Neural Control of Immunity in Hypertension: Council on Hypertension Mid Career Award for Research Excellence, 2019

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Abstract—The nervous system and the immune system share the common ability to exert gatekeeper roles at the interfaces between internal and external environment. Although interaction between these 2 evolutionarily highly conserved systems has been recognized for long time, the investigation into the pathophysiological mechanisms underlying their crosstalk has been tackled only in recent decades. Recent work of the past years elucidated how the autonomic nervous system controls the splenic immunity recruited by hypertensive challenges. This review will focus on the neural mechanisms regulating the immune response and the role of this neuroimmune crosstalk in hypertension. In this context, the review highlights the components of the brain-spleen axis with a focus on the neuroimmune interface established in the spleen, where neural signals shape the immune response recruited to target organs of high blood pressure.

Key Words: autonomic nervous system ■ blood pressure ■ immune system ■ spleen

The past decades assisted to the development of several therapeutic approaches for controlling arterial hypertension. Nonetheless, high blood pressure still represents one of the most impacting causes of morbidity and mortality worldwide, remaining the main risk factor for stroke, heart failure, and kidney diseases.[1–3] The current guidelines for treatment of hypertension consist in combination therapies comprising drugs interfering with the renin-angiotensin system, diuretics, calcium channel blockers, and anti-adrenergic drugs.[3] However, a quite consistent proportion of patients with essential hypertension does not reach the target blood pressure levels,[4,5] making hypothesize that additional pathophysiological mechanisms contribute to sustaining blood pressure elevation. Yet, even in subjects with acceptable blood pressure levels, an elevated residual risk of cardiovascular events may persist.[6,7]

All the current antihypertensive strategies are the result of research conducted over the last century, which showed an unquestionable role of the renin-angiotensin system, the sympathetic nervous system, and the mechanisms contributing to increased peripheral resistances. Many factors, including genetic susceptibility and environmental challenges, together with perturbations of neural, mechanical, and hormonal regulatory mechanisms, have been shown to regulate blood pressure and determine hypertension.[8]

In the past 10 years, there has been an increasing surge in the observations reporting that alterations in immune system functions likely contribute to the onset of hypertension and related target organ damage.[9]

This review summarizes the major findings identifying a role of the immune system in hypertension, and how the autonomic nervous system participates in shaping the immune responses involved in blood pressure regulation.

The Role of Immune System in Hypertension

Although observations showing that inflammatory and immune mechanisms were involved in hypertension and related target organ damage date back to long time ago,[10–12] the first mechanistic evidence that changed the scenario was the finding that mice devoid of lymphocytes (*Rag1*$^{-/-}$ mice) are protected from blood pressure increase in response to various stimuli.[13] More recently, provocative findings by Seniuk et al[14] showed that the same colony of *Rag1*$^{-/-}$ mice unexpectedly lost the phenotype of blunted hypertensive response to angiotensin II and high-salt diet. Although suggesting the need of further studies investigating these controversial results, the notion that the immune system has a role in hypertension is proven by a myriad of published studies.[9] Subsequent experiments further clarified that CD8$^{-/-}$ mice, but not CD4$^{-/-}$ mice, are protected from the typical blood pressure response observed upon chronic infusion of angiotensin II,[15] thus hypothesizing that mechanisms related to CD8 T-cell activation are crucial for hypertension. Further supporting an involvement of T cells in hypertension, a subsequent work found that mice with a severe combined immunodeficiency were protected from the development of hypertension.[16] Parallel findings in rats demonstrated that the role of adaptive immune system in hypertension is shared among species.[17]

The process of activation into effector T cells consists in a cascade of signaling events determining oligoclonal expansion, cytokine production, alteration in surface proteins that overall permit the egression from secondary lymphoid organs, like the

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spleen, toward peripheral tissues. Homing of effector T cells in sites of tissue infection or damage is driven by a regulated process of chemokines and cytokines released from the target tissues. In turn, when infiltrating peripheral target tissues, effector T cells produce mediators that further contribute to the local inflammatory response. As an example, in the context of hypertension, T-cells activated by hypertensive stimuli, become competent to infiltrate the typical organs regulating the peripheral resistances and renal function, thus contributing to the rising of blood pressure and target organ damage.[13,15,18–20] On this notice, gene knockout and antibody-mediated neutralization of the IL (interleukin)-17 cytokine proved as an effective strategy limiting the development angiotensin II–dependent hypertension in mice.[21]

A typical paradigm of immunologic activation of naïve T cells consists in the encounter with antigen-presenting cells. Interestingly, it has been shown that the prototype hypertensive stimulus, angiotensin II, increases the expression of CD86, a crucial mediator of T-cell costimulation by antigen-presenting cell, in the spleen.[18,22] Moreover, mice lacking CD86 costimulation molecule displayed a reduced number of activated T cells in the circulation and aorta and a reduced hypertensive response.[22] The above findings made it clear that the crosstalk between innate and adaptive immune cells is necessary in hypertension, like in many other immunologic processes. Monocyte-derived dendritic cells are among the classical subtypes of antigen-presenting cell and have been involved in hypertension, both in experimental models and in humans.[23] Isolevuglandins are the product of free radical-dependent oxidation of arachidonate containing lipids,[24] a process typically observed in hypertension. Interestingly, it was found that isolevuglandin-modified proteins may represent a key stimulating factor for $CD11c^+$ dendritic cells in hypertension.[24] At the molecular level, it was shown that the administration of angiotensin II or deoxycorticosterone acetate-salt to mice caused activation of NADPH oxidase enzymes in $CD11c^+$ cells, leading to increased formation of reactive oxygen species, lipid oxidation, and the deposition of isolevuglandin adducts on self-proteins. In vitro studies indicated that this mechanism may also be activated by direct exposure of dendritic cells to a high-salt environment.[25] Isolevuglandin-modified proteins were also increased in monocytes and dendritic cells of patients with hypertension.[24] Moreover, dendritic cells activated in this manner promoted CD8 T-cell proliferation, release of proinflammatory cytokines like IFN (interferon)-γ and IL-17A, and hypertension when transferred into normotensive mice.[24]

Further supporting the role of innate immune cells in hypertension, another elegant study in mice demonstrated that myeloid cells have a crucial role in hypertension. By using the lysozyme M-targeted strategy to delete cells expressing the diphtheria toxin receptor, the authors found a protection from vascular alterations and blood pressure increase in response to chronic infusion of angiotensin II.[26]

More recently, another work also demonstrated that B cells and immunoglobulins are mechanistically involved in the development of angiotensin II–induced hypertension and vessel remodeling in mice.[27]

However, it was also found that T-regulatory cells, with suppressor functions of T-effector lymphocytes, have protective functions in hypertension. In more details, it was shown that the selective depletion of T-regulatory cells aggravates hypertension in mice.[28] Conversely, a strategy aimed at increasing T-regulatory cell numbers through adoptive transfer displayed protective effects against hypertension and consequent cardiovascular damage developed in response to angiotensin II.[29] Taken together the above findings suggest the existence of friend and foe immune cells in hypertension and, hence, the appealing perspective of designing novel therapeutic approaches to reduce blood pressure and target organ damage, by boosting the appropriate protective T-regulatory cell subset.

The Role of Autonomic Nervous System in Hypertension

The autonomic nervous system refers to the combination of afferent and efferent neurons that connect the brain with visceral effectors throughout the body.[30] The 2 branches of the autonomic nervous system, generally described as the parasympathetic and the sympathetic arms, comprise parallel but differentially regulated neural pathways. It is usually recognized that the sympathetic and parasympathetic nervous systems correspond to 2 counteracting forces whereby the parasympathetic arm establishes the "rest and digest" response and the sympathetic one the "flight and fight" reaction. Therefore, it was quite common figuring out that sympathetic and parasympathetic actions of the autonomic nervous system were largely limited to extreme and acute stimuli. It became later apparent that sympathetic and parasympathetic nervous systems can also deeply modulate chronic responses in target organs.[31–33] Actually, imbalance of the autonomic nervous system regulation contributes to cardiovascular disease.[30,34]

Investigations conducted in experimental models of hypertension and in patients led to define a role of elevated sympathetic nervous system outflow in hypertension, a phenomenon usually accompanied with enhanced vascular reactivity that manifests as greater vasoconstrictor responses to norepinephrine.[30] On another notice, even environmental factors, ranging from the exposure to mental stress, to obesity and humoral factors, proved capable of increasing the susceptibility to hypertension by enhancing the activity of the sympathetic nervous system.[35,36] Hence, pharmacological treatments targeting the sympathetic nervous system effectively lower blood pressure in quite vast proportion of patients.[37]

Physiologists have been interested in studying the functions of sympathetic nervous system activity in hypertension, and for many decades, this stream of research took over the stage of hypertension pathophysiology.[38] A consequent necessity was the requirement of tools able to tackle the study of sympathetic nervous system both in experimental and clinical settings. A significant progression came from the establishment of experimental techniques, which made accessible analyzing the role of peripheral nerves in the development of essential hypertension and related cardiovascular outcomes. As an example, microneurographic techniques allowed direct recording of the electrical activity of postganglionic sympathetic efferent innervating the vasculature of skeletal muscle, a gold standard procedure to assess human regional sympathetic activity.[39–41] When combined with the measurement of organ-specific norepinephrine release in the plasma through norepinephrine spillover,[40] clinical

microneurography helped in identifying patients with an overactive sympathetic outflow to the skeletal muscle vasculature, heart, and kidneys.[34,40–44] However, microneurographic techniques proved useful in the investigation of sympathetic outflow to the kidneys in various experimental models of hypertension.[31] In fact, despite providing little advancements in the treatment of hypertension, the pathophysiological studies on the autonomic nervous system gave great improvements in demonstrating a causal role of excessive sympathetic activation in blood pressure elevation.[37]

Additional advances derived from the evidence that physiological and pathophysiological responses of the sympathetic nervous system may show a regional pattern of activation and regulation.[33,45] Indeed, several experimental data reported that, both in humans and in animal models, variations in sympathetic nervous system activity differently affect the numerous organs involved in blood pressure regulation, that is, kidneys, vasculature, heart, and the splanchnic district.[31,32] However, target organs of the sympathetic nervous system are also able to influence reflex responses by signaling to the brain feedback messages from the periphery.[46,47] Such a complex organization gives a signature of how the autonomic nervous system is activated in hypertension, as a result of a wide variety of factors as regionality, timing, and intensity.[33,45]

The Pathophysiological Basis of the Inflammatory Reflex

Immune organs are directly innervated by the autonomic nervous system.[48] The parenchyma of primary lymphoid organs, like the bone marrow and thymus, as well as that of the secondary lymphoid organs, that is, spleen and lymph nodes, are densely entangled by noradrenergic fibers. Data collected thus far support the concept that the sympathetic nervous system influences immune and inflammatory responses, with some reports describing proinflammatory effects and others indicating anti-inflammatory or immunomodulatory actions.[18,19,49–51]

A major discovery was the identification of the neurophysiological basis of the inflammatory reflex.[52] A series of experiments conducted by electrical stimulation of the vagus nerve demonstrated that efferent fibers of the vagus nerve dampen the endotoxemia induced by the bacterial toxin lipopolysaccharide through an inhibitor effect on TNF (tumor necrosis factor) release.[53] In pathophysiological conditions, mice subjected to peripheral injection of lipopolysaccharide activate an afferent activity signaling the danger to the brain and, in turn, integrated with an efferent activity of the vagus nerve, which controls peripheral cytokine levels and inflammation.[53–55] The effector arm of this neuronal circuit is represented by the vagus nerve, which transmits action potentials to the celiac ganglion that, in turn, gives origin to the noradrenergic splenic nerve.[54,55] Once activated, splenic neurons prime a specific T-cell subset in the white pulp, responsive to norepinephrine and producing acetylcholine, the choline acetyltransferase-CD4 T cells.[56] The acetylcholine produced by these T cells signal to a specific subtype of macrophages of the red pulp and marginal zone, expressing α7 nicotinic acetylcholine receptors (α7nAChR), which inhibit the production of TNF and other proinflammatory cytokines.[55] Henceforth, the resulting neuronal reflex was termed the cholinergic inflammatory pathway.

The Brain to Spleen Axis in Hypertension

A link between the central effects of hypertensive stimuli, like angiotensin II, and the activation of immune response was already suggested some years ago, when it was found that the vasoactive peptide angiotensin II exerted immune-modulating functions through brain-mediated circuits.[57] In fact, earlier observations highlighted the existence of a pathophysiological connection between the brain and the spleen. As an example, it was demonstrated that direct intracerebral ventricular infusion of angiotensin II raised central sympathetic nerve activation and concomitantly enhanced the peripheral immune response through release of proinflammatory cytokines.[57] The first mechanistic work demonstrating brain control of immune activation in hypertension was later published. Mice were subjected to a selective lesion of the anteroventral third cerebral ventricle, that is, the region of the brain that comprises the subfornical organ and is characterized by a leaky blood-brain barrier.[58] Interestingly, when challenged with angiotensin II these mice did not show increased blood pressure, T-cell activation, and infiltration in the vasculature, thus indicating a crucial role of central actions of angiotensin II in hypertension.[58]

A typical schematic pathway of the autonomic nervous system would predict that a peripheral sympathetic overdrive involved in the modulation of cardiovascular function would establish a brain to periphery connection through the intermediolateral gray column of the spinal cord that coveys the vast majority of sympathetic nerves entangling vasculature, kidney, and heart.[30]

Interestingly, while dissecting the potential connection established by hypertensive challenges to signal the spleen to produce PlGF (placental growth factor), we found that the immediate preganglionic neuron activating the splenic nerve upon angiotensin II infusion is the celiac efferent branch of the vagus nerve.[19]

Taken together, these findings demonstrate that the effects of angiotensin II on the cardiovascular and immune systems are not only due to direct actions of the hormone on vasculature and immune cells but rather mediated and integrated by neural signals.

Splenic Neuroimmune Interactions in Hypertension

Human hypertension has essential neurogenic components, being the control of blood pressure classically attributed to the sympathetic regulation of key physiological parameters, including vascular tone and renal sodium excretion.[1,44] In fact, the sympathetic nervous system is one of the fine regulators of blood pressure control.[31,38]

The most important sympathetic innervation for blood pressure regulation has been considered the one entangling kidneys. Indeed, renal denervation was one of the outmost innovative approaches to fight hypertension.[32] However, the discovery of novel roles of the sympathetic nervous system in controlling the activity of immune system, arises new questions to be addressed. Interestingly, it has also been shown that renal denervation, besides the more classically known renal effects, modulates previously unknown immune functions and inflammatory responses associated with the pathophysiological alterations induced by hypertension in kidney.[59,60]

The discovery of a cholinergic inflammatory reflex in hypertension, whereby the brain is linked to the spleen through a vagus-sympathetic connection, to prime immune cells later recruited to target organs where they exert crucial effects for blood pressure regulation,[18,19] changed the perspective. At the molecular level, a new pathway emerged as a neuroimmune mediator of hypertension: the angiogenic growth factor, PlGF.[18] It was found that, upon angiotensin II infusion, PlGF is expressed in the spleen[18] and that PlGF knockout mice are protected against hypertension,[18] by exerting immuno-modulating functions. Moreover, PlGF$^{-/-}$ mice were also protected from angiotensin II–induced T-cell priming and egress toward target organs.[18] A similar protection from hypertension was observed in mice subjected to splenectomy.[18] More mechanistic, chimeric mice generated by reimplanting spleen from wild-type donors in PlGF$^{-/-}$ mice restored the hypertensive response to angiotensin II.[18] Conversely, wild-type mice reimplanted with spleen from PlGF$^{-/-}$ donors continued to be protected from the hypertensive effects of angiotensin II, thus highlighting a crucial role of splenic PlGF in hypertension.[18]

On the above premises, PlGF emerges as a new molecular target for designing therapeutic strategies. In fact, interesting to notice, tools clinically available for targeting this pathway already exist.[61,62] Based on evidence collected in experimental models of diseases, monoclonal antibodies raised against PlGF have been developed in the past years as a potential therapeutic approach to reduce tumor growth and for age-related macular degeneration.[61,62] The results obtained in the setting of experimental hypertension prompt to hypothesize the design of targeted therapies inhibiting PlGF, as the mediator of neuroimmune activation involved in blood pressure regulation and target organ damage due to excessive immune activation.

These findings also encourage an additional consideration about the frequently reported hypertensive side effects of antiangiogenic therapies used in patients with cancer, particularly agents that inhibit the signaling of VEGF (vascular endothelial growth factor).[63] Despite showing ameliorated outcomes in patients with cancer, and thus used as first-line therapies at least in certain tumors, VEGF inhibitors often manifest hypertension as a dose-dependent complication of treatment. The molecular mechanisms underlying increased blood pressure and cardiovascular risk in cancer patients receiving antiangiogenic therapies are still under investigation, but it is conceivable to hypothesize that vascular and angiogenic pathways are involved.[63] The newly identified role of PlGF as a neuroimmune mediator of hypertension, let us envisage that oncological strategies aimed at counteracting this pathway, instead of the VEGF-mediated one, would lead to effective anti-tumor effects and less hypertensive side effects. Future studies should be designed to test this hypothesis.

Clinical Perspective

The key concept proposed by this review is a change in the way we look at the autonomic nervous system in hypertension, mainly known to exert preeminently direct hemodynamic effects, as a fine modulator of the immune response (Figure). A consequent inference is the translational challenge that ensues from the evidence that the autonomic nervous system is the hard-wired circuit that connects priming of immune responses to cardiovascular effects.

Although interventional strategies targeted to sympathetic nervous system for the treatment of hypertension have been explored in the renal district, this review suggests that it could be time to speculate that therapeutic manipulation of neuroimmune circuits and mechanisms are worth of further investigation for future translational strategies.

While the above findings support convincing evidence that inflammatory/immune dysregulation ensues during hypertension, there is a certain reluctance about the introduction of therapies targeting the immune system in primary hypertension. A major concern emerges in the analysis of risk/benefit ratio that cannot fail to consider the potential side-effects related to modulation of the immune system. On this notice, innovative strategies modulating the neural control of the immune system would allow more targeted interventions. In fact, findings highlighting the importance of neural control of splenic immunity in hypertension sets the basis for a new interventional strategy for resistant hypertension, manipulating celiac vagus nerve or splenic nerve activities to inhibit

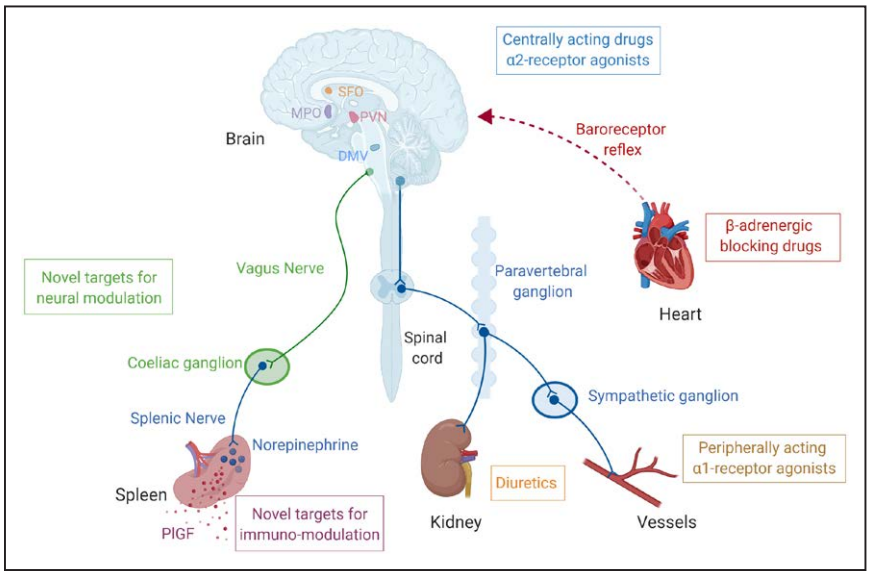

Figure. The neuroimmune pathway in hypertension. The schematic representation of hard-wired neural connections established by the autonomic nervous system at the different peripheral cardiovascular organs, integrates the newly identified neuroimmune reflex linking the brain to the spleen. The cholinergic (green)-sympathetic (blue) efferent nerves are intertwined at the level of the celiac mesenteric ganglion. Norepinephrine release at the splenic neuroimmune interface is in turn responsible for the activation of PlGF (placental growth factor). The integration of the classical autonomic nervous system reflexes, regulating cardiac, renal, and vascular function, with the neuroimmune pathway identifies new avenues of therapeutic strategies for hypertension (Created with BioRender.com). SFO indicates subfornical organ.

hypertension-related immune mechanisms.[19] Additionally, further investigations into the molecular mechanisms regulating the neuroimmune interface established in the spleen may help elucidating new directions for tailored strategies avoiding the generalized effects of immunosuppressive therapies.

On another notice, clinical and preclinical studies underline a robust association between hypertension and disorders concerning sustained activation of the immune system, such as systemic lupus erythematosus, psoriasis, periodontitis, systemic sclerosis, and rheumatoid arthritis.[64] These observations prompted to investigate potential effects of immune-targeting therapies on hypertension as a comorbidity of classical autoimmune diseases, finding favorable reports in some cases[64,65] but not in others.[66]

The favorable balance of risk to benefit ratio of drugs targeting the immune system might be further enhanced by exploiting a precision-medicine approach, whereby any decision to use such therapies would be based on evidence of increased immune activity. This kind of approach has been successfully leveraged in the recent CANTOS trial (Canakinumab Anti-inflammatory Thrombosis Outcome Study), which used plasma levels of hsCRP (high-sensitivity C-reactive protein) as inclusion criteria for receiving anti-IL-1β for secondary risk prevention.[67] Conversely, a parallel trial, the CIRT (Cardiovascular Inflammation Reduction Trial), where the effects of low-dose methotrexate were examined without considering any marker of the immune status as inclusion/exclusion criterion, found no positive impact on the incidence of cardiovascular events.[68] Building on these premises, PlGF and its related signaling pathway may emerge as a marker of immune activation in hypertension and, as such, might be worthy of further investigation in various clinical settings for developing precision-medicine based therapeutic approaches. Furthermore, once released, PlGF determines the activation of a downstream signaling that may be recruited through one of its 2 major receptors: VEGFR-1 and Nrp-1 (neuropilin 1), being the former the only known tyrosine kinase receptor for PlGF,[62] and the latter being usually known to act as a coreceptor that enhances responses to several growth factors.[69,70] Future studies focusing on the introspection in the signaling pathway activated by PlGF could be important to clarify the molecular hierarchy in the splenic neuroimmune interface, thus driving the translational advancements in a targeted way. Indeed, biological therapies selectively targeting the molecular axes PlGF/VEGFR-1 or PlGF/Nrp-1 have been already developed and may be used for trialing in patients with primary hypertension with clear markers of enhanced immune activation.

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Disclosures

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