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REVIEW

Gut Microbiome and Neuroinflammation in Hypertension

Elaine M. Richards, Jing Li, Bruce R. Stevens, Carl J. Pepine, Mohan K. Raizada

ABSTRACT: Hypertension is a worldwide problem with major impacts on health including morbidity and mortality, as well as consumption of health care resources. Nearly 50% of American adults have high blood pressure, and this rate is rising. Even with multiple antihypertensive drugs and aggressive lifestyle modifications, blood pressure is inadequately controlled in about 1 of 5 hypertensive individuals. This review highlights a hypothesis for hypertension that suggests alternative mechanisms for blood pressure elevation and maintenance. A better understanding of these mechanisms could open avenues for more successful treatments. The hypothesis accounts for recent understandings of the involvement of gut physiology, gut microbiota, and neuroinflammation in hypertension. It includes bidirectional communication between gut microbiota and gut epithelium in the gut-brain axis that is involved in regulation of autonomic nervous system activity and blood pressure control. Dysfunction of this gut-brain axis, including dysbiosis of gut microbiota, gut epithelial dysfunction, and deranged input to the brain, contributes to hypertension via inflammatory mediators, metabolites, bacteria in the circulation, afferent information alterations, etc resulting in neuroinflammation and unbalanced autonomic nervous system activity that elevates blood pressure. This in turn negatively affects gut function and its microbiota exacerbating the problem. We focus this review on the gut-brain axis hypothesis for hypertension and possible contribution to racial disparities in hypertension. A novel idea, that immunoglobulin A-coated bacteria originating in the gut with access to the brain could be involved in hypertension, is raised. Finally, minocycline, with its anti-inflammatory and antimicrobial properties, is evaluated as a potential antihypertensive drug acting on this axis.

Key Words: blood pressure ■ dysbiosis ■ communication ■ epithelium ■ hypertension

Hypertension is the leading modifiable risk factor for cardiovascular disease and related morbidity and mortality, worldwide. Over the past 30 years the number of adults with hypertension has almost doubled and accounts for ≈8 million deaths each year worldwide.[1] Racial and ethnic disparities, sedentary lifestyles and diets with high fat and low fiber content, have all contributed to this ever-rising prevalence of hypertension. More troubling, rates of blood pressure (BP) control are stagnant, if not declining, in the United States.[2] Despite recent advances in use of a combination of antihypertensives, titration to the most effective doses for individual patients, and assertive guidance for lifestyle changes, successful control and treatment of hypertension remains an art rather than an evidence-based practice. This situation is further complicated by evidence that about one in 5 of all hypertension patients do not achieve BP goals even when treated with ≥3 antihypertensive medications, thus have treatment-resistant hypertension.

Therefore, there is a compelling need for the development of innovative mechanism-based concepts that would provide novel targets for hypertension treatment. Involvement of the gut and its microbiota in BP homeostasis and their dysfunction in hypertension have been important discoveries in the field and could offer one such innovation. Rapid progress in the past 6 to 7 years implicating an impaired gut-brain axis in the pathophysiology of hypertension and validation of this basic concept by multiple investigators emphasizes the implications for new therapeutic targets. Therefore, our objective in this review is to present succinct summaries of involvement of the gut and its microbiota in hypertension, particularly human hypertension. We will also address racial/ethnic changes in microbiota and the role of gut

Correspondence to: Mohan K. Raizada, PhD, Department of Physiology and Functional Genomics, University of Florida College of Medicine, PO Box 100274, Gainesville, Florida 32610. Email mraizada@ufl.edu
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Nonstandard Abbreviations and Acronyms

5-HT	5-hydroxytryptamine
AMP	antimicrobial peptide
Ang II	Angiotensin II
ARIC	Atherosclerosis Risk in Communities
BBB	blood brain barrier
BP	blood pressure
CMT-3	chemically modified tetracycline 3
IBS	irritable bowel syndrome
IgA	Immunoglobulin A
IL-1β	interleukin 1β
MS	multiple sclerosis
PVN	paraventricular nucleus of the hypothalamus
SCFA	short chain fatty acid
SHR	spontaneously hypertensive rat
TNF-α	tumor necrosis factor α
WA:	non-Hispanic White American

epithelium-microbiota communication in hypertension. We also discuss several new concepts in the hypertension field, that while still in their infancy, may guide future research and treatment efforts. Our overall approach is to synthesize available literature into critical issues that we believe important in translating the mechanism-based knowledge in this field to development of new strategies for hypertension management.

INVOLVEMENT OF THE GASTROINTESTINAL TRACT

The involvement of the gastrointestinal tract tract in hypertension should have been realized perhaps sooner considering that long-known risk factors for hypertension such as low-fiber diet, high-salt diet, alcohol consumption, and constipation (in older women) directly impact the gastrointestinal tract and its microbiota. Nevertheless, this is now an area of active research and has the potential to offer key insights for hypertension mitigation.

The gastrointestinal tract is able to digest and absorb organic nutrients, electrolytes and water necessary for health of the host while maintaining a tight barrier between the outside world of its lumen and the host. It accomplishes this by several means. A mucus layer, involving goblet cells, separates the contents of the gut lumen from the gut epithelium. Few members of the microbiota penetrate or live in this layer under healthy conditions. The epithelial cells form a barrier with tight junctions and other mechanisms and secrete antimicrobial factors to further protect the host. The gut has an immune system that actively surveilles the luminal contents and epithelium and initiates immune responses against pathogens, while maintaining tolerance to the core commensal microbiome of the host that is central to host health by a variety of mechanisms such as vitamin production, bile salt cycling, short chain fatty acid synthesis etc. Meanwhile, it has a complex array of transporters that move nutrients etc into the host.

The earliest studies of the gut in hypertension were performed in hypertensive rats and described dysbiosis in the gut microbiome that was confirmed in hypertensive people.[3,4] In rats, this was associated with gut pathology including changes in blood flow indicative of inflammation,[5] increased gut stiffness,[6] increased fibrosis in the muscular layers of the gut, decreased goblet cells, increased norepinephrine containing fibers in the mucosal layer, decreased villus length in the small intestine, changes in the proteins involved in sealing the gut barrier and altered sympathetic flow to the gut. The leaky gut barrier in Ang II (angiotensin II)–induced hypertensive mice could be improved with butyrate supplementation.[7] Studies in hypertensive rats suggest that the gut epithelium has altered immune function. Intestinal epithelial cells act as antigen presenting cells, transcriptomic analyses suggest decreased presentation in hypertension, likewise T-cell receptor-mediated responses were decreased.[8,9] Furthermore, these transcriptional changes are maintained in colon organoid cultures made from hypertensive rats.[9] These data suggest gut pathology is present in hypertension and could contribute to initiation and or maintenance of hypertension by the interruption in barrier function (mucus and epithelial cells involvement), altered immune responses, altered sympathetic input, blood flow, and stiffness.

ALTERED GUT MICROBIOTA IN ANIMAL MODELS OF HYPERTENSION

There is general agreement for an altered gut microbiota in many animal models of hypertension including the spontaneously hypertensive rat,[3] stroke-prone spontaneously hypertensive rats,[10] Ang II–induced hypertensive rats and mice,[3,5,7] Dahl-salt sensitive rats,[11] mice made hypertensive by high-salt diet,[12] deoxycorticosterone acetate-salt induced hypertensive rats,[13] obstructive sleep apnea-induced hypertensive rats and mice,[14,15] and mice made hypertensive by transfer of fecal matter from hypertensive humans.[4] In most cases where it has been tried fecal matter transfer from these animals causes hypertension in the normotensive recipient, except for the Dahl-salt sensitive and resistant rat strains that responded paradoxically.[11]

Mice hypertensive due to a high-salt diet[16] have specific depletion of *Lactobacillus murinus*, and supplementation with this bacterium prevented hypertension through mechanisms related to Th17-mediated immunity. As the name implies, these bacteria are found only in the gut of mice. Rodents have a stratified, squamous

epithelia in their gut that allows specific adhesion of lactobacilli in biofilms. Similar colonization of the gut by lactobacilli is not observed in humans, though it is in oral cavity, etc, where this type of epithelium is found.[17] Depletion of lactobacilli in the gut microbiota in some people on high-salt diets has been observed.[16] But there is debate about whether lactobacilli are normal residents of the human gut or occur there from diet or the use of proton pump inhibitors, that allow these acid-intolerant bacteria to descend lower into the gastrointestinal tract than usual.

It would be useful if studies revealed a specific bacterium that was either overabundant or depleted in hypertension so that tailored therapy could be performed to correct the imbalance. However, in animal models and humans with hypertension, specific bacteria were not usually associated with hypertension, rather the function of the entire gut microbiota was altered. For example, dysbiotic microbiota of hypertension are depleted of short chain fatty acid-producing bacteria, but since many bacteria have this function and different ones populate individuals' microbiomes, specifically depleted or overabundant bacteria species or strains were not observed in hypertension.[1–3,5,8–14]

Our studies, and those of others[4,18] showed that functions of the gut microbiota are more different in hypertensive and normotensive animals and people than the bacteria present.[7] This suggests that the gut environment in hypertension either promotes or permits shifts in what the microbiota does and how the bacterial communities interact. Most available information about gut microbiota function relates to metabolism; hypertension has been associated with increases in microbial membrane transport, lipopolysaccharide biosynthesis and steroid degradation, but decreases in vitamin synthesis, amino acid, and co-factor metabolism.[18] This information is cost prohibitive sometimes, requiring whole genome (shotgun) sequencing rather than the cheaper 16S rRNA sequencing. However, as the cost of sequencing continues to decline, combined with recognition of their value, these data and their inferences should become more widely available.

Metabolic function of the gut microbiota can also be assessed by measurement of circulating metabolites. This is an emerging field that is restricted by the lack of databases attributing metabolites origins to the gut microbiota and the many unidentified metabolites that are measured by the techniques. Metabolites of the gut microbiota, the result of its digestion of multiple energy sources, nonetheless are important in hypertension.[4,19–22] Gut microbiota-dependent metabolites were altered in hypertension induced by Ang II in mice. Some metabolites were altered in a fashion suggesting they were host-protective and produced by the gut microbiota in response to hypertension, while others were potentially harmful.[23]

BRAIN, NEUROINFLAMMATION, AND AUTONOMIC NERVOUS SYSTEM IN HYPERTENSION

The autonomic nervous system controls BP via sympathetic and parasympathetic outflow. Signals are integrated in the brain in the central autonomic network[24] to affect autonomic nervous system activity and, therefore, BP. Neuroinflammation is particularly apparent in the hypothalamic paraventricular nucleus (PVN) of hypertensive rodents,[23,24] part of the central autonomic network that houses preautonomic neurons. But it is also seen in brain stem areas of the central autonomic network, for example, in the nucleus of the solitary tract and rostroventrolateral medulla in animal models of hypertension. The preautonomic neurons in the PVN receive input from multiple higher brain areas, including areas outside of the blood brain barrier. Thus, they are responsive to circulating factors, as well as afferent inputs via, for example, the nucleus of the solitary tract from organs including the gastrointestinal tract. Information is integrated in the PVN and passed to brain stem nuclei housing autonomic neurons. Functional magnetic resonance imaging is now being used to confirm components of the central autonomic network in humans that previously were only understood through lesion and autopsy information. Brain areas previously not thought to be involved in BP regulation are also being discovered with functional magnetic resonance imaging, particularly those related to, for example, stress-related BP reactivity.[27,28] It will be interesting to understand if neuroinflammation is also present in these brain areas in human hypertension. This is an area of research that could be highly relevant but is poorly explored to date.

Increasing evidence supports the concept that neuroinflammation plays an important role in hypertension by a multifaceted mechanism.[29–36] Enhanced sympathetic nervous system activity is one such facet. Evidence supporting this include: neuroinflammation is intimately linked with elevated sympathetic drive in animal models of hypertension.[32,37,38] Additionally, increased sympathetic drive is implicated in human hypertension.[39–43] Central administration of proinflammatory cytokines (eg, IL1-β [interleukin 1β] and TNF [tumor necrosis factor]-α) increases sympathetic nerve activity and BP in animal models.[25,44–46] Furthermore, increased expression of proinflammatory cytokines in cardio-regulatory brain regions has been noted in hypertensive animals.[25,47,48] In contrast, administration of the anti-inflammatory cytokine, IL-10, or minocycline exerts an antihypertensive response.[25] Also, central IL-10 overexpression reduces TNF-α, IL-1β in the PVN and attenuates hypothalamic inflammation and sympathoexcitation in a rat heart failure model.[25,49]

Disruption of the blood brain barrier (BBB) is another aspect that contributes to neuroinflammation. This allows access of peripheral inflammatory molecules and signals

to autonomic brain regions. The integrity of the BBB is tightly regulated by interplay among the participants of the neurovascular unit involving neurons, astrocytes, pericytes, endothelial, and microglial cells. Hypertensive stimuli such as Ang II, are known to interrupt neurovascular unit integrity to increase BBB permeability in hypertension.[50–52] For example, gut dysbiosis and a dysregulated gut epithelial-microbiota-mediated generation of bacterial and other gut metabolites in hypertension would have free access to the brain to initiate neuroinflammation. This view is supported by evidence that bacterial products (ie, tryptophan metabolites, lipopolysaccharides, cytokines, butyrate, etc) that are altered in hypertension, are also known to influence neuroinflammation, thus, affecting the glial-neuronal functional unit.

Microglia activation is an integral part of neuroinflammation in hypertension. Hypertensive stimuli directly, or indirectly via microbiota-influenced metabolites and a leaky BBB, activate microglia to impair neuronal-microglial communication. In fact, prolonged microglial activation results in their destruction of astrocytic end feet, an important component of the BBB, thus exacerbating this process.[53] Any brain insult activates microglia to the M1 state, M1-activated microglia release proinflammatory cytokines and many cytotoxic substances such as quinolinic acid and glutamate. Thus, the M1 microglial phenotype is responsible for creating an inflammatory milieu.[36] This phenotype consequently shifts to M2 anti-inflammatory phase, which is responsible for directing tissue repair, extracellular matrix formation, debris clearance to return to normal homeostatic balance following insults. We propose that the transition of M1 to M2 activation state of microglia is disrupted creating a persistent inflammatory environment in hypertension and would be an important line of further investigation. A similar transition has been reported for other neural diseases including Alzheimer disease, multiple sclerosis (MS), and encephalomyelitis.[54–56] The concept of involvement of activated microglia in hypertension has been gaining strength in recent years, and our studies were among the first to provide experimental support.[25] They showed that induction of hypertension by chronic Ang II infusion resulted in an increase in activated microglia predominantly in the PVN, a finding now supported by others.[57] Inhibition of microglia activation by central minocycline infusion attenuated hypertension.[25] This antihypertensive effect of minocycline is unlikely to be due to its antibiotic activity since central administration of CMT-3 (chemically modified tetracycline 3), an anti-inflammatory tetracycline derivative devoid of antimicrobial activity, also ameliorates hypertension.[33] Finally, depletion of microglia attenuates hypertension.[58] Despite this strong evidence in animal models, the role of microglia in human hypertension is only now evolving. Our recent pilot trial suggested that treatment with minocycline results in a significant decrease in BP in treatment-resistant hypertension patients. This appears to also decrease activated microglia in brain sympathetic region in positron emission tomography scans.[59] This needs to be confirmed by a larger double-blind randomized study.

Finally, it will be important to investigate whether neuroinflammatory pathways are causative or consequential in hypertension. The decrease in BP and attenuation of hypertension by anti-inflammatory drugs such as minocycline and CMT-3, the microglia depletion experiment and the increase in BP by intracerebroventricular administration of inflammatory cytokines all support a causative relationship. Additional study is warranted to solidify the concept.

HYPOTHESIS: GUT-BRAIN AXIS DYSFUNCTION CAUSES HYPERTENSION

The previous sections of this review highlighted strong evidence for roles of the gut, its microbiota and neuroinflammation in hypertension. These studies led us to propose a gut-brain bidirectional communication hypothesis for BP control and impaired interactions within this axis in hypertension and is presented in Figures 1 and 2. Hypertensive stimuli, such as stress, diet, salt, maternal factors, environmental toxins, etc, influence autonomic brain regions to affect neural, endocrine, and immune pathways. During hypertension, the neural pathway exhibits increased sympathetic and attenuated parasympathetic activities. The prohypertensive endocrine pathway primarily results from activation of the hypothalamic-pituitary-adrenal axis while the immune pathway is disordered interplay of the complex interactions of bone marrow, gut, spleen, and other immune regulatory organs. These pathways can act rapidly to activate blood vessel tone and contraction of vascular smooth muscle increasing peripheral resistance and BP. Increased neural activity could also trigger neuroinflammatory events that activate microglia, increase proinflammatory molecules, and create an inflammatory milieu in autonomic brain regions, primarily in the hypothalamic paraventricular region.

Hypertensive stimuli, either directly or via their effects on the brain or in a combination of the two, have profound effects on the gut causing pathophysiology associated with development and establishment of hypertension. Key changes in the gut include microbial dysbiosis, impaired epithelial gene expression, wall pathology, and inflammation. These changes are the basis of a dysfunctional gut-microbiota communication that consequently leads to altered gut motility and increased permeability. These events alter metabolites of host, bacteria, and other organisms in the microbiota, immune-modulating cells and molecules in the circulation where they can negatively affect neural activity, peripheral inflammation and other cardiovascular-relevant organs to contribute to the establishment of hypertension.

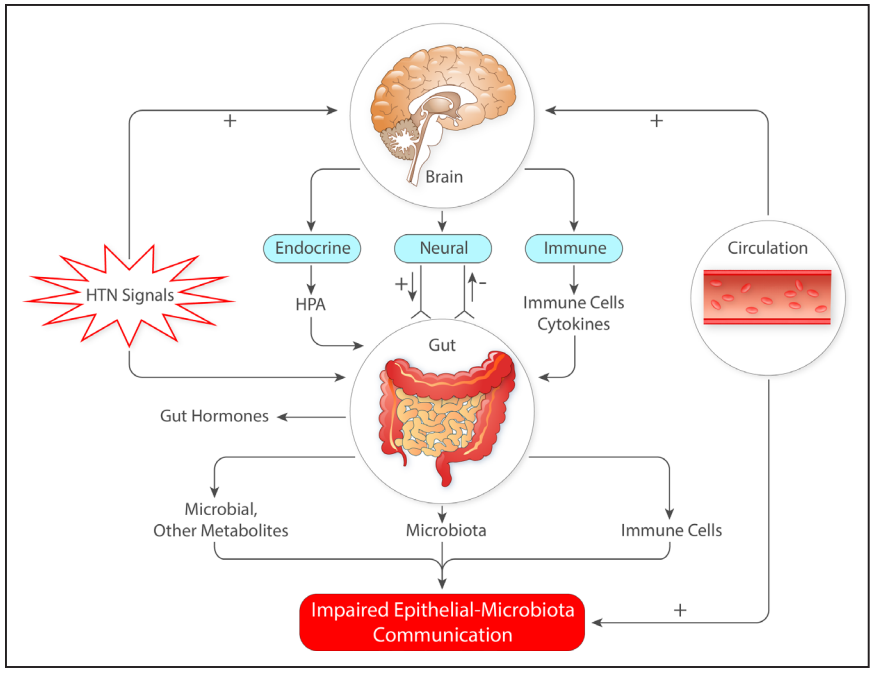

Figure 1. Bidirectional gut-brain axis in hypertension (HTN).
HTN stimuli influence both autonomic brain regions and the gut in the regulation of blood pressure (BP). They stimulate brain endocrine, neural, and immune system associated pathways which converge on to the gut to regulate secretion of gut hormones, microbial communities, metabolites, and gut immune status. These culminate in impaired epithelial-microbiota communication in HTN. Furthermore, HTN-associated dysbiosis-linked altered gut metabolites are released into the blood through leaky gut, access the brain through leaky blood brain barrier (BBB), and influence neuronal-microglial communication increasing neuroinflammation. HTN stimuli can also influence the gut directly to initiate alteration in epithelial-microbiota interactions. Illustration Credit: Ben Smith.

The bidirectional aspect of the hypothesis proposes a coordinated communication between the gut and the brain following hypertensive stimuli in perpetuation of hypertension. For example, metabolic products circulating as a result of dysbiosis and impaired gut-microbiota interaction, could influence neuronal activity, neuroinflammation, heart, kidney, and blood vessels as well as autonomic nervous system-driven results of hypertensive stimuli.

GUT MICROBIAL DYSBIOSIS AND GUT-BRAIN AXIS DYSFUNCTION IN HUMAN HYPERTENSION

Normotensive Versus Hypertensive

Since the first suggestion of a role for gut microbial dysbiosis in hypertension,[3] numerous studies have supported and expanded upon its role and were reviewed recently.[60] Evidence was initially correlative, such as studies of exercise that both decreases BP and alters the gut microbiome[61] but a secure footing developed when it became clear that transfer of dysbiotic gut microbiota also transferred the hypertensive phenotype.[4] This was found to be true when transfer was from human hypertensive subjects to animals and animal to animal. Transfer of a healthy microbiome to hypertensive animals decreases BP and attenuates many detrimental outcomes of hypertension, (eg, neuroinflammation, increased sympathetic activity, vascular dysfunction, etc).[62,63]

One study shows that fecal matter transfer from healthy donors lowers BP in hypertensive subjects by either nasogastric tube or lower gastrointestinal tract administration.[64] This is now being tested in a pilot study as a multicenter, randomized, double-blinded, placebo-controlled clinical trial (URL: https://www.clinicaltrials.gov; Unique identifier: NCT04406129). These findings suggest that gut microbial dysbiosis is a key feature of hypertension and open possibilities for more widespread exploitation to treat hypertension.

Dysbiosis

What is the difference between the microbiome of normotensive individuals and those with hypertension? One common difference is the depletion of short chain fatty acid-producing bacteria, such as *Eubacterium rectale*[7] *Roseburia* and *Ruminococcaceae*,[4,18,65,66] in gut microbiota of hypertensive subjects as compared with that of normotensive subjects and reduction of bacterial genes for butyrate production in hypertension.[67] Interestingly, lower fecal content of short chain fatty acid (SCFA) is associated with lower BP.[66]

SCFA are important in maintaining the health of gut epithelial cells, in turn maintaining the gut barrier against the outside world of the gut contents. Gut epithelial cells use SCFAs as an energy source, consuming most of the SCFAs, especially butyrate, produced by the gut microbiota. Those SCFA that reach the circulation have receptors located in organs important for the maintenance of BP homeostasis, including the kidney, sympathetic nervous system, and blood vessels[19–21,68] and activating these receptors affects BP. Supplementation with SCFAs, either directly or via a high-fiber diet (the raw material for gut microbial production of SCFAs) decreases BP.[69–72] SCFAs such as

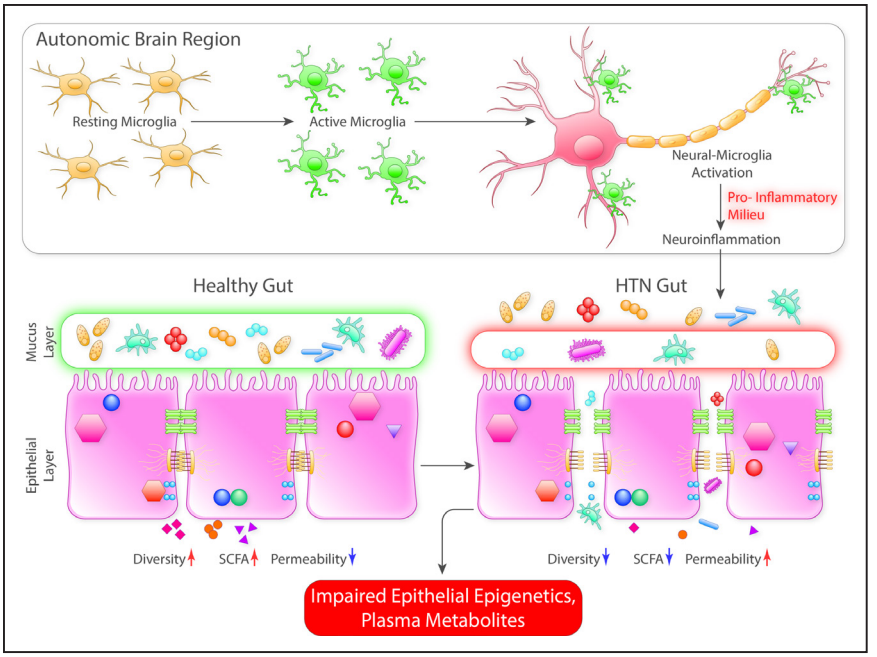

Figure 2. Salient steps associated with a dysfunctional gut-brain axis in hypertension.
In the autonomic brain regions, primarily in the hypothalamic paraventricular nucleus, hypertension stimuli initiate a cascade of signaling events that include activation of resident microglia, altered microglia-neurons interactions that results in an increase in proinflammatory milieu. Establishment of neuroinflammation would be associated with impaired hormonal, neural and immune influences impacting gut pathology and functions. This would include dysbiosis, reduction in mucin and mucus layer, increased gut wall permeability, pathology and decrease in short chain fatty acid (SCFA). This would result in a proinflammatory plasma profile that would directly or indirectly influence autonomic brain areas to perpetuate hypertensive responses. Illustration Credit: Ben Smith.

acetate act as histone acetylases, and thereby modulate gene expression important for BP control[73,74] via inhibition of histone deacetylase activity and expression. SCFA are also neuroprotective,[75,76] reducing lipopolysaccharides-induced neuroinflammation.[77] They decrease gut inflammation by reducing inflammatory T cells that home to the gut.[78] Finally, butyrate signals the brain via the vagal nerve to affect BP.[79]

Another difference between microbiota of normotensive and hypertensive subjects is the increased potential for lipopolysaccharides production.[4,18] *Veillonellae* are examples of lipopolysaccharides-producing bacteria that are enriched in human hypertensives' gut microbiota.[80] This bacterium also impacts biofilm formation. Biofilms encase communities of bacteria in bacteria-produced extracellular matrix, increasing their adherence and allowing the community to create an environment more suited to their growth, and where they are protected from outside influences such as antibiotics. Lipopolysaccharides-producing bacteria in biofilms are more inflammatory than gram negative bacteria without the protection of a biofilm.[81,82] This suggests that the hypertensive microbiota is more inflammatory both systemically and since lipopolysaccharides reaches the brain, centrally, contributing to the potential for autonomic nervous system activation and increased BP.

Depletion of lactobacilli from the gut microbiota of both humans and animals with high-salt diet–induced hypertension has been reported.[16,83,84] Although this was not confirmed in other studies.[66] Lactobacilli are not detected in the gut microbiota of all healthy individuals, thus depletion of lactobacilli cannot be the common pathway of high-salt diet dependent-hypertension.[85,86] But, lactobacilli were shown to be associated with the lowest quartile of ambulatory aortic stiffness index, generally considered to be indicative of lower BP.[87] Taken together, although there is consensus for the bacterial communities involved in biofilm formation, SCFA production and lipopolysaccharides in hypertension, characterization of specific bacterial genera, and species remains to be established.

Few studies have examined functions associated with the gut microbiome as permitted by whole genome sequencing. Our studies showed that examination of function distinguished hypertensive from normotensive gut microbiomes better than microbial composition.[7] Others using similar methods showed enrichment of membrane transport, lipopolysaccharides biosynthesis and steroid degradation in the gut microbiota of hypertensive subjects.[4,18] Along with these increases, there were significant decreases in essential functions associated with health, including branch-chain amino acid biosynthesis and transport, ketone body biosynthesis, 2 component regulatory system, degradation of methionine and purine,[4,60] and Vitamin D3 production.[88] These data suggest that functions of the gut microbiota community may be more important in determining hypertensive status than bacterial composition.

HYPERTENSION IN BLACKS AND GUT MICROBIOTA

The prevalence of hypertension in Black non-Hispanic Americans remains significantly higher (56%) than non-Hispanic White (WA, 47%), non-Hispanic Asian (45%) and Hispanic (44%) Americans.[89] Black non-Hispanic Americans develop high BP at an earlier age, and the resulting hypertension tends to be more severe and

resistant to treatment. Treatment-resistant hypertension has been defined as BP above the goal of ≥130/90 mm Hg, despite lifestyle modifications and adequate doses of ≥3 antihypertensive drugs, including a diuretic.[90] In addition, Black patients are more likely to manifest nondipping BP, increased prevalence of left ventricular hypertrophy, and increased stroke risk. As a result, ≈50% Black non-Hispanic Americans hypertensives have uncontrolled hypertension.[91] Thus, Black non-Hispanic Americans accumulate significantly greater burdens from cardiovascular disease, end-stage renal disease, and stroke leading to higher morbidity and mortality.[91]

Genetics, obesity, higher salt sensitivity, low-plasma renin, abnormal vascular function, and environmental factors (diet, sedentation, stresses related to racial inequality and poverty, exposure to particulate matter air pollution) are all important contributors to this disparity. For example, a study of 30 000 participants followed for 9 years showed that consumption of a typical southern diet that includes fried food, organ, and processed meats was the greatest mediator of differences in hypertension incidence between Black and WA subjects.[92] The Dietary Approach to Stop Hypertension diet, rich in fruits, vegetables, whole grains, and low fat, lowers BP more effectively in Black compared with WA.[93] Additionally, the Jackson Heart Study, a large ongoing study, suggests genetic and environmental risk factors disproportionally burden Black with cardiovascular disease and hypertension. Its principal observations, with those of the Barbershop cluster-randomized trial, suggest that public health efforts to increase awareness and treatment help control hypertension in Black non-Hispanic Americans.[94,95] None the less, because of the multifactorial influences on BP and difficulties adapting currently available therapies to this group of patients, challenges to successful control and management of hypertension in Black non-Hispanic Americans remain. Interestingly, recent advances in metabolomics and metagenomics coupled with evidence of gut dysbiosis in hypertension begs the question: Is gut microbiota and its communication with the gut epithelium the Holy Grail in delineating ethnic disparities in Black Americans? Below, we summarize current knowledge on microbiota and hypertension in Black patients and provide our perspective of its clinical implications.

The initial indication of the involvement of the gut and its microbiota was derived from studies of Zheng et al who determined metabolomic profiles in a subsample of randomly selected Black participants in ARIC (Atherosclerosis Risk in Communities) study. The key observation was that each increment of 1 SD from baseline in 4-hydroxyhippurate, a product of the gut microbiota and host metabolism, was associated with 18% higher risk of hypertension. Our small cohort study that combined metabolomics and microbiota analyses showed that HBP in Black patients was associated with higher oxidative stress, insulin resistance, and gut ischemia.[96] Furthermore, multivariate analyses reveal significant differences in gut bacterial populations of Black versus WA hypertensives (Figure 3). Chen et al[97,98] tested the concept that reduction in BP due to dietary sodium reduction was associated with beneficial effects on SCFAs that are primarily products of gut microbiota. The basis for this prediction was evidence that salt exerts significant effects on gut microbiota, and there is a greater salt sensitivity and impaired salt handling by Black hypertension patients.[16,91] The randomized, double blind, placebo-controlled cross-over trial with 145 participants (42% Black) showed that sodium reduction increased 8 SCFAs including butyrate and isobutyrate.

EPITHELIA-MICROBIOTA COMMUNICATION IN HYPERTENSION

Intestinal epithelium is paramount in regulation of overall body homeostasis involving the gut-brain axis. Normal epithelial cell function confers protection of vital organs from harmful effects by maintaining gut barrier properties and sequestering adverse molecules, microbial metabolites, immune cells, and inflammatory molecules. Thus, any structural or functional compromise of epithelial cells leads to loss of the epithelial seal of the gut wall and increased leakiness. Indeed, increased gut leakiness has been associated with pathogenesis of chronic diseases such as Parkinson and Alzheimer diseases, obesity, inflammatory bowel disease (IBD), other gut inflammatory conditions, and even aging.[99–102] Parts of the gut microbial community are in intimate contact with the gut thus, ideally positioned to exert epigenetic influences on epithelial cells.[103] This gut microbiota communication is proposed to be bidirectional. For example, multiple signaling and metabolic pathways from microbiota have been shown to influence epithelial genes and functions. Microbial metabolites such as SCFA and catabolic products of tryptophan are 2 established candidates in microbiota's repertoire that effect epithelial cells. SCFA influence tight junction proteins expression, inhibit histone deacetylase, and immune signaling-relevant genes affecting epithelial cell turnover, growth and cellular functions[104] while tryptophan metabolites effect anti-inflammatory and protective barrier functions. Regulation of mucin secretion and production of AMP (antimicrobial peptides) are additional means of microbial communication that involve pattern recognition receptor/MYD88.[105] Another signal transmission component is the inflammasome. These are multimeric, enzymatically active protein complexes assembled in the cytosol following perception of bacterial-derived molecular patterns and activate inflammatory cytokines within innate immune cells. Furthermore, a diverse, healthy microbiota provides signals for activation of the NLRP6 (NLR Family Pyrin Domain Containing 6) inflammasome class, a process likely to be impaired with dysbiosis, thus initiating a cascade of signals decreasing production of AMPs and promoting

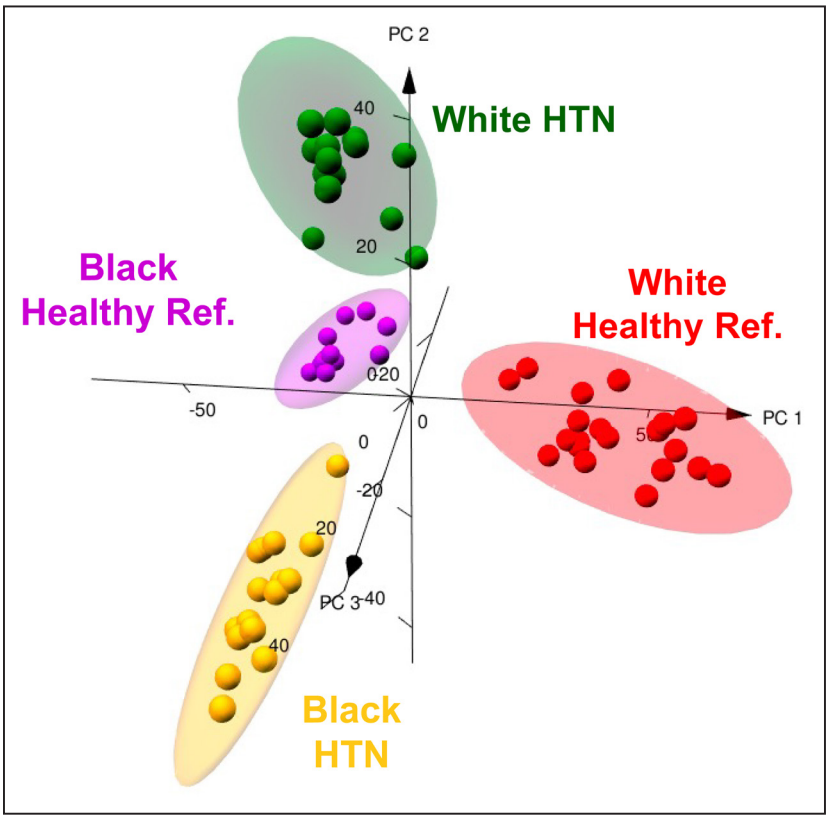

Figure 3. Principle component analysis of gut microbiome shows distinct clustering of gene differences among Black and White American (WA) reference and hypertensive subjects. Three-dimentional PCA is shown with 95% ellipsoids based on centered log ratio transformed de-noised prevalence data from shotgun sequencing (1817 total genes pooled from all 63 patients) obtained by machine learning. Each point represents an individual subject. Mean blood pressure (BPs) were WA-REF=121/73 mm Hg, WA-hypertension (HTN)=149/79 mm Hg, Black-REF=125/77 mm Hg, and Black-HTN=151/85 mm Hg.

inflammation.[106] Finally, microbiota is important source of polyamines (spermine, putrescine, histamine, etc) whose anti-inflammatory activity is counteracted by other microbial products such as taurine.[107] Taken together, these observations form the basis for the concept of microbiota-driven influences on gut epithelial functions.

There is limited but important evidence for epithelium-initiated gut to microbiota communication. Epithelial cells are among the first to receive environmental, physical, and neural cues with drastic effects on gut microbial homeostasis. One such effector is 5-hydroxytryptamine (5-HT), ≈95% of which is housed in the gut. Predominantly produced by enterochromaffin cells in the gut epithelium, 5-HT is an important regulator of enteric nervous system, gut motility, and inflammation primarily by dampening gut vagal afferents.[108] The fact that altered levels of this biogenic amine are found in many gut inflammatory diseases involving dysbiosis has led to the suggestion that 5-HT could have a direct influence on microbial communities.[109] This is supported by evidence that tryptophan hydroxylase-deficient mice with significantly reduced gut 5-HT demonstrate altered gut microbiota and reduced severity of colitis.[110] In addition, 5-HT is shown to inhibit certain and stimulate other commensal bacterial communities.[111]

Finally, the role of microRNAs in epithelial-microbiota cross talk is a rapidly emerging area of investigation. The following findings support this view: (1) miRNAs enter bacteria and co-localize with bacterial nucleic acid suggesting their influence on microbiota[112]; (2) miRNA profiles in epithelial cells were demonstrated to be related to microbial status[113]; (3) human miRNAs such as miR-515-5p and miR-1226-5p were shown to induce changes in 16S RNA transcripts when introduced into *F. nucleatum* and *E. coli*[112]; and (4) fecal miRNA transplantation induced significant microbial changes.[112] miR-21 aggravates DSS-induced colitis by effects on gut microbiota[114] and differentially expressed miRNAs (miR-199a, miR-1226, miR-548a, and miR-515-5p) affect growth of *F. nucleatum* and *E. coli* that are linked to increased IBD.[114]

Is there evidence of an impaired bidirectional epithelial-microbiota communication in hypertension? Emerging data supports this contention: (1) Gut leakiness had been observed in both animal models and in human hypertension establishing an important clue for dysfunctional sequalae. This is associated with altered gene expression for tight junction proteins.[5,7,29,66] (2) Consequences of leaky gut are increased translocation of certain metabolites, toxins, and IgA-coated bacteria into the systemic circulation with profound adverse outcomes on vascular, inflammatory and neural mechanisms. Decreased bacterial communities associated with butyrate and subsequent effects on epithelium, plasma butyrate, and BP are well-established.[3,7,78] Increased lipopolysaccharides and a positive correlation between plasma levels and hypertension has been found.[7,66] Altered tryptophan metabolites including 5-HT have been implicated in high BP and chronic kidney disease although detailed mechanisms remain elusive.[96,111,115] (3) Transcriptomics of colon and colon organoids are beginning to shed light on hypertension-linked epigenetic changes in the epithelium and

identification of candidate genes with potential to interact/influence microbiota. Our data show that colonic epithelium from the spontaneously hypertensive rat (SHR) exhibits a distinct gene expression profile compared with its normotensive controls.[8,9] For example, genes associated with T-cell receptor signaling and those involved in immunity, barrier function, and dysbiosis were decreased implicating, for the first time, their potential role in altered bacterial communities in hypertension. We next used colon organoid cultures to set the stage for identification of epithelial genes interacting with microbiota.[9] We found significant decreases in genes relevant to antigen presentation, immune response, virus defense response pathways in the SHR.[9] This observation led us to suggest that decreased expression of genes of these pathways likely compromises mounting immune and other defense responses, altered epithelial cell proliferation, differentiation and metabolism in the SHR.[9] We performed correlation analyses to investigate associations between gene expression and abundance of gut bacterial communities relevant to hypertension in the SHR. We observed that enrichment of *Allobaculum*, *Bifdobacterium*, *Blautia*, and *Gordonibacter* in Wistar Kyoto was significantly correlated with increased expression of most gene sets such as antigen presentation (RT.Ba, RT.Bb), immune response (Cd69, Ccl20) and cell junction organization (Cldn10, Plec) in gut epithelium compared with SHR (Figure 4; correlation coefficients (r) cutoff value is 0.81, $P<0.05$). Interestingly, *Bifdobacterium*, *Allobaculum*, and *Blautia* are butyrate-producing bacteria whose populations are depleted in hypertension and this is consistent with evidence of decreased hypertension-associated plasma butyrate, butyrate-producing bacterial communities, and alleviation of hypertension pathophysiology by butyrate treatment.[3,7,116,117] In contrast, the enrichment of *Turicibacter* could be linked to downregulation of antigen presentation genes in the SHR. Collectively, such correlation analysis points out the potential of bioinformatics in combination with metagenomics of organoids in identification of key targets in epithelial-microbiota crosstalk (Figure 4). In conclusion, although limited data support the view of altered epithelial-microbiota communication in hypertension, much more investigation is needed to delineate details.

GUT MICROBIOTA-IMMUNOGLOBULIN A TRAFFICKING: EVOLVING CONCEPT FOR NEURO-INFLAMMATION IN HYPERTENSION

Immunoglobulin A (IgA) is emerging as an important molecule in dynamic gut microbiota communication and regulation of neuroinflammatory signaling in diseases involving dysfunction in the gut-brain axis. IgA isotype antibody-producing cells are B lymphocytes that originate from hematopoietic stem cells in bone marrow. Immature B cells leave bone marrow and migrate to lymphoid organs such as spleen, lymph nodes, and gut-associated lymphoid tissues where they are activated and differentiate into plasma cells in response to antigens. These plasma cells reside in the lamina propria of the gut where they produce large quantities of antibodies in response to the gut environment including bacterial communities.[118,119] Secretory IgA is generated basolaterally, transported across epithelial cells, and released into the lumen where it is in an ideal position to interact with bacteria (Figure 5). IgA and IgA$^+$ plasma cells circulate in the blood and can target relevant organs such as lungs, kidneys, blood vessels, and brain. Thus, plasma cells and IgA are important in regulating gut mucosal immunity. IgA's binding to specific gut bacterial taxa, termed IgA coating serves multiple functions. This includes providing protection against pathogens and toxins, facilitating host colonization with beneficial bacteria and altering bacterial and viral adhesion to epithelial cells influencing epithelial-microbiota communication.[120,121] Recent studies combining bacterial flow cytometry with high throughput 16S sequencing have led to the characterization of bacterial taxa coated by IgA.[122–126] They are diverse with four major phyla (actinobacteria, bacteroidetes, firmicutes, and proteobacteria) suggesting that this diversity in IgA-coating may be important in defining immunopathology of gut-linked diseases.

Studies of Rojas et al[127] were among the first to demonstrate the role of IgA$^+$ plasma cells and dysbiosis in experimental autoimmune encephalomyelitis and in MS. They demonstrated that some plasma cells in the brain of mice with experimental autoimmune encephalomyelitis originate from the gut and produced high levels of IgA. A significant decrease in IgA$^+$ plasma cells in the gut of mice and reduced IgA-coated fecal bacteria was observed and further depletion exacerbated disease. This suggested their involvement in microbial dysbiosis and brain inflammatory processes.[127] Interestingly, the study also showed that patients with MS exhibited a reduction in fecal bacterial-bound IgA during MS relapses due to mobilization of IgA$^+$ cells out of the gut. Together, the evidence supports the contention that IgA$^+$ plasma cells are involved in dampening experimental autoimmune encephalomyelitis-associated neuroinflammation. Recent evidence from Pröbstel et al[128] supports this view. The proportion of IgA bound bacterial taxa (operational taxonomic units) was significantly greater in MS and translocation of gut-associated IgA$^+$ B cells and plasma cells to the CNS attenuated neuroinflammation.

Evidence is also emerging implicating IgA and plasma cells in type-1 diabetes. Huang et al[129] demonstrated that newly diagnosed individuals with type-1 diabetes have altered microbiota and associated increase in IgA bound to gut bacteria. Fecal transplant

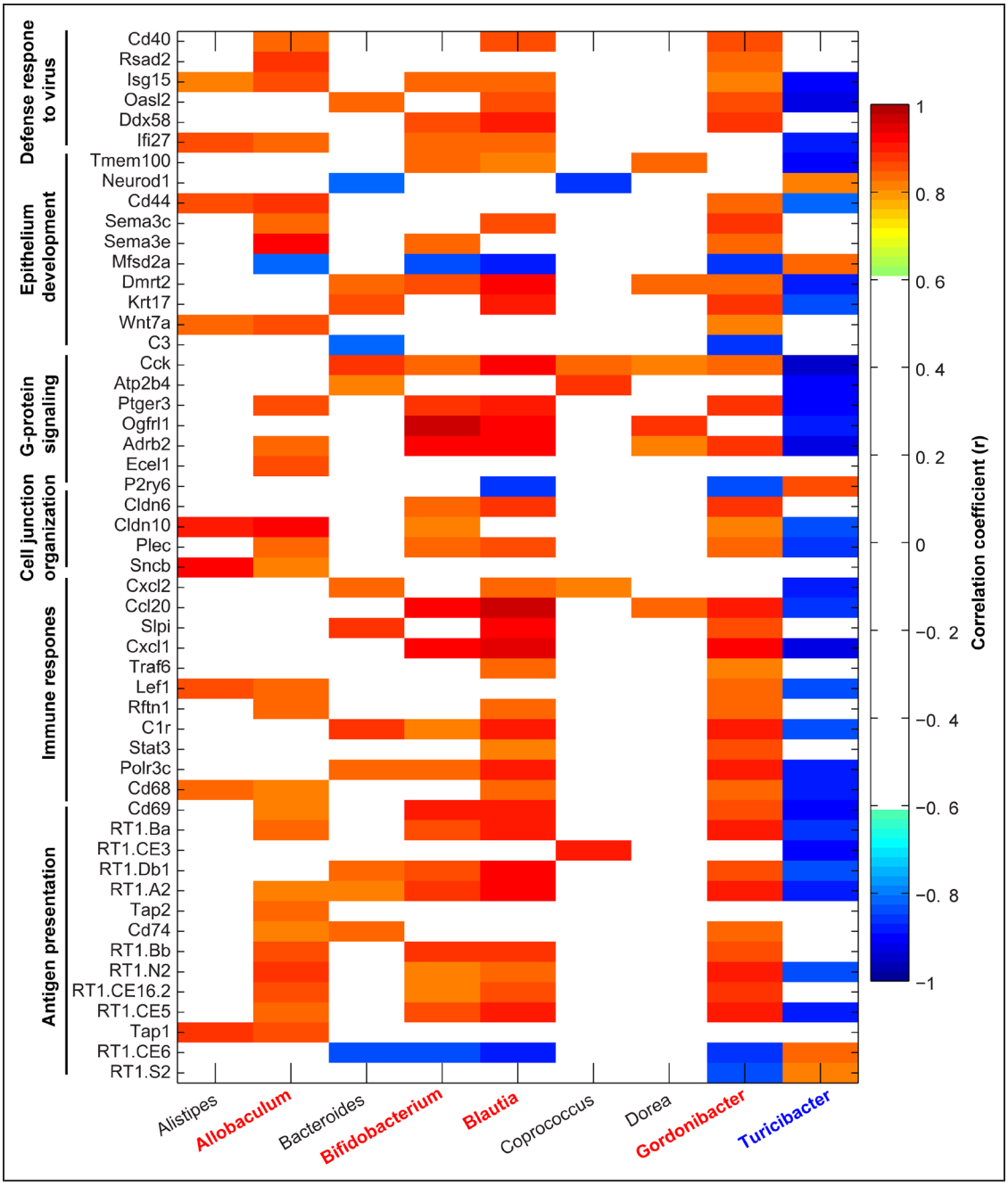

Figure 4. Alteration of gut microbiota composition correlates with gene expression for functional gene sets in gut epithelium of Wistar Kyoto (WKY) rats and spontaneously hypertensive rat (SHR).

Correlation analyses were performed on the normalized abundance of bacterial genera data[3] and gene expression values from RNA-seq data.[8] Pearson correlation coefficients (r) were generated for all samples regardless of group using MATLAB software with a cutoff value of 0.811 (n=5, $P<0.05$) and displayed using pixel maps. Red-highlighted genera are positively correlated, and blue-highlighted genera are negatively correlated.

from type-1 diabetes donors into germ-free nonobese diabetic mice enhanced gut permeability, gut antimicrobial peptide expression, decreased IgA^+ B cells in pancreas, and increased IgA bound bacteria in cecum, colon, and stool. Together, this was the first body of evidence linking IgA immune responses and gut microbiota regulated by SCFA (acetate) in the pathogenesis of type-1 diabetes. Finally, the role of IgA in regulation of gut homeostasis in irritable bowel syndrome (IBS) has been extensively studied.[120,130–132] Increases in fecal IgA and altered microbiota composition was demonstrated in 44 patients with diarrhea-predominant IBS.[133] This was associated with increases in IgA-coated bacteria particularly *Escherichia-Shigella*, *Granulicatella,* and *Hemophilus* in IBS compared with healthy controls. These findings suggest that dysbiosis in IBS results in higher IgA production, increased IgA-coated bacteria leading to a local inflammatory

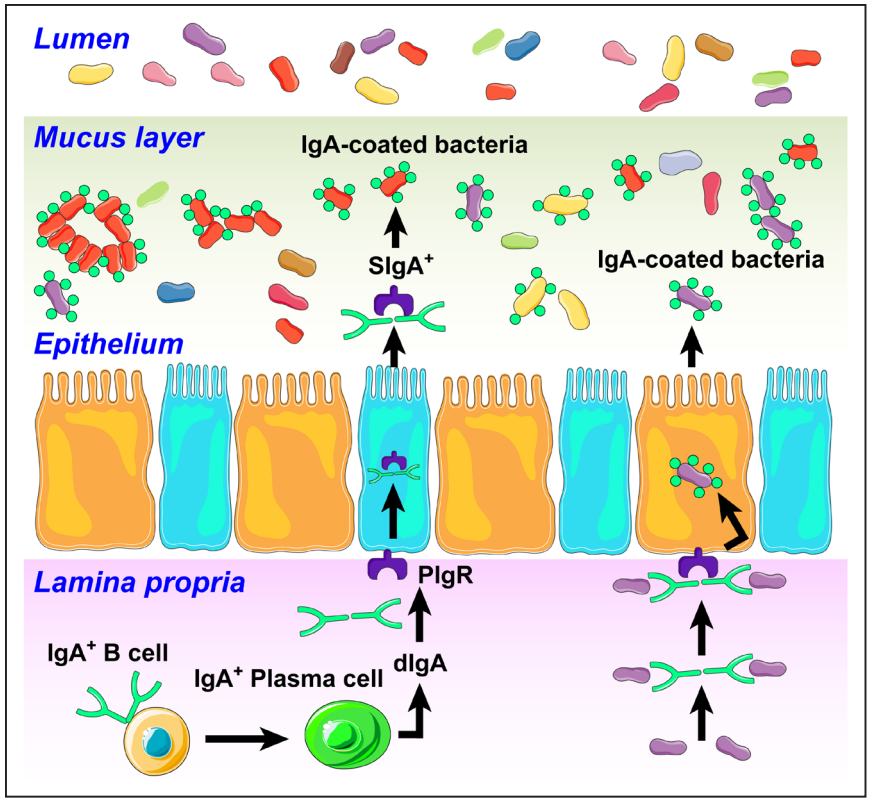

Figure 5. Influence of immunoglobulin A (IgA) on gut microbiota in hypertension.
In response to antigenic signals, in part generated by an altered microbiota, IgA B cells are activated into plasma cells to produce dimeric IgA (dIgA). dIgAs bind to polymeric Ig receptors (PIgR) expressed on the basolateral side of the epithelial cells. This complex is then transported to the apical surface, where PIgR is cleaved to generate secretory IgA (SIgA). SIgA binds and coats bacteria in an antigen-specific manner altering their functions. This could include growth inhibition, clustering, and elimination of beneficial bacteria and affecting the proliferation of harmful bacteria and production of toxins. We also propose that hypertension-specific IgA bound bacteria taxa could translocate to the brain through leaky gut and blood brain barrier (BBB) to induce neuroinflammation.

responses and manifestations of IBS. A common theme is emerging from the above studies regarding involvement of IgA and plasma cells in the gut-linked neural and other chronic diseases. Altered microbiota seems to be an important triggering step in activation of gut PB cells into plasma cells that produce large quantities of IgA. This selectively coats responsible bacterial taxa altering their composition and microbiota-epithelial interaction. Alternatively, the possibility of dysfunctional plasma cells overproducing IgA resulting in greater IgA coating of bacteria cannot be ruled out. Increased IgA and plasma cells could also enter the circulation because of leaky gut, translocate into the brain, and other relevant organs to influence immune status.

Are IgA and IgA-producing plasma cells relevant in hypertension since both dysbiosis and neuroinflammation are key factors of the disease? Despite an early report of increased circulating IgA in the SHR little progress has been made in delineating their involvement.[134] Increases in IgA in prehypertensive SHR clearly indicate that its contribution is not secondary to hypertension. Contribution of plasma cells to hypertension was demonstrated by a depletion experiment. Taylor et al[135] showed that depletion of plasma cells by bortezomib attenuated hypertension, renal injury, and B- and T-cell infiltration into the kidneys. A cross-sectional study with 12 373 participants showed that increased circulating IgA was associated with higher prevalence of hypertension and correlated with BP.[136] Another study with 61 hypertensive and 55 control subjects demonstrated significantly increased IgA among other immunoglobulins.[137] Despite these tantalizing data a major gap in knowledge exists in delineating the involvement and importance of IgA and plasma cells in the pathophysiology of hypertension, including whether the primary event is gut microbial dysbiosis or malfunctioning plasma cells. We have conducted a proof of principle study with a small cohort of hypertensive (N=13) and normal (N=9) subjects. IgA-coated bacteria from stool samples revealed a trend towards an increase in hypertensive subjects, (Figure 6) providing first indication of similar involvement of IgA and associated bacteria in hypertension.

MINOCYCLINE: AN ANTIHYPERTENSIVE DRUG?

Minocycline is an antibiotic of the tetracycline family that also possess anti-inflammatory properties. Its ability to penetrate the BBB, rapid absorbability from the gut, very good safety record over several decades of clinical use and its dual antibiotic and anti-inflammatory properties, have resulted in its evaluation to mitigate many neural diseases involving neuroinflammatory pathways.[138] Interestingly, despite success in animal models of stroke, MS, Parkinson disease, Amyotropic lateral sclerosis (ALS) and Huntington disease, clinical trials of minocycline for neuroinflammatory disorders have met with limited success.

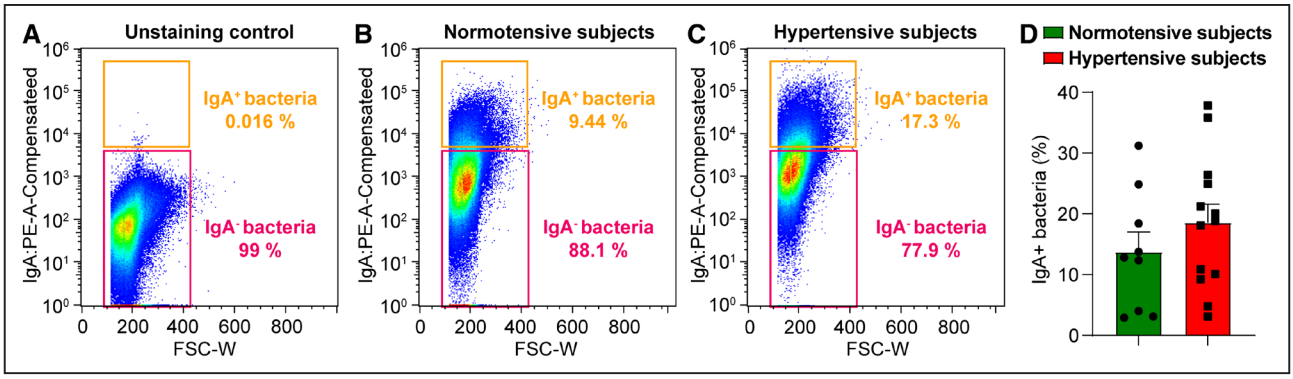

Figure 6. IgA-coated bacteria are enriched in hypertensive subjects.
Fecal bacteria isolated as described elsewhere[131] from stool samples from normotensive (N=9, systolic blood pressure [SBP]: 115 mm Hg) and hypertensive (N=13, SBP: 145 mm Hg) subjects were stained with PE-conjugated anti-human IgA to measure the percentage of IgA-coated bacteria in stool by flow cytometry. **A**, Unstained control. **B** and **C**, Representative IgA-staining of fecal bacteria from a normotensive (**B**) and a hypertensive subject (**C**). **D**, Cumulative data, percentage of IgA-coated, and noncoated bacteria in stool of 9 normotensive and 13 hypertensive subjects determined by flow cytometry (mean±SEM).

We decided to test the efficacy of minocycline in treatment-resistant hypertension based on the following: (1) extensive animal experiments have established that minocycline attenuates high BP and hypertension pathophysiology.[25,32,33] These effects are associated with decreases in active microglia in the PVN and other autonomic brain regions and decrease in sympathetic nerve activity. (2) Minocycline rebalances the gut microbiota composition of hypertensive rats towards that found in normotensive animals. The effect on microbiota in hypertension is consistent with minocycline's influences on gut microbiota and neuroinflammation in other diseases including depression, anxiety and Alzheimer disease.[139–143] (3) Promising results of this drug are reported in experimental colitis and other gut inflammatory diseases[144] where it works by inducing mucosal healing and reducing gut inflammation.[145] (4) Minocycline prevents development of diabetic retinopathy in rodent models.[146] Additionally, an observational study of morbidly obese type-2 diabetic patients demonstrated that it decreased hemoglobin A1c, reduced BP and improved visual acuity and symptoms associated with neuropathy in a small cohort of morbidly obese type 2 diabetic patients.[147] Taken together, these observations demonstrate that minocycline could be beneficial against neuroinflammation, gut microbiota and its pathophysiology, gut immunity, and proinflammatory mechanisms, processes all also associated with hypertension.

An open label study conducted in high-risk treatment-resistant hypertension patients supports this contention.[59] We observed that 16 of 26 patients (62%) responded to minocycline with a mean reduction in daytime ambulatory systolic BP (135/74 to 124/68 mm Hg).[59] Decreased daytime ambulatory systolic BP was associated with reductions in circulating $CD4^+$ cells containing $CD161^+IL17^+$ and $CCR6^+ITGb7^+CD161^+IL17^+$ markers. This suggested that gut inflammatory cell populations were decreased by minocycline treatment.[59] These data are tantalizing but clearly more work is needed to clarify the usefulness of minocycline in treatment-resistant hypertension.

Is minocycline uniquely poised compared with other drugs with only anti-inflammatory or antibiotic properties for its antihypertensive effects in treatment-resistant hypertension patients? There is little evidence to support this contention. One would predict that these classes of drugs would influence BP since inflammation and gut microbial dysbiosis are hallmark events in hypertension. However, results have been discouraging, in part because NSAIDSs have been implicated in adverse cardiovascular effects,[148] leading to caution in testing other anti-inflammatory drugs with BP-reducing properties in animal models in human hypertension.[149] Therefore, the usefulness of anti-inflammatory/immunosuppressive drugs for hypertension remains questionable at the present time.

Evidence for the influence of antibiotics on BP is primarily derived from animal studies, mostly indirect, and sometimes disparate. An early study showed that antibiotic treatment significantly attenuated the corticosterone-induced increase in BP.[150] Doxycycline treatment reduced BP in SHR in one,[151] but had no effect in another[152] study although the antibiotic attenuated vascular remodeling. Vancomycin decreased while neomycin did not change BP in the SHR.[153] Recently, Galla et al[154] demonstrated that amoxicillin treatment lowered BP in the SHR and altered gut microbiota. Interestingly, this BP-lowering effect persisted after antibiotic treatment was discontinued. They concluded that amoxicillin reshapes gut microbiota with long-term antihypertensive effects extending even to their offspring. The concept of transmission of gut microbiota imprinting from dams to offspring is supported by our study.[30] A few studies have implicated a BP-lowering effect of certain antibiotics, although indirectly, in human hypertension. For example, cotreatment with ampicillin caused an enhanced antihypertensive response of amlodipine, possibly by increasing its bioavailability.[155] In a retrospective study of 7100 older patients receiving

a calcium channel blocker, erythromycin, and clarithromycin were strongly associated with increased risk of hypotension.[156] Finally, our case study showed a pronounced BP-lowering effect with a combination of antibiotics in a 69-year-old woman with a long history of hypertension.[157]

In conclusion, it is apparent that drugs that target both inflammation and gut microbiota may have greater probability to decrease BP in hypertension, particularly in patients with treatment-resistant hypertension. Would minocycline, with its long safety record, be the drug of choice? This remains an open question and 2 ongoing clinical trials (NCT02133872 and NCT04478500) are likely to resolve this issue.

CONCLUSIONS AND FUTURE DIRECTIONS

Significant progress has been made and new concepts have been presented since the first demonstration of dysbiosis in hypertension less that 10 years ago. The evidence that microbiota is involved in the initiation of high BP has been presented. Also, the supposition for bidirectional communication in BP control and a dysfunctional gut-brain axis in hypertension has gained basic support. Evidence is also emerging in support of an active crosstalk between gut epithelium and bacterial communities which may be impaired in hypertension. We have presented provocative ideas for epithelial-bacterial communication involving antigen presenting genes and IgA^+ cells in systemic and neuroinflammation. Human studies are increasingly implicating microbiota in ethnic hypertension disparity. Finally, translational implications of a dysfunctional gut-brain axis have been realized by a small trial showing that minocycline treatment decreased BP in treatment-resistant hypertension patients.

Moving forward, we propose that microbiome and hypertension research should focus on the following critical issues to continue advancing basic science mechanisms and to develop innovative strategies for the control of hypertension:

- Epithelial epigenetics certainly plays an important role in physiological homeostasis. How are these epigenetic influences altered in hypertension?
- Involvement of IgA and plasma cells in the transmission of inflammatory signals to cardiovascular-relevant organs (ie, heart, kidneys, brain, etc) would be an important avenue to investigate.
- Experiments with animal models are unquestionably important and provide the backbone of the mechanistic information. However, considering insurmountable limitations with animal models, efforts should be continued to validate concepts in human hypertension. For example, can one utilize tissues from colonic biopsies and make 3-dimensional organoid cultures or colon-on-a-chip to investigate epithelial genes and their interacting bacterial partners in hypertension subjects? IgA bacteria can be analyzed from the stool of subjects to home-in on taxa involved in systemic and neuroinflammation.
- Sex-linked differences in BP regulation are well-established but little is known about the involvement of gut microbiota in this. It would be a relevant future direction in view of the evidence for (1) differential regulation of microbiota by sex hormones in men and women and different metabolism of sex hormones by their gut microbiota. (2) the heavy influence of a mother's gut microbiota on her offspring's that may play a role in transgenerational transmission of hypertension susceptibility.
- An appropriately powered, trial is needed to confirm beneficial effects of minocycline in treatment-resistant hypertension.

ARTICLE INFORMATION

Affiliations

Department of Physiology and Functional Genomics (E.M.R., J.L., B.R.S., M.K.R.) and Division of Cardiovascular Medicine, Department of Medicine (C.J.P.), University of Florida College of Medicine, Gainesville, Florida.

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Disclosures

None.

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