in hypertension is multi-faceted. Thus far, our current therapies target hypertension from a multitude of directions: vasodilators, diuretics, angiotensin converting enzyme (ACE) inhibitors, Ang II receptor blockers (ARBs), central sympatholytic alpha receptor agonists, and aldosterone antagonists. The majority of these agents target the RAAS, which appears to play an important role in the modulation of the M1/M2 ratio. New therapies that target the signaling cascade of the M1 and M2 macrophage, such as nAChR and intracellular signaling pathways of the RAAS, may harbor a new frontier in combating hypertension and helping us understand the benefit or lack of benefit on morbidity/mortality of current and future therapies. For example, the beneficial effects of aldosterone antagonism in hypertension and

cardiovascular disease may be explained by its ability to induce a “switch” from the M1 to the M2 phenotype, limiting the amount of tissue inflammation. Another example is the development of hypertension in patients being treated with anti-VEGF therapies for malignancies. Knowing that M2 macrophages express VEGF, we may begin to understand the mechanisms responsible for the pro-hypertensive effects of these therapies. More importantly, currently therapies for hypertension such as renal denervation may be able to modulate other inflammatory disease processes. More broadly, neural denervation and/or stimulation may serve as important therapies in inflammatory disorders, including hypertension, if we can determine which neural pathways can modulate macrophage polarization so as to limit inflammation. Based on the model proposed here, we would hypothesize that these therapies will have differential effects based on the timing of their implementation in the disease process, i.e. they may be more beneficial earlier than later in the disease process.

Closing Thoughts

Macrophages are ubiquitous and universally present in all tissues. This includes the organ systems involved in essential hypertension: nervous, renal, and cardiovascular. Our understanding of macrophage biology and its role in hypertension, as well as other disease processes, is quickly advancing. The ability of macrophages to polarize into numerous phenotypes with functional versatility places them in the unique position to serve as mediators and agents between various tissues. It is clear that the basic elements involved in polarization of macrophages (such as NO, ROS, sodium balance, and regulation of T-lymphocytes) have direct applicability and relevance to the development of essential hypertension. It is also readily apparent that the influence of the “neuro-immuno” axis on macrophages is profound and also versatile. Our evidence indicates a possible model for the role of macrophages in the development of hypertension, demonstrating a critical role for their ability to “switch” between phenotypes, almost as if on a “time-release program”.

Perhaps one of the most exciting and promising areas to be explored is the manipulation of macrophage polarization via the “neuro-immuno” axis. Although, results regarding renal denervation in patients are inconsistent (165, 166), a better understanding of the “neuro-immuno” axis may allow revisions to this or other therapies, including the development of novel pharmacological agents. In this pursuit, it will be extremely important to take into consideration the dynamic nature of macrophages and their ability to “switch” between phenotypes. This transition between phenotypes appears to be time-dependent, linked to the chronological course of the disease process. Thus, future studies should take into account the fact that although one phenotype, e.g. M1, dominates early in the course of the disease, another phenotype such as the M2 may dominate the later stages of a chronic disease process such as hypertension. Hence, the administration of therapies to patients targeting macrophage polarization will also be time-dependent. Although much remains to be discovered regarding the cholinergic, adrenergic, and neurohormonal receptor influence on macrophages in essential hypertension, novel therapeutic strategies that harness the power of macrophage polarization to reverse, control, or prevent essential hypertension and/or its devastating cardiovascular consequences have great promise.

Acknowledgments

This review was funded by a grant from the National Institute of Health (K08 HL119588).

We gratefully thank Shawn Roach (Design Center, Department of Internal Medicine, University of Iowa) for assistance in preparing the illustrations, Ms. Angela Hester for her administrative support, Dr. Francois Abboud for his mentorship and our laboratory personnel (Cameron Braverman, Nandita Raikwar, and Jason Ratcliff) for their efforts in performing experiments, as well as discussion and comments. The author has read the journal's authorship agreement and has reviewed and approved the manuscript.

Abbreviations

IFN-γ	interferon-gamma
IL-1b	interleukin-1 beta
IL-2	interleukin-2
IL-4	interleukin-4
IL-6	interleukin-6
IL-8	interleukin-8
IL-10	interleukin-10
IL-12	interleukin-12
IL-13	interleukin-13
IL-17	interleukin-17
IL-18	interleukin-18
IL-23	interleukin-23
TNF-α	tumor necrosis alpha
Ang II	angiotensin II
AT1R	angiotensin type 1 receptor
AT2R	angiotensin type 2 receptor
nAChR	nicotinic acetylcholine receptor
AR	adrenergic receptor
TLR	toll-like receptor
iNOS	inducible nitric oxide synthase
eNOS	endothelial nitric oxide synthase
nNOS	neuronal nitric oxide synthase
RAG	Recombination activating gene

DTR	Diphtheria toxin receptor
NO	nitric oxide
ROS	reactive oxygen species
TAM	tumor associated macrophages
MHC-II	major histocompatibility complex II
VEGF	vascular endothelial growth factor
TGF-β	tumor growth factor-beta
SHR	Spontaneously Hypertensive Rats
WKY	Wistar Kyoto
NK	Natural Killer Cell
LLT1	lectin like transcript 1
PPAR-γ	peroxisome proliferator-activated receptor-gamma
m-CSF	monocyte colony stimulating factor
PRR	pathogen-recognition receptor
PAMPs	pathogen-associated molecular patterns
DAMPs	danger associated molecular patterns
CNS	Central Nervous System
PNS	parasympathetic nervous system
SNS	sympathetic nervous system
α7-nAChR	alpha7-nicotinic acetylcholine receptor
klf4	kruppel-like factor 4
BBB	Blood-Brain Barrier
CP	Choroid Plexus
DC	dendritic cells
CVO	circumventricular organs
OVLT	organum vasculosum lamina terminalis
SFO	subfornical organs
AP	area postrema
PVN	paraventricular nucleus

RVLM	rostromedullary lateral medulla
AV3V	anteroventral third ventricle
SCO	subcommissural organ
NTS	nucleus tractus solitarius
RAAS	renin-angiotensin-aldosterone system
MCP-1	monocyte chemoattractant protein-1
TONEBP	tonicity-enhancer binding protein
CRP	C-reactive protein
ICAM-1	intercellular adhesion molecule – 1
PBMC	peripheral blood monocytes
LPS	lipopolysaccharide
ACE	angiotensin converting enzyme

References

1. Dzik JM. The ancestry and cumulative evolution of immune reactions. *Acta Biochim Pol.* 2010; 57(4):443–66. [PubMed: 21046016]
2. Kaufmann SH. Immunology's foundation: the 100-year anniversary of the Nobel Prize to Paul Ehrlich and Elie Metchnikoff. *Nat Immunol.* 2008; 9(7):705–12. [PubMed: 18563076]
3. Nathan CF, Murray HW, Wiebe ME, Rubin BY. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J Exp Med.* 1983; 158(3):670–89. [PubMed: 6411853]
4. Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med.* 1992; 176(1):287–92. [PubMed: 1613462]
5. Mantovani A, Sozzani S, Locati M, et al. Infiltration of tumours by macrophages and dendritic cells: tumour-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Novartis Found Symp.* 2004; 256:137–45. discussion 46–8, 259–69. [PubMed: 15027487]
6. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol.* 2000; 164(12):6166–73. [PubMed: 10843666]
7. Ji Q, Cheng G, Ma N, et al. Circulating Th1, Th2, and Th17 Levels in Hypertensive Patients. *Disease Markers.* 2017; 2017:12.
8. Guzik TJ, Hoch NE, Brown KA, et al. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J Exp Med.* 2007; 204(10):2449–60. [PubMed: 17875676]
9. Madhur MS, Lob HE, McCann LA, et al. Interleukin 17 promotes angiotensin II-induced hypertension and vascular dysfunction. *Hypertension.* 2010; 55(2):500–7. [PubMed: 20038749]
10. Schiffrin EL. Immune mechanisms in hypertension and vascular injury. *Clin Sci (Lond).* 2014; 126(4):267–74. [PubMed: 24144355]
11. Svendsen UG. Influence of neonatal thymectomy on blood pressure and hypertensive vascular disease in rats with renal hypertension. *Acta Pathol Microbiol Scand A.* 1975; 83(2):199–205. [PubMed: 1096529]
12. Svendsen UG. Evidence for an initial, thymus independent and a chronic, thymus dependent phase of DOCA and salt hypertension in mice. *Acta Pathol Microbiol Scand A.* 1976; 84(6):523–8. [PubMed: 998251]

13. Svendsen UG. The importance of thymus in the pathogenesis of the chronic phase of hypertension in mice following partial infarction of the kidney. *Acta Pathol Microbiol Scand A*. 1977; 85(4): 539–47. [PubMed: 331861]
14. Mills CD. Macrophage arginine metabolism to ornithine/urea or nitric oxide/citrulline: a life or death issue. *Crit Rev Immunol*. 2001; 21(5):399–425. [PubMed: 11942557]
15. Nava E, Noll G, Luscher TF. Nitric oxide in cardiovascular diseases. *Ann Med*. 1995; 27(3):343–51. [PubMed: 7546624]
16. Shen YH, Wang XL, Wilcken DE. Nitric oxide induces and inhibits apoptosis through different pathways. *FEBS Lett*. 1998; 433(1–2):125–31. [PubMed: 9738946]
17. Hermann M, Flammer A, Luscher TF. Nitric oxide in hypertension. *J Clin Hypertens (Greenwich)*. 2006; 8(12 Suppl 4):17–29. [PubMed: 17170603]
18. Herrera M, Garvin JL. Recent advances in the regulation of nitric oxide in the kidney. *Hypertension*. 2005; 45(6):1062–7. [PubMed: 15753231]
19. Llorens S, Jordan J, Nava E. The nitric oxide pathway in the cardiovascular system. *J Physiol Biochem*. 2002; 58(3):179–88. [PubMed: 12603012]
20. Loscalzo J. Salt-sensitive hypertension and inducible nitric oxide synthase: form-function dichotomy of a coding region mutation, *Mutatis mutandis*. *Circ Res*. 2001; 89(4):292–4. [PubMed: 11509443]
21. Ramseyer VD, Ortiz PA, Carretero OA, Garvin JL. Angiotensin II-mediated hypertension impairs nitric oxide-induced NKCC2 inhibition in thick ascending limbs. *Am J Physiol Renal Physiol*. 2016; 310(8):F748–F54. [PubMed: 26887831]
22. Zhang Y, Choksi S, Chen K, Pobezińska Y, Linnoila I, Liu ZG. ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages. *Cell Res*. 2013; 23(7):898–914. [PubMed: 23752925]
23. Majzunova M, Dovinova I, Barancik M, Chan JY. Redox signaling in pathophysiology of hypertension. *J Biomed Sci*. 2013; 20:69. [PubMed: 24047403]
24. Montezano AC, Touyz RM. Reactive oxygen species, vascular Noxs, and hypertension: focus on translational and clinical research. *Antioxid Redox Signal*. 2014; 20(1):164–82. [PubMed: 23600794]
25. Datla SR, Griendling KK. Reactive oxygen species, NADPH oxidases, and hypertension. *Hypertension*. 2010; 56(3):325–30. [PubMed: 20644010]
26. Wiig H, Schroder A, Neuhofer W, et al. Immune cells control skin lymphatic electrolyte homeostasis and blood pressure. *J Clin Invest*. 2013; 123(7):2803–15. [PubMed: 23722907]
27. Hashimoto-Kataoka T, Hosen N, Sonobe T, et al. Interleukin-6/interleukin-21 signaling axis is critical in the pathogenesis of pulmonary arterial hypertension. *Proc Natl Acad Sci U S A*. 2015; 112(20):E2677–86. [PubMed: 25941359]
28. Li SN, Wang W, Fu SP, et al. IL-21 modulates release of proinflammatory cytokines in LPS-stimulated macrophages through distinct signaling pathways. *Mediators Inflamm*. 2013; 2013:548073. [PubMed: 24574581]
29. Martin-Fernandez B, Rubio-Navarro A, Cortegano I, et al. Aldosterone Induces Renal Fibrosis and Inflammatory M1-Macrophage Subtype via Mineralocorticoid Receptor in Rats. *PLoS One*. 2016; 11(1):e0145946. [PubMed: 26730742]
30. Roszer T. Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. *Mediators Inflamm*. 2015; 2015:816460. [PubMed: 26089604]
31. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol*. 2008; 8(12):958–69. [PubMed: 19029990]
32. Duluc D, Delneste Y, Tan F, et al. Tumor-associated leukemia inhibitory factor and IL-6 skew monocyte differentiation into tumor-associated macrophage-like cells. *Blood*. 2007; 110(13): 4319–30. [PubMed: 17848619]
33. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell*. 2010; 141(1):39–51. [PubMed: 20371344]
34. Chavez-Galan L, Olleros ML, Vesin D, Garcia I. Much More than M1 and M2 Macrophages, There are also CD169(+) and TCR(+) Macrophages. *Front Immunol*. 2015; 6:263. [PubMed: 26074923]

35. Ikezumi Y, Hurst LA, Masaki T, Atkins RC, Nikolic-Paterson DJ. Adoptive transfer studies demonstrate that macrophages can induce proteinuria and mesangial cell proliferation. *Kidney Int.* 2003; 63(1):83–95. [PubMed: 12472771]
36. Ikezumi Y, Suzuki T, Hayafuji S, et al. The sialoadhesin (CD169) expressing a macrophage subset in human proliferative glomerulonephritis. *Nephrol Dial Transplant.* 2005; 20(12):2704–13. [PubMed: 16169862]
37. Karasawa K, Asano K, Moriyama S, et al. Vascular-resident CD169-positive monocytes and macrophages control neutrophil accumulation in the kidney with ischemia-reperfusion injury. *J Am Soc Nephrol.* 2015; 26(4):896–906. [PubMed: 25266072]
38. Zhang J, Crowley SD. Role of T lymphocytes in hypertension. *Curr Opin Pharmacol.* 2015; 21:14–9. [PubMed: 25523165]
39. Barhoumi T, Kasal DA, Li MW, et al. T regulatory lymphocytes prevent angiotensin II-induced hypertension and vascular injury. *Hypertension.* 2011; 57(3):469–76. [PubMed: 21263125]
40. Kasal DA, Barhoumi T, Li MW, et al. T regulatory lymphocytes prevent aldosterone-induced vascular injury. *Hypertension.* 2012; 59(2):324–30. [PubMed: 22146512]
41. Kassan M, Wecker A, Kadowitz P, Trebak M, Matrougui K. CD4+CD25+Foxp3 regulatory T cells and vascular dysfunction in hypertension. *J Hypertens.* 2013; 31(10):1939–43. [PubMed: 23881298]
42. Pozo D, Vales-Gomez M, Mavaddat N, Williamson SC, Chisholm SE, Reyburn H. CD161 (human NKR-P1A) signaling in NK cells involves the activation of acid sphingomyelinase. *J Immunol.* 2006; 176(4):2397–406. [PubMed: 16455998]
43. Steiniger B, Stehling O, Scriba A, Grau V. Monocytes in the rat: phenotype and function during acute allograft rejection. *Immunol Rev.* 2001; 184:38–44. [PubMed: 12086320]
44. Scriba A, Grau V, Steiniger B. Phenotype of rat monocytes during acute kidney allograft rejection: increased expression of NKR-P1 and reduction of CD43. *Scand J Immunol.* 1998; 47(4):332–42. [PubMed: 9600314]
45. Kamishikiryo J, Fukuhara H, Okabe Y, Kuroki K, Maenaka K. Molecular basis for LLT1 protein recognition by human CD161 protein (NKR-P1A/KLRB1). *J Biol Chem.* 2011; 286(27):23823–30. [PubMed: 21572041]
46. Harwani SC, Chapleau MW, Legge KL, Ballas ZK, Abboud FM. Neurohormonal Modulation of the Innate Immune System is Pro-inflammatory in the Pre-hypertensive Spontaneously Hypertensive Rat, a Genetic Model of Essential Hypertension. *Circ Res.* 2012; 111(9):1190–7. [PubMed: 22904093]
47. Harwani SC, Ratcliff J, Sutterwala FS, et al. Nicotine Mediates CD161a+ Renal Macrophage Infiltration and Premature Hypertension in the Spontaneously Hypertensive Rat. *Circ Res.* 2016; 119(10):1101–15. [PubMed: 27660287]
48. Lin CH, Lee SY, Zhang CC, et al. Fenretinide inhibits macrophage inflammatory mediators and controls hypertension in spontaneously hypertensive rats via the peroxisome proliferator-activated receptor gamma pathway. *Drug Des Devel Ther.* 2016; 10:3591–7.
49. Bouhrel MA, Staels B, Chinetti-Gbaguidi G. Peroxisome proliferator-activated receptors--from active regulators of macrophage biology to pharmacological targets in the treatment of cardiovascular disease. *J Intern Med.* 2008; 263(1):28–42. [PubMed: 18042221]
50. Ndisang JF, Mishra M. The heme oxygenase system selectively suppresses the proinflammatory macrophage m1 phenotype and potentiates insulin signaling in spontaneously hypertensive rats. *Am J Hypertens.* 2013; 26(9):1123–31. [PubMed: 23757400]
51. Gomolak JR, Didion SP. Angiotensin II-induced endothelial dysfunction is temporally linked with increases in interleukin-6 and vascular macrophage accumulation. *Front Physiol.* 2014; 5:396. [PubMed: 25400581]
52. Wenzel P, Knorr M, Kossmann S, et al. Lysozyme M-positive monocytes mediate angiotensin II-induced arterial hypertension and vascular dysfunction. *Circulation.* 2011; 124(12):1370–81. [PubMed: 21875910]
53. De Ciuceis C, Amiri F, Brassard P, Endemann DH, Touyz RM, Schiffrin EL. Reduced vascular remodeling, endothelial dysfunction, and oxidative stress in resistance arteries of angiotensin II-infused macrophage colony-stimulating factor-deficient mice: evidence for a role in inflammation

in angiotensin-induced vascular injury. *Arterioscler Thromb Vasc Biol.* 2005; 25(10):2106–13. [PubMed: 16100037]

54. Lieschke GJ, Grail D, Hodgson G, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood.* 1994; 84(6):1737–46. [PubMed: 7521686]
55. Wikstrom AK, Stephansson O, Cnattingius S. Tobacco use during pregnancy and preeclampsia risk: effects of cigarette smoking and snuff. *Hypertension.* 2010; 55(5):1254–9. [PubMed: 20231527]
56. Yoshida H, Hayashi S, Kunisada T, et al. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature.* 1990; 345(6274):442–4. [PubMed: 2188141]
57. Gallego C, Golenbock D, Gomez MA, Saravia NG. Toll-like receptors participate in macrophage activation and intracellular control of *Leishmania (Viannia) panamensis*. *Infect Immun.* 2011; 79(7):2871–9. [PubMed: 21518783]
58. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest.* 2012; 122(3):787–95. [PubMed: 22378047]
59. Harrison DG, Guzik TJ, Lob H, et al. Inflammation, Immunity, and Hypertension. *Hypertension.* 2010
60. Farrugia M, Baron B. The Role of Toll-Like Receptors in Autoimmune Diseases through Failure of the Self-Recognition Mechanism. *Int J Inflam.* 2017; 2017:8391230. [PubMed: 28553556]
61. Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature.* 2000; 405(6785):458–62. [PubMed: 10839541]
62. Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor $\alpha 7$ subunit is an essential regulator of inflammation. *Nature.* 2003; 421(6921):384–8. [PubMed: 12508119]
63. Nance DM, Sanders VM. Autonomic innervation and regulation of the immune system (1987–2007). *Brain Behav Immun.* 2007; 21(6):736–45. [PubMed: 17467231]
64. Felten SY, Olschowka J. Noradrenergic sympathetic innervation of the spleen: II. Tyrosine hydroxylase (TH)-positive nerve terminals form synapticlike contacts on lymphocytes in the splenic white pulp. *J Neurosci Res.* 1987; 18(1):37–48. [PubMed: 2890771]
65. Zaldivia MT, Rivera J, Hering D, et al. Renal Denervation Reduces Monocyte Activation and Monocyte-Platelet Aggregate Formation: An Anti-Inflammatory Effect Relevant for Cardiovascular Risk. *Hypertension.* 2017; 69(2):323–31. [PubMed: 27956575]
66. de Jonge WJ, van der Zanden EP, The FO, et al. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. *Nat Immunol.* 2005; 6(8):844–51. [PubMed: 16025117]
67. Yuan F, Fu X, Shi H, Chen G, Dong P, Zhang W. Induction of murine macrophage M2 polarization by cigarette smoke extract via the JAK2/STAT3 pathway. *PLoS One.* 2014; 9(9):e107063. [PubMed: 25198511]
68. Ganta CK, Lu N, Helwig BG, et al. Central angiotensin II-enhanced splenic cytokine gene expression is mediated by the sympathetic nervous system. *Am J Physiol Heart Circ Physiol.* 2005; 289(4):H1683–91. [PubMed: 15908469]
69. Carnevale D, Pallante F, Fardella V, et al. The angiogenic factor PlGF mediates a neuroimmune interaction in the spleen to allow the onset of hypertension. *Immunity.* 2014; 41(5):737–52. [PubMed: 25517614]
70. Carnevale D, Perrotta M, Pallante F, et al. A cholinergic-sympathetic pathway primes immunity in hypertension and mediates brain-to-spleen communication. *Nat Commun.* 2016; 7:13035. [PubMed: 27676657]
71. Xiao L, Kirabo A, Wu J, et al. Renal Denervation Prevents Immune Cell Activation and Renal Inflammation in Angiotensin II-Induced Hypertension. *Circ Res.* 2015; 117(6):547–57. [PubMed: 26156232]
72. Liao X, Sharma N, Kapadia F, et al. Kruppel-like factor 4 regulates macrophage polarization. *J Clin Invest.* 2011; 121(7):2736–49. [PubMed: 21670502]
73. Grisanti LA, Perez DM, Porter JE. Modulation of immune cell function by $\alpha(1)$ -adrenergic receptor activation. *Curr Top Membr.* 2011; 67:113–38. [PubMed: 21771488]

74. Grailer JJ, Haggadone MD, Sarma JV, Zetoune FS, Ward PA. Induction of M2 regulatory macrophages through the beta2-adrenergic receptor with protection during endotoxemia and acute lung injury. *J Innate Immun.* 2014; 6(5):607–18. [PubMed: 24642449]
75. Lee RH, Vazquez G. Evidence for a prosurvival role of alpha-7 nicotinic acetylcholine receptor in alternatively (M2)-activated macrophages. *Physiol Rep.* 2013; 1(7):e00189. [PubMed: 24744866]
76. Li DJ, Evans RG, Yang ZW, et al. Dysfunction of the cholinergic anti-inflammatory pathway mediates organ damage in hypertension. *Hypertension.* 2011; 57(2):298–307. [PubMed: 21173343]
77. Ge Q, Ruan CC, Ma Y, et al. Osteopontin regulates macrophage activation and osteoclast formation in hypertensive patients with vascular calcification. *Sci Rep.* 2017; 7:40253. [PubMed: 28091516]
78. Wang Y, Zhang F, Yang W, Xue S. Nicotine Induces Pro-inflammatory Response in Aortic Vascular Smooth Muscle Cells Through a NFkappaB/Osteopontin Amplification Loop-Dependent Pathway. *Inflammation.* 2011
79. Changeux JP. The nicotinic acetylcholine receptor: the founding father of the pentameric ligand-gated ion channel superfamily. *J Biol Chem.* 2012; 287(48):40207–15. [PubMed: 23038257]
80. Reichrath S, Reichrath J, Moussa AT, Meier C, Tschernig T. Targeting the non-neuronal cholinergic system in macrophages for the management of infectious diseases and cancer: challenge and promise. *Cell Death Discov.* 2016; 2:16063. [PubMed: 27785369]
81. Ganong WF. Circumventricular organs: definition and role in the regulation of endocrine and autonomic function. *Clin Exp Pharmacol Physiol.* 2000; 27(5–6):422–7. [PubMed: 10831247]
82. Wolburg H, Paulus W. Choroid plexus: biology and pathology. *Acta Neuropathol.* 2010; 119(1):75–88. [PubMed: 20033190]
83. Shi P, Raizada MK, Sumners C. Brain cytokines as neuromodulators in cardiovascular control. *Clin Exp Pharmacol Physiol.* 2010; 37(2):e52–7. [PubMed: 19566837]
84. Besedovsky H, del Rey A, Sorkin E, Dinarello CA. Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science.* 1986; 233(4764):652–4. [PubMed: 3014662]
85. Goehler LE, Relton JK, Dripps D, et al. Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull.* 1997; 43(3):357–64. [PubMed: 9227848]
86. Labus J, Hackel S, Lucka L, Danker K. Interleukin-1beta induces an inflammatory response and the breakdown of the endothelial cell layer in an improved human THBMEC-based in vitro blood-brain barrier model. *J Neurosci Methods.* 2014; 228:35–45. [PubMed: 24631939]
87. Rochfort KD, Collins LE, Murphy RP, Cummins PM. Downregulation of blood-brain barrier phenotype by proinflammatory cytokines involves NADPH oxidase-dependent ROS generation: consequences for interendothelial adherens and tight junctions. *PLoS One.* 2014; 9(7):e101815. [PubMed: 24992685]
88. Farina C, Aloisi F, Meinl E. Astrocytes are active players in cerebral innate immunity. *Trends Immunol.* 2007; 28(3):138–45. [PubMed: 17276138]
89. Takeshita Y, Ransohoff RM. Inflammatory cell trafficking across the blood-brain barrier: chemokine regulation and in vitro models. *Immunol Rev.* 2012; 248(1):228–39. [PubMed: 22725965]
90. Kim WK, Alvarez X, Fisher J, et al. CD163 identifies perivascular macrophages in normal and viral encephalitic brains and potential precursors to perivascular macrophages in blood. *Am J Pathol.* 2006; 168(3):822–34. [PubMed: 16507898]
91. Goldmann T, Wieghofer P, Jordao MJ, et al. Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat Immunol.* 2016; 17(7):797–805. [PubMed: 27135602]
92. Laflamme N, Lacroix S, Rivest S. An essential role of interleukin-1beta in mediating NF-kappaB activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. *J Neurosci.* 1999; 19(24):10923–30. [PubMed: 10594073]
93. Faraco G, Sugiyama Y, Lane D, et al. Perivascular macrophages mediate the neurovascular and cognitive dysfunction associated with hypertension. *J Clin Invest.* 2016; 126(12):4674–89. [PubMed: 27841763]

94. Liu Y, Jacobowitz DM, Barone F, et al. Quantitation of perivascular monocytes and macrophages around cerebral blood vessels of hypertensive and aged rats. *J Cereb Blood Flow Metab.* 1994; 14(2):348–52. [PubMed: 8113330]
95. Prinz M, Erny D, Hagemeyer N. Ontogeny and homeostasis of CNS myeloid cells. *Nat Immunol.* 2017; 18(4):385–92. [PubMed: 28323268]
96. Meeker RB, Williams K, Killebrew DA, Hudson LC. Cell trafficking through the choroid plexus. *Cell Adh Migr.* 2012; 6(5):390–6. [PubMed: 22902764]
97. Winkowski PJ, Radkowski M, Wszedybyl-Winkowska M, Demkow U. Brain inflammation and hypertension: the chicken or the egg? *J Neuroinflammation.* 2015; 12:85. [PubMed: 25935397]
98. Dampney RA, Horiuchi J, Killinger S, Sheriff MJ, Tan PS, McDowall LM. Long-term regulation of arterial blood pressure by hypothalamic nuclei: some critical questions. *Clin Exp Pharmacol Physiol.* 2005; 32(5–6):419–25. [PubMed: 15854152]
99. Esler M. The sympathetic nervous system through the ages: from Thomas Willis to resistant hypertension. *Exp Physiol.* 2011; 96(7):611–22. [PubMed: 21551268]
100. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci.* 2006; 7(5):335–46. [PubMed: 16760914]
101. Ginhoux F, Greter M, Leboeuf M, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science.* 2010; 330(6005):841–5. [PubMed: 20966214]
102. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci.* 2007; 10(12):1538–43. [PubMed: 18026097]
103. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. *Nat Rev Immunol.* 2014; 14(7):463–77. [PubMed: 24962261]
104. Kang YM, Ma Y, Zheng JP, et al. Brain nuclear factor-kappa B activation contributes to neurohumoral excitation in angiotensin II-induced hypertension. *Cardiovasc Res.* 2009; 82(3):503–12. [PubMed: 19246475]
105. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. *Physiol Rev.* 2011; 91(2):461–553. [PubMed: 21527731]
106. Shen XZ, Li Y, Li L, et al. Microglia participate in neurogenic regulation of hypertension. *Hypertension.* 2015; 66(2):309–16. [PubMed: 26056339]
107. Zubcevic J, Jun JY, Kim S, et al. Altered inflammatory response is associated with an impaired autonomic input to the bone marrow in the spontaneously hypertensive rat. *Hypertension.* 2014; 63(3):542–50. [PubMed: 24366083]
108. Orihuela R, McPherson CA, Harry GJ. Microglial M1/M2 polarization and metabolic states. *Br J Pharmacol.* 2016; 173(4):649–65. [PubMed: 25800044]
109. Miyoshi M, Miyano K, Moriyama N, Taniguchi M, Watanabe T. Angiotensin type 1 receptor antagonist inhibits lipopolysaccharide-induced stimulation of rat microglial cells by suppressing nuclear factor kappaB and activator protein-1 activation. *Eur J Neurosci.* 2008; 27(2):343–51. [PubMed: 18190523]
110. Waki H, Gouraud SS, Maeda M, Raizada MK, Paton JF. Contributions of vascular inflammation in the brainstem for neurogenic hypertension. *Respir Physiol Neurobiol.* 2011; 178(3):422–8. [PubMed: 21601658]
111. Wu CY, Zha H, Xia QQ, et al. Expression of angiotensin II and its receptors in activated microglia in experimentally induced cerebral ischemia in the adult rats. *Mol Cell Biochem.* 2013; 382(1–2):47–58. [PubMed: 23754618]
112. Biancardi VC, Stranahan AM, Krause EG, de Kloet AD, Stern JE. Cross talk between AT1 receptors and Toll-like receptor 4 in microglia contributes to angiotensin II-derived ROS production in the hypothalamic paraventricular nucleus. *Am J Physiol Heart Circ Physiol.* 2016; 310(3):H404–15. [PubMed: 26637556]
113. Perry VH, Teeling J. Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Semin Immunopathol.* 2013; 35(5):601–12. [PubMed: 23732506]

114. Chen XL, Tummala PE, Olbrych MT, Alexander RW, Medford RM. Angiotensin II induces monocyte chemoattractant protein-1 gene expression in rat vascular smooth muscle cells. *Circ Res.* 1998; 83(9):952–9. [PubMed: 9797345]
115. Cheng ZJ, Vapaatalo H, Mervaala E. Angiotensin II and vascular inflammation. *Med Sci Monit.* 2005; 11(6):RA194–205. [PubMed: 15917731]
116. Ruiz-Ortega M, Ruperez M, Lorenzo O, et al. Angiotensin II regulates the synthesis of proinflammatory cytokines and chemokines in the kidney. *Kidney Int Suppl.* 2002; (82):S12–22. [PubMed: 12410849]
117. Hilgers KF, Hartner A, Porst M, et al. Monocyte chemoattractant protein-1 and macrophage infiltration in hypertensive kidney injury. *Kidney Int.* 2000; 58(6):2408–19. [PubMed: 11115074]
118. Liao TD, Yang XP, Liu YH, et al. Role of inflammation in the development of renal damage and dysfunction in angiotensin II-induced hypertension. *Hypertension.* 2008; 52(2):256–63. [PubMed: 18541733]
119. Ozawa Y, Kobori H, Suzuki Y, Navar LG. Sustained renal interstitial macrophage infiltration following chronic angiotensin II infusions. *Am J Physiol Renal Physiol.* 2007; 292(1):F330–9. [PubMed: 16804106]
120. Schiffrin EL, Touyz RM. Inflammation and vascular hypertrophy induced by angiotensin II: role of NADPH oxidase-derived reactive oxygen species independently of blood pressure elevation? *Arterioscler Thromb Vasc Biol.* 2003; 23(5):707–9. [PubMed: 12740221]
121. Moore JP, Vinh A, Tuck KL, et al. M2 macrophage accumulation in the aortic wall during angiotensin II infusion in mice is associated with fibrosis, elastin loss, and elevated blood pressure. *Am J Physiol Heart Circ Physiol.* 2015; 309(5):H906–17. [PubMed: 26071547]
122. Aki K, Shimizu A, Masuda Y, et al. ANG II receptor blockade enhances anti-inflammatory macrophages in anti-glomerular basement membrane glomerulonephritis. *Am J Physiol Renal Physiol.* 2010; 298(4):F870–82. [PubMed: 20071465]
123. Gabbai FB, De Nicola L, Peterson OW, et al. Renal response to blood pressure elevation in normal and glomerulonephritic rats. *J Am Soc Nephrol.* 1996; 7(12):2590–9. [PubMed: 8989737]
124. Jurewicz M, McDermott DH, Sechler JM, et al. Human T and natural killer cells possess a functional renin-angiotensin system: further mechanisms of angiotensin II-induced inflammation. *J Am Soc Nephrol.* 2007; 18(4):1093–102. [PubMed: 17329576]
125. Nataraj C, Oliverio MI, Mannon RB, et al. Angiotensin II regulates cellular immune responses through a calcineurin-dependent pathway. *J Clin Invest.* 1999; 104(12):1693–701. [PubMed: 10606623]
126. Ma LJ, Corsa BA, Zhou J, et al. Angiotensin type 1 receptor modulates macrophage polarization and renal injury in obesity. *Am J Physiol Renal Physiol.* 2011; 300(5):F1203–13. [PubMed: 21367915]
127. Zhang JD, Patel MB, Griffiths R, et al. Type 1 angiotensin receptors on macrophages ameliorate IL-1 receptor-mediated kidney fibrosis. *J Clin Invest.* 2014; 124(5):2198–203. [PubMed: 24743144]
128. Kato H, Ishida J, Nagano K, et al. Deterioration of atherosclerosis in mice lacking angiotensin II type 1A receptor in bone marrow-derived cells. *Lab Invest.* 2008; 88(7):731–9. [PubMed: 18490898]
129. Crowley SD, Song YS, Sprung G, et al. A role for angiotensin II type 1 receptors on bone marrow-derived cells in the pathogenesis of angiotensin II-dependent hypertension. *Hypertension.* 2010; 55(1):99–108. [PubMed: 19996062]
130. Merino A, Alvarez-Lara MA, Ramirez R, Carracedo J, Martin-Malo A, Aljama P. Losartan prevents the development of the pro-inflammatory monocytes CD14+CD16+ in haemodialysis patients. *Nephrol Dial Transplant.* 2012; 27(7):2907–12. [PubMed: 22247233]
131. Kim J, Padanilam BJ. Renal nerves drive interstitial fibrogenesis in obstructive nephropathy. *J Am Soc Nephrol.* 2013; 24(2):229–42. [PubMed: 23264683]
132. Machnik A, Dahlmann A, Kopp C, et al. Mononuclear phagocyte system depletion blocks interstitial tonicity-responsive enhancer binding protein/vascular endothelial growth factor C expression and induces salt-sensitive hypertension in rats. *Hypertension.* 2010; 55(3):755–61. [PubMed: 20142563]

133. Machnik A, Neuhofer W, Jantsch J, et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med*. 2009; 15(5):545–52. [PubMed: 19412173]
134. Hucke S, Eschborn M, Liebmann M, et al. Sodium chloride promotes pro-inflammatory macrophage polarization thereby aggravating CNS autoimmunity. *J Autoimmun*. 2016; 67:90–101. [PubMed: 26584738]
135. Kleinewietfeld M, Manzel A, Titze J, et al. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. *Nature*. 2013; 496(7446):518–22. [PubMed: 23467095]
136. Buxade M, Lunazzi G, Minguillon J, et al. Gene expression induced by Toll-like receptors in macrophages requires the transcription factor NFAT5. *J Exp Med*. 2012; 209(2):379–93. [PubMed: 22312110]
137. Choi SY, Lee HH, Lee JH, et al. TonEBP suppresses IL-10-mediated immunomodulation. *Sci Rep*. 2016; 6:25726. [PubMed: 27160066]
138. Chobanian AV. Shattuck Lecture. The hypertension paradox--more uncontrolled disease despite improved therapy. *N Engl J Med*. 2009; 361(9):878–87. [PubMed: 19710486]
139. Ebringer A, Doyle AE. Raised serum IgG levels in hypertension. *Br Med J*. 1970; 2(5702):146–8. [PubMed: 4985868]
140. Gudbrandsson T, Hansson L, Herlitz H, Lindholm L, Nilsson LA. Immunological changes in patients with previous malignant essential hypertension. *Lancet*. 1981; 1(8217):406–8. [PubMed: 6110039]
141. Kristensen BO, Solling K. Serum concentrations of immunoglobulins and free light chains before and after vascular events in essential hypertension. *Acta Med Scand*. 1983; 213(1):15–20. [PubMed: 6402888]
142. Mirhafez SR, Mohebbati M, Feiz Disfani M, et al. An imbalance in serum concentrations of inflammatory and anti-inflammatory cytokines in hypertension. *J Am Soc Hypertens*. 2014; 8(9): 614–23. [PubMed: 25224864]
143. Kirabo A, Fontana V, de Faria AP, et al. DC isoketal-modified proteins activate T cells and promote hypertension. *J Clin Invest*. 2014; 124(10):4642–56. [PubMed: 25244096]
144. Olsen F, Loft B. Delayed hypersensitivity directed against arterial antigens in the hypertensive disease in man. *Acta Pathol Microbiol Scand A*. 1973; 81(4):498–500. [PubMed: 4779701]
145. Srivastava K, Narang R, Bhatia J, Saluja D. Expression of Heat Shock Protein 70 Gene and Its Correlation with Inflammatory Markers in Essential Hypertension. *PLoS One*. 2016; 11(3):e0151060. [PubMed: 26989902]
146. Sesso HD, Wang L, Buring JE, Ridker PM, Gaziano JM. Comparison of interleukin-6 and C-reactive protein for the risk of developing hypertension in women. *Hypertension*. 2007; 49(2): 304–10. [PubMed: 17159088]
147. Sesso HD, Jimenez MC, Wang L, Ridker PM, Buring JE, Gaziano JM. Plasma Inflammatory Markers and the Risk of Developing Hypertension in Men. *J Am Heart Assoc*. 2015; 4(9):e001802. [PubMed: 26391130]
148. Heptinstall RH. Renal biopsies in hypertension. *Br Heart J*. 1954; 16(2):133–41. [PubMed: 13160264]
149. Sommers SC, Relman AS, Smithwick RH. Histologic studies of kidney biopsy specimens from patients with hypertension. *Am J Pathol*. 1958; 34(4):685–715. [PubMed: 13559400]
150. Hughson MD, Gobe GC, Hoy WE, Manning RD Jr, Douglas-Denton R, Bertram JF. Associations of glomerular number and birth weight with clinicopathological features of African Americans and whites. *Am J Kidney Dis*. 2008; 52(1):18–28. [PubMed: 18514988]
151. Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension*. 2005; 46(5):1118–22. [PubMed: 16216991]
152. Pietri P, Vyssoulis G, Vlachopoulos C, et al. Relationship between low-grade inflammation and arterial stiffness in patients with essential hypertension. *J Hypertens*. 2006; 24(11):2231–8. [PubMed: 17053545]
153. Tsioufis C, Thomopoulos K, Dimitriadis K, et al. The incremental effect of obstructive sleep apnoea syndrome on arterial stiffness in newly diagnosed essential hypertensive subjects. *J Hypertens*. 2007; 25(1):141–6. [PubMed: 17143185]

154. Dorffle Y, Latsch C, Stuhlmüller B, et al. Preactivated peripheral blood monocytes in patients with essential hypertension. *Hypertension*. 1999; 34(1):113–7. [PubMed: 10406833]
155. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med*. 2017; 377(12):1119–31. [PubMed: 28845751]
156. Youn JC, Yu HT, Lim BJ, et al. Immunosenescent CD8+ T cells and C-X-C chemokine receptor type 3 chemokines are increased in human hypertension. *Hypertension*. 2013; 62(1):126–33. [PubMed: 23716586]
157. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood*. 2010; 116(16):e74–80. [PubMed: 20628149]
158. Stulc T, Svobodova H, Krupickova Z, Dolezalova R, Marinov I, Ceska R. Rosiglitazone influences the expression of leukocyte adhesion molecules and CD14 receptor in type 2 diabetes mellitus patients. *Physiol Res*. 2014; 63(Suppl 2):S293–8. [PubMed: 24908235]
159. Nadar SK, Blann A, Beevers DG, Lip GY. Abnormal angiopoietins 1&2, angiopoietin receptor Tie-2 and vascular endothelial growth factor levels in hypertension: relationship to target organ damage [a sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT)]. *J Intern Med*. 2005; 258(4):336–43. [PubMed: 16164572]
160. Hofer E, Schweighofer B. Signal transduction induced in endothelial cells by growth factor receptors involved in angiogenesis. *Thromb Haemost*. 2007; 97(3):355–63. [PubMed: 17334501]
161. Marketou ME, Kontaraki JE, Tsakountakis NA, et al. Arterial stiffness in hypertensives in relation to expression of angiopoietin-1 and 2 genes in peripheral monocytes. *J Hum Hypertens*. 2010; 24(5):306–11. [PubMed: 20072144]
162. Berg KE, Ljungcrantz I, Andersson L, et al. Elevated CD14++CD16-monocytes predict cardiovascular events. *Circ Cardiovasc Genet*. 2012; 5(1):122–31. [PubMed: 22238190]
163. Ulrich C, Heine GH, Garcia P, et al. Increased expression of monocytic angiotensin-converting enzyme in dialysis patients with cardiovascular disease. *Nephrol Dial Transplant*. 2006; 21(6):1596–602. [PubMed: 16476718]
164. Zhao Z, Ni Y, Chen J, et al. Increased migration of monocytes in essential hypertension is associated with increased transient receptor potential channel canonical type 3 channels. *PLoS One*. 2012; 7(3):e32628. [PubMed: 22438881]
165. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med*. 2014; 370(15):1393–401. [PubMed: 24678939]
166. Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Bohm M. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet*. 2010; 376(9756):1903–9. [PubMed: 21093036]

Figure 1. Tree of Macrophage Polarization

Macrophage polarization has been simplified into the M1 and M2 subsets. However, new markers are continually being discovered and add to the complexity of the paradigm of macrophage polarization. Here we represent a simplified over view of the macrophage subsets, in addition to the M1 and M2 phenotypes, that have been discovered. This includes the tumor associated macrophage (TAM), the T-cell receptor-positive (TCR+), CD169-positive, CD161a-positive, and various known subpopulations of the M2 (M2a, M2b, M2c, and M2d). Although new markers are continually being discovered, it is likely that the various macrophage subsets are related to each other and their relatedness will need to be reflected in a hierarch such as this phylogenetic tree.

Figure 2. Imbalance of M1 and M2 macrophage populations in SHR

A) Splenocytes from young (3–5 week old) pre-hypertensive Spontaneously Hypertensive Rats (SHR) and their age-matched Wistar Kyoto (WKY) controls were isolated and cultured in the absence (media alone) or presence of nicotine (10 micromolar) in vitro for 48 hours. Splenocytes were then harvested and stained with antibodies against CD161a and CD68 (ED1) and underwent flow cytometry. Nicotine induced a significant increase in the CD161a +/CD68+ M1 macrophage population, compared to the WKY; B) Splenocytes were isolated from young SHR and WKY following an 2-week in vivo infusion of nicotine bitartrate (15mg/kg/day) and stained for CD4 and CD163, a marker of the M2 macrophage. There was over a 50% reduction in the nicotine-infused SHR, compared to WKY.

Figure 3. Induction of M2 Macrophages Leads to Decrease in the M1/M2 Ratio and Decrease in Blood Pressure

Data from Ndisang and Mishra 2013 (46) redrawn to demonstrate the decrease in blood pressure (■) (mmHg) following induction of the M2-macrophage (■) and the decrease in the M1-macrophage (■)(with consequent decrease in the M1/M2 ratio) in the adult Spontaneously Hypertensive Rat with established hypertension.

Figure 4.

Figure 4A. Overview of the “Neuro-Immuno” Axis in Hypertension.

The autonomic nervous system is comprised of the sympathetic/adrenergic and parasympathetic/cholinergic arms. Activation of the adrenergic component leads to direct pro-inflammatory influence; where the cholinergic neural arm mediates an anti-inflammatory response on macrophages and other innate immune cells. Pro-inflammatory innate immune regulation leads to an pro-inflammatory adaptive immune (T- and B-lymphocytes) response and the development of hypertension, marked by end-organ inflammation.

Figure 4B. The Neuronal “Neuro-Immuno” Axis in Macrophage Polarization.

Activation of the sympathetic autonomic nervous system mediates polarization of macrophages towards the M1 phenotype. In contrast, activation of the parasympathetic autonomic nervous system mediates polarization of macrophages towards the M2 phenotype.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 5. Central Nervous System Interfaces and Hindbrain/Forebrain Neural Centers Involved in the Regulation of Blood Pressure

Schematic depicts the Blood-Brain Barrier (BBB) and Choroid Plexus (CP) along with the associated structural (astrocytes and pericytes) and immune cells. Immune cells include microglia, perivascular/infiltrating macrophages, dendritic cells (DC), monocytes, and macrophages. Hindbrain and forebrain structures include the circumventricular organs (CVO), neurohypophysis (pituitary & hypothalamus), organum vasculosum lamina terminalis (OVLT), subfornical organs (SFO), area postrema (AP), paraventricular nucleus (PVN), rostroventrolateral medulla (RVLM), anteroventral third ventricle (AV3V), subcommissural organ (SCO), and nucleus tractus solitarius (NTS).

Figure 6. Role of the “Neuro-Immuno” and Neurohormonal Axis as a force in macrophage polarization in the Development of Hypertension

Angiotensin II (Ang II) activates the microglia through the angiotensin type 1 receptor (AT1R) and promotes their polarization towards an M1 phenotype. Minocycline is a known inhibitor of Ang II mediated M1 polarization of microglia. M1 polarization of the microglia leads to activation of the sympathetic outflow from the central nervous system (CNS). Increased sympathetic outflow leads to 1) Increased expression of chemoattractant molecules (e.g. monocyte chemoattractant protein-1, MCP-1) in end-organs, such as the kidney. Simultaneously, increased sympathetic outflow mobilizes macrophages from immune cell reservoirs (e.g. spleen), while priming them for polarization towards an M1 phenotype. In response to increased expression of the chemoattractants in the kidney, macrophages infiltrate the renal medulla and are activated to polarize into the M1 phenotype. Renal and vascular inflammation leads to the development of hypertension. As hypertension develops, engagement of the AT1R directly on the surface of the macrophage results in M1 polarized macrophages “switching” into M2 macrophages, responsible for renal/vascular fibrosis and remodeling.