Despite decades of advancements in the management and treatment, the prevalence of hypertension continues to remain high, as it has been difficult to achieve recommended blood pressure (BP) goals in a large proportion of this patient population at-risk for adverse outcomes.\textsuperscript{1-3} Accumulating evidence indicates that bone marrow (BM) plays critically important regulatory roles in both peripheral- and neuroinflammation associated with many pathophysiological conditions. This has led us to investigate the neural communication between the BM and the brain in hypertension. Thus, the objectives of this review are to summarize recent advances in knowledge that link neuroinflammation with hypertension, and the role of the autonomic nervous system–bone marrow communication in hematopoietic cell homeostasis and their impact on hypertension pathophysiology. In addition, we discuss the novel and emerging field of intestinal microbiota and roles of gut permeability and dysbiosis in cardiovascular disease and hypertension. Finally, we propose a brain–gut–bone marrow triangular interaction hypothesis and discuss its potential in the development of novel therapies for hypertension. (Circ Res. 2016;118:1327-1336. DOI: 10.1161/CIRCRESAHA.116.307709.)

**Key Words:** blood pressure ■ bone marrow ■ dysbiosis ■ hypertension ■ microbiota

**Neuroinflammation**

Our understanding of the involvement of neuroinflammation in the pathogenesis of hypertension has become more robust in the recent years. Multiple proinflammatory cytokines, such as leukotriene-B\textsubscript{4},\textsuperscript{4} C–C chemokine ligand 2,\textsuperscript{5} nuclear factor-xb,\textsuperscript{6} high-mobility group box 1,\textsuperscript{7} tumor necrosis factor-α, interleukin (IL)-1β, and IL-6,\textsuperscript{4} have all been documented to be elevated in the brains of animal models of hypertension. Although the mechanisms remain under investigation, an enhanced vasodeleterious axis of the renin–angiotensin system,\textsuperscript{9} such as angiotensin type 1 receptors brain–gut–BM triangular interaction hypothesis, which we think contributes to persistent and chronic hypertension, and may provide an opportunity to develop novel therapeutic strategies for this disease.
and angiotensin-converting enzyme, have been shown to be involved in driving central proinflammatory pathways. In contrast, central overexpression of angiotensin-converting enzyme 2, a member of the vasoprotective axis of the renin–angiotensin system, beneficially attenuates neurogenic hypertension. Consistent with this knowledge is the observation that shedding of membrane-bound angiotensin-converting enzyme 2 is involved in neurogenic hypertension. Finally, mice that have an overactive brain renin–angiotensin system also have elevated production of several proinflammatory cytokines in the brain. These findings highlight the importance of the renin–angiotensin system in neuroinflammation and hypertension.

How neuroinflammation modulates BP is an area of active investigation. Evidence indicates that increased inflammation in cardioregulatory brain centers is associated with elevated sympathetic nervous system drive that leads to increased BP; conversely, inhibiting these central proinflammatory pathways dampens the increase in BP. In addition, central nervous system injection of proinflammatory cytokines, including IL-1β and tumor necrosis factor-α, increases sympathetic nerve activity (SNA) and BP. Furthermore, inhibition of brain tumor necrosis factor-α attenuates the development of hypertension in animal models. Thus, although there is a general consensus of the role of proinflammatory cytokines in central BP control, the origin of these cytokines remains unclear. Although it is true that circumberventricular organs may respond to systemic cytokines, these cytokines can also be produced centrally and released by neurons and microglial cells to induce proinflammatory processes.

The contribution of microglial cells is of particular relevance in this regard. Microglial cells are the innate immune cells of the brain, constantly surveying the brain environment, promoting immune homeostasis, and producing neurotrophic factors. They become activated in response to pathological insults and alterations in brain homeostasis. Our group was among the first to demonstrate the involvement of brain microglial cells in hypertension, an observation now supported by others. Inhibition of microglia activation is associated with attenuation of hypertension, sympathetic activation, and peripheral inflammation. Furthermore, specific deletion of brain microglia attenuates angiotensin II (Ang II)–induced hypertension. A particularly interesting issue is whether these microglia are directly responding to Ang II, or if this response is mediated by other neuronal factors. There is no consensus on this issue at present. Evidence indicates that resting microglia in the adult brain do not express angiotensin type 1 receptor, although others have demonstrated effects of Ang II on these cells both in vitro and in vivo. This discrepancy could be because of the fact that microglial cells do not express angiotensin type 1 receptor in normal physiological conditions unless primed by certain stimuli, such as prohypertensive stress signals. Thus, it would be reasonable to suggest that prohypertensive signals, such as Ang II, that are known to activate autonomic neurons, could generate mediators that could cause microglial cell activation/differentiation leading to their responsiveness to Ang II. Support for this contention is the evidence that Ang II causes generation of reactive oxygen species and cytokines, such as C–C chemokine ligand 2 and high-mobility group box 1, that are known to affect microglia. Further investigation is needed to clarify this area of neuronal-mediated microglial activation.

### Brain–BM Communication

As mentioned above, neuroinflammation contributes to peripheral sympathoexcitation. Our group has shown that the femoral sympathetic nerve is activated, and BM norepinephrine contents are elevated during hypertension. BM is highly innervated by the sympathetic nervous system. This sympathetic innervation regulates hematopoiesis and the stem-cell niche homeostasis. Sympathetic signals from the brain are thought to travel through adrenergic nerve fibers to BM releasing neurotransmitters that affect hematopoietic stem and progenitor cell (HSPC) mobilization and release into the general circulation. For example, catecholaminergic neurotransmitters control the release of HSPCs from BM by G-CSF (granulocyte-colony stimulating factor)–induced osteoblast suppression and bone CXCL12 regulation, or by directly modulating the Wnt-β–catenin pathway in HSPCs. BM sympathetic fibers can also regulate HSPC mobilization through substance P-mediated nociceptive signaling. Although this mechanism remains to be investigated in hypertension, it has been characterized in patients with diabetes mellitus.

Dysfunction of BM sympathetic tone or complete sympathetic denervation has been associated with impaired HSPC mobilization and loss of circadian rhythmicity of HSPC release. In addition, inhibition of norepinephrine reuptake is associated with enhanced HSPC mobilization. Sympathetic innervation to BM has also been shown to be important in several cardiovascular pathologies. In myocardial infarction and stroke, enhanced sympathetic drive to BM mobilizes HSPCs and increases the output of inflammatory monocytes. This process has been specifically attributed to CCR2+ HSPCs. Our research indicates that in hypertension, enhanced sympathetic tone to BM is associated with loss of circadian rhythmicity possibly associated with altered adrenergic signaling. In addition, we have recently observed that Ang II regulates HSPC proliferation in BM and enhances production of inflammatory monocytes in the spleen also via CCR2+ HSPCs. These studies provide convergent evidence that Ang II–induced increases of CCR2+ HSPCs and myeloid progenitors in BM and spleen could contribute to the development

<table>
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<th>Nonstandard Abbreviations and Acronyms</th>
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<td>BP</td>
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of hypertension through increased sympathetic drive to BM. However, it remains to be determined if CCR2+ HSPCs are also activated in other models of hypertension and if CCR2+ HSPCs are rhythmically released from BM in response to sympathetic drive.

In addition to regulating HSPC mobilization from BM, the sympathetic nervous system regulates the immune system in a circadian manner. Adrenergic nerves have been shown to regulate recruitment of leukocytes to tissues. Norepinephrine stimulates immune cells to regulate proliferation, differentiation, maturation, and effector functions. Specifically, adrenergic receptor stimulation of immune cells has been implicated in both anti- and proinflammatory responses. Autonomic modulation of the immune system seems to be important in development of hypertension-related pathology and seems to be specifically biased toward enhanced proinflammatory responses. Autonomic modulation of the immune system seems to be important in the hypothalamic paraventricular nucleus. Similar findings have been observed in the spleen and peripheral blood, suggesting that there is constant trafficking of myeloid progenitors from BM to the spleen that contribute to monocyte-mediated inflammation in hypertension. It seems that in hypertension, autonomic modulation of the immune system is altered such that anti-inflammatory cholinergic modulation of the innate immune system becomes proinflammatory. In addition, sympathetic drive seems to promote hypertension by norepinephrine-mediated T-cell activation. Interestingly, norepinephrine preferentially activates memory T-cells to release proinflammatory cytokines, which is particularly important because memory T-cells accumulate in the kidney and vasculature of hypertensive animals. Our data indicate that norepinephrine application into BM increases mobilization of immune cells, and this response is attenuated by application of acetylcholine. Furthermore, renal denervation has been shown to prevent immune cell activation and reduce renal inflammation in Ang II–induced hypertension. Therefore, it becomes evident that autonomic regulation of the immune system is not only important but also altered toward enhancing proinflammatory responses in hypertension.

Another important aspect to consider is a positive feedback loop, where neuroinflammation contributes to sympathoexcitation, which then promotes activation of the immune system and stem/progenitor cells in BM. In turn, this can feedback to the brain and exacerbate central inflammation generating a vicious proinflammatory cycle. Using BM-chimeric rats, our group has shown that proinflammatory progenitors from BM extravasate and enter the hypothalamic paraventricular nucleus to contribute to neuroinflammation in Ang II–induced hypertension. In addition, treatment with minocycline, an anti-inflammatory antibiotic, not only attenuates central microglial activation and hypertension but also decreases the number of BM-derived microglia/macrophages in the hypothalamic paraventricular nucleus. Similar findings in the hypothalamic paraventricular nucleus were reported in a mouse model of chronic stress, an important risk factor for the development of hypertension. Others have shown the presence of T-cell infiltration in the subfornical organ during Ang II–induced hypertension. Taken together, these observations indicate that sympathetic activation has a profound impact on BM proinflammatory progenitors, as some of these cells extravasate into the brain, and contribute to neuroinflammation.

The specific mechanisms underlying extravasation of BM cells into the brain remain a subject of extensive investigation. Studies indicate that a C–C chemokine ligand 2 gradient, leading to its increased concentration in the brain, could be one of these signals. In addition, proinflammatory Ly6C+ CCR2+ monocytes have been suggested to be the monocyte progenitor responsible for these extravasated cells in the brain. Although this view has not yet been evaluated in hypertension studies, there is evidence both from our group (Figure 1) and others indicating that Ang II–induced hypertension in mice models is associated with elevated Ly6C+ monocytes mostly expressing CCR2. Finally, a leaky blood–brain barrier associated with hypertension could also contribute to extravasation of BM cells into the brain.

**Brain–Gut Communication**

The gut enteric nervous system is complex and capable of functioning independent of extrinsic inputs. Communication between this system and the central nervous system has been extensively reviewed elsewhere. Therefore, we will focus on the effects of autonomic innervation on immune responses and gut function, as well as the effects of gut microbial factors on the central nervous system.

Autonomic input to the gut plays an important role in regulating the local immune response. Norepinephrine and sympathetic nerves are key in regulating lymphocyte migration and accumulation in the gut. Resident intestinal macrophages are closely regulated by both the vagus nerves and the sympathetic varicosities. Vagal nerve stimulation and blocking sympathetic drive prevent breakdown of the intestinal lumen–blood barrier and enhances epithelial cell barrier function, respectively. These observations suggest that both the sympathetic and the parasympathetic arms of the autonomic nervous system are important in regulating the gut’s immune response as well as function of the gut epithelial barrier. However, whether intestinal inflammation and barrier permeability are altered in hypertension remains to be determined.

The brain–gut communication seems to be bidirectional where gut microbiota and their products are implicated in sympathetic activation that maintains an influx of lymphocytes to intestinal tissue. This view is supported by evidence from germ-free (GF) mice, where lacking gut microbes seem to have a less anxious phenotype in both the elevated plus maze and the light dark box tests. Relevant to cardiovascular physiology, GF mice also have an exaggerated hypothalamic–pituitary–adrenal stress response, in addition to a disruption of the blood–brain barrier. It is interesting to note that these mice also display global microglial defects. Taken together with the evidence that microglia homeostasis is regulated by bacterial short-chain fatty acids (SCFAs), it is tempting to suggest that gut microbiota and its products hold the potential to regulate neural control mechanisms in hypertension.

**Intestinal Microbiota and Hypertension**

The gut microbiota has become one of the most active areas of research in cardiovascular and metabolic diseases. The human
Microbiota research is enabled by metagenomics, which allows high-resolution and culture-independent sequencing of bacterial DNA using either amplicons sequencing or whole-metagenome shotgun sequencing. Bioinformatics analysis of sequencing data is usually performed in 2 ways: first, for taxonomic classification and second, for functional profiling by microbial product identification/prediction. Resulting data provide information on the presence of different species in a microbial population and their potential metabolic functions. However, the involvement of these microbial populations in pathophysiology requires more complex experimental design, such as microbial depletion by antibiotics, or fecal transplantation studies between control and experimentally diseased animals and perhaps fecal transplantation from human samples into GF mice. The latter approach using humanized mice has led to important discoveries, indicating that gut microbiota modulate metabolism and obesity. The added benefit of using human intestinal microbiota samples for these experiments is that they may have superior benefits for patients. For example, a recent study indicates that transplants from lean donors to patients with metabolic syndrome led to an increase in the recipient’s insulin sensitivity.

To date, there are limited studies indicating a direct association between gut microbiota and hypertension in both animal models and human disease. Early studies have shown an elevated BP in GF rats by ≈20 mm Hg, implicating a role for gut microbiota in BP regulation. Availability of metagenomic technology has accelerated investigation linking hypertension and gut microbiota. The first evidence for such a link was the demonstration that SCFAs modulate BP through the renal and vascular olfactory receptor (Olfr) 78 and G-protein–coupled receptor 41 in mice. These receptors are mutually antagonistic and respond to SCFAs, products of bacterial metabolism found in the circulation. This is supported by a series of studies using Olfr78 and G-protein–coupled receptor 41 knockout mice, demonstrating that stimulation of Olfr78 elevates BP, whereas stimulation of G-protein–coupled receptor 41 lowers BP. Therefore, we sought to determine whether there were alterations in mRNA expression of the rat orthologs of Olfr78 and G-protein–coupled receptor 41, namely Olfr59 and free fatty-acid receptor 3 (Ffar3), comparing Wistar Kyoto and spontaneously hypertensive rat models. Interestingly, we found a 2-fold upregulation of...
Olfr59 in the small intestine accompanied by a 60% down-regulation of Ffar3 (Figure 2). Because Olfr59 elevates BP and Ffar3 opposes this action to lower BP, these data support the suggestion that altered SCFA receptors in the small intestine may play a role in elevated BP of the spontaneously hypertensive rat. Clearly, additional studies are necessary to confirm this conclusion and provide a mechanism for the differential regulation of these SCFA receptors throughout the gastrointestinal tract.

Two simultaneous studies reported a link between gut microbial composition and hypertension.81,82 Using Dahl rats, Mell et al81 demonstrated significant differences in cecal microbiota comparing salt-sensitive and salt-resistant strains. In addition, changes in several SCFAs in plasma after cecal transplantation were observed. They concluded that microbial composition does indeed affect plasma SCFA levels. Our study compared alterations in the fecal microbiota in the spontaneously hypertensive rat and chronic Ang II infusion rat models of hypertension.82 We observed a significant dysbiosis as a result of decreases in microbial richness, diversity, evenness, and increased Firmicutes/Bacteroidetes ratio in hypertensive animals. This dysbiosis was associated with decreased acetate- and butyrate-producing bacterial colonies.82 In addition, we found that the antihypertensive effects of minocycline were modulated by various gut immune and microbial mechanisms, including interferon-γ, IL-10, and myeloid differentiation primary response gene 88.83 Interestingly, enhancing the gut barrier to reduce lipopolysaccharide leakage improves insulin sensitivity in mice.84 In addition, a gut-specific anti-inflammatory agent that targets peroxisome proliferator-activated receptor-γ3.85 5-aminosalicylic acid, has been suggested to be beneficial in insulin resistance and obesity by reducing gut permeability, increasing bacterial diversity, and reversing bowel inflammation.85 Hence, the question becomes whether these gut pathologies are also observed in the hypertensive state. Future work in the field must determine whether low-grade inflammation and enhanced barrier permeability are present during or preceding hypertension development, and importantly, whether a gut-specific anti-inflammatory agent such as 5-aminosalicylic acid would lower BP in hypertensive animal models with gut dysbiosis.

In addition to directly modulating intestinal immunity and barrier function, gut bacteria can affect peripheral immune cells and BM HSPCs. The presence of reduced numbers of myeloid progenitors in both the BM and the spleen in GF mice supports this view.86 In addition, Rag1-deficient mice have lower numbers and proportions of HSPCs, an effect which is reversed by fecal transplantation from wild-type mice.87 In obesity, gut microbiota have been shown to regulate HSPC differentiation by impairing BM niche function.88 Therefore, it could be suggested that hypertension, which exhibits gut dysbiosis, is likely to have an impact on BM stem-cell niche and HSPC function.

The mechanism by which gut microbiota could modulate the immune system relative to hypertension is an area of active investigation. One possibility is through bacterial products that enter the circulation. For example, SCFAs such as acetate and butyrate have been shown to have anti-inflammatory effects on myeloid cells as well as intestinal epithelial cells.89 Through histone deacetylase inhibition, butyrate regulates intestinal macrophage function,90 whereas acetate promotes T-helper 17 cell development.91 T-helper 17 cells are modulated by various gut immune and microbial mechanisms and have been associated with the development of intestinal inflammation.92 We recently observed that T-helper 17 cells (CD4+CD17+) are elevated in hypertensive patients,93 which is particularly relevant because activation of these cells is regulated by gut-intrinsic mechanisms.94,95 It would be important to determine in future experiments if the increase in these T-helper 17 cells is mediated by gut-derived factors as a result of dysbiosis in hypertension.

**Implications for Human Hypertension**

It could be summarized from the above discussion that the brain, BM (immune system), and gut microbiota are potentially intertwined functionally to control BP, and their dysfunctions could be associated with hypertension. Key questions to be addressed are whether there is interplay among these organs in hypertension and if so, what is the contribution of this interplay in the overall hypertensive state? We propose the following brain–gut–BM triangular interaction hypothesis (Figure 3). This working hypothesis has been
developed by synthesizing available evidence from the literature, including our own work. We propose that hypertensive stimuli (such as Ang II, salt, stress, and other hypertension risk factors) trigger autonomic neural pathways resulting in increases in sympathetic and dampening of parasympathetic activities. This directly affects cardiovascular-relevant organs (such as blood vessels, heart, kidney, etc) to increase BP. We propose that increases in sympathetic drive to the gut and BM may also set in motion a sequence of signaling events that ultimately contribute to an overall increase of BP and establishment of hypertension. For example, increased SNA to the gut could result in increased gut permeability, gut inflammatory status, and dysbiosis, leading to an imbalance in microbial-derived metabolites in the plasma. These metabolites, working together with elevated sympathetic drive to the BM, may act as modulators for BM cell activity by increasing production and release of myeloid progenitors and other proinflammatory cells, and decrease in angiogenic progenitors. This could be a critical event for establishment of hypertension because increases in myeloid progenitors contribute to an overall increase in peripheral inflammation and neuroinflammation by differentiating into brain macrophages/microglia. Decreases in angiogenic cells also can lead to a compromised vascular repair capacity, a hallmark of hypertension. Neuroinflammation-associated increases in cytokines, chemokines, and reactive oxygen species accentuate autonomic neuronal activity and SNA to the gut and BM, thus perpetuating hypertension. Therefore, although there are multiple factors that cause high BP, we propose that involvement of SNA-mediated gut dysbiosis, BM proinflammatory cell activity, and neuroinflammation all play an important role in elevating BP.

The concept of gut microbiota affecting BP is novel and represents a paradigm shift in the hypertension field. However, several important gaps in knowledge remain in support of this hypothesis as outlined in the Table. If proven, this may have great implications for treatment of hypertension with the use of dietary supplements, such as pre- and probiotics, as well as appropriate fecal/bacterial transplantation. Some evidence already exists supporting a beneficial role for Lactobacillus probiotics in BP regulation.

Furthermore, a meta-analysis of 9 randomized trials demonstrated a significant decrease in both systolic and diastolic BP in patients who consumed a daily dose of ≥10¹¹ CFU of probiotics.

In summary, evidence in this review emphasizes that gut microbial composition holds the potential for playing an important role in BP control. This field is in its rudimentary stage, at present, and many important questions, as outlined in the Table, must be addressed before its full clinical and translational potential is recognized. First, a full-scale clinical study must be conducted to confirm dysbiosis in hypertensive patients; second, fecal transplantation studies would be useful in animals to establish the proof of concept of the role of gut microbial dysbiosis in hypertension and resistant hypertension; and finally, metabolic profiles of plasma must be conducted in patients to determine if there are bacterial metabolites unique to resistant hypertension.

### Summary and Future Directions

In this review, we have presented a working hypothesis involving the brain, gut, and BM, whose dysfunctional interactions may be critical in persistent neuroinflammation and key in the development and establishment of hypertension. Of course, the story is just beginning and an extensive amount of research must be undertaken, and critical issues addressed, to further support, modify, or even refute this hypothesis. Some of these issues are as follows: (1) what are the characteristics of extravasated cells into the brain? Are they all activated microglia? How long do they remain in the brain? What is the role of the spleen in the extravasation of myeloid progenitors? Is there an increase in activated microglia in human hypertension? (2) Is there a unique signature of metabolite(s), such as SCFAs, or bacterial DNA in plasma of hypertensive animals or patients that could be considered as a marker for early detection? (3) Extensive studies must be conducted to further
**Table. Summary of Gaps in Knowledge in the Brain–Gut–BM Triangular Interaction Hypothesis**

<table>
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<th>Critical questions for further establishing mechanism and proof of concept</th>
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<tr>
<td>Do changes in SNA to gut and BM precede, accompany, or result in hypertension?</td>
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<tr>
<td>Are altered gut permeability, pathophysiology and dysbiosis universal for animal models of hypertension?</td>
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<tr>
<td>Do gut permeability and dysbiosis initiate hypertension or are they a consequence of hypertension? Does gut-targeted therapy reverse hypertension?</td>
</tr>
<tr>
<td>What is the contribution rate of BM-derived proinflammatory myeloid progenitors vs resident microglia? What is the involvement of spleen?</td>
</tr>
<tr>
<td>What are the molecular/cellular signals in extravasation of peripheral proinflammatory progenitors into the brain leading to persistent neuroinflammation and hypertension?</td>
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Clinical/Translational gaps

- Large-scale clinical trial to establish gut dysbiosis link to high BP and a unique microbial profile with hypertension.
- Evaluate contribution of brain–gut–BM axis on overall hypertension status.
- Attempt to establish metabolite(s) and bacterial signature profile(s) that could be used as biomarker(s) for resistant hypertension.
- Fecal transplantation and anti-inflammatory drugs (e.g., minocycline) in combination with current antihypertensive medication improve BP control.
- What is the relative importance of the brain–gut–BM axis in human hypertension?

BM indicates bone marrow; BP, blood pressure; and SNA, sympathetic nerve activity.

Characterize the autonomic regulation of SNA to the gut and BM. (4) The role of SCFAs, their receptors, and their targets (blood vessels, gut, BM, brain, etc) requires investigation. Answers to these and other related questions entrust optimism toward the development of a novel therapeutic approach for the control of hypertension.

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**Disclosures**

None.

**References**


Brain–Gut–Bone Marrow Axis: Implications for Hypertension and Related Therapeutics
Monica M. Santisteban, Seungbum Kim, Carl J. Pepine and Mohan K. Raizada

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