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Brain-Splenic Immune System Interactions in Hypertension: Cellular and Molecular Mechanisms

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ABSTRACT: Hypertension represents a major worldwide cause of death and disability, and it is becoming increasingly clear that available therapies are not sufficient to reduce the risk of major cardiovascular events. Various mechanisms contribute to blood pressure increase: neurohormonal activation, autonomic nervous system imbalance, and immune activation. Of note, the brain is an important regulator of blood pressure levels; it recognizes the peripheral perturbation and organizes a reflex response by modulating immune system and hormonal release to attempt at restoring the homeostasis. The connection between the brain and peripheral organs is mediated by the autonomic nervous system, which also modulates immune and inflammatory responses. Interestingly, an increased autonomic nervous system activity has been correlated with an altered immune response in cardiovascular diseases. The spleen is the largest immune organ exerting a potent influence on the cardiovascular system during disease and is characterized by a dense noradrenergic innervation. Taken together, these aspects led to hypothesize a key role of neuroimmune mechanisms in the onset and progression of hypertension. This review discusses how the nervous and splenic immune systems interact and how the mechanisms underlying the neuroimmune cross talk influence the disease progression.

Key Words: autonomic nervous system ■ cardiovascular diseases ■ cardiovascular system ■ immune system ■ neuroimmunomodulation

STRUCTURE AND ORGANIZATION OF THE SPLEEN

The spleen is a secondary lymphoid organ characterized by specialized microenvironments that contribute to hematopoiesis, erythrocyte turnover, and immune protection against pathogens and disturbances of homeostasis during disease progression.[1] These functions are performed thanking to the highly organized compartmentalization, like the red pulp, the white pulp, and the marginal zone. The red pulp is the splenic area with functions related to the process of blood filtering and of old erythrocytes that need to be replenished. The white pulp is the lymphoid structure of the spleen characterized by a specific organization of T- and B-cell clusters, whose compartmentalization is controlled and maintained by specific chemokines produced by fibroblastic stromal cells (fibroblast reticular cells [FRCs]) that also provide a physical scaffold to the spleen.[2] The marginal zone is a layer of cells surrounding T-cell zone and follicles (B-cell zone) predominantly composed by endothelial cells, FRCs, and 2 subsets of resident macrophages, the marginal zone macrophages and the metallophilic macrophages located near to the white pulp. The marginal zone is an important transit area for T, B, and dendritic cells that migrate from the bloodstream to the compartments of the white pulp. In addition, it acts as the first immune barrier where myeloid cells are activated and the immune response starts.[1]

SPLENIC FIBROBLASTIC RETICULAR CELLS

FRCs are stromal cells of mesodermal origin that synthesize the extracellular matrix component like collagen,

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Nonstandard Abbreviations and Acronyms

Ang II	angiotensin II
ANS	autonomic nervous system
AT1a	angiotensin II type 1a
CCL	CC-chemokine ligand
ChAT	choline acetyltransferase
FRC	fibroblast reticular cell
IL	interleukin
MAdCAM-1	mucosal vascular addressing cell adhesion molecule-1
PlGF	placental growth factor
SNS	sympathetic nervous system

Highlights

- Splenic fibroblast reticular cells provide the structural support of the spleen and contribute to tissue homeostasis by interacting with immune cells.
- Autonomic nervous system connects the brain and the immune cells in lymphoid and nonlymphoid organs establishing a neuroimmune cross talk that influences hypertensive disease.
- Once activated by peripheral challenges like hypertension, the immune and the autonomic nervous system can stimulate each other by releasing neurohormonal and inflammatory mediators modulating immune and neuronal functions.
- The splenic neuroimmune cross talk recruited by hypertension is mediated by the soluble factor PlGF (placental growth factor) that, in homeostatic conditions, is produced by fibroblast reticular cells in the spleen.

reticular fibers, and elastic fibers to provide the structural support of the spleen. They also produce cytokines and growth factors to guide the migration and interaction of immune cells, contributing to tissue homeostasis.[3] Four major FRC subsets organize and maintain the dedicated compartments that compose the spleen: the marginal reticular cells, the T-cell zone reticular cells, the B-cell zone reticular cells, and the perivascular reticular cells.[4] The marginal reticular cells and lymphatic endothelial cells form a border region of lymphoid tissue with the ability to capture and deliver antigens from the marginal zone to B-cell follicles. The expression of the extracellular marker MAdCAM-1 (mucosal vascular addressing cell adhesion molecule-1) distinguishes these cells from other subpopulations of FRCs.[5] The T-cell zone reticular cells form the scaffold of the splenic white pulp compartment and produce CCL (CC-chemokine ligand) 19 and 21 to attract T cells in the T-cell zone where they interact with dendritic cells and migrating B cells.[6,7] The T-cell zone reticular cells express podoplanin and maintain T cell homeostasis and support their survival.[8] B-cell zone reticular cells are the most abundant reticular cells in the B-cell follicles. These reticular cells achieve the important function of capturing and presenting the antigen to B cells through FCγ receptors, such as CD16/CD32, on their surface to promote their maturation and the activation of the immune response.[9] The center of B-cell follicles contains the follicular dendritic cells, identified by the expression of the complement receptors CD21 and CD35 and by the secretion of CXCL13, a B cell–attracting chemokine that binds to CXCR5.[10] In the B-cell zone, the processes of B-cell clonal expansion, isotype switching, and somatic hypermutation take place.[11] The perivascular reticular cells are a subset of FRCs located along the vasculature of all compartments of the spleen that express Ly6a (Sca-1), Pdgfra (CD140a), and Vcam1 (CD106) markers.[12] The perivascular reticular cells form the perivascular niche where multipotent periarterial progenitor cells give origin to the different FRC subsets by receiving stimuli from the microenvironment.[12]

SENSORY AND NORADRENERGIC FIBERS INNERVATING THE SPLEEN

Increasing evidence suggests that the cross talk between the autonomic nervous system (ANS) and the spleen plays an essential role in the regulation of the immune response. The spleen—the largest immune organ with a potent influence on the cardiovascular system during disease[13,14]—is directly connected with the celiac plexus ganglia via the splenic nerve.[15–18] The celiac ganglion represents the anatomic location where both vagal and sympathetic preganglionic fibers connect to the splenic nerve. Sympathetic noradrenergic fibers enter the spleen via the splenic nerve and release the main neurotransmitter norepinephrine that interacts with immune cells and parenchymal cells to modulate immune response.[19,20] Also, sensory neurons have been detected in the spleen where they perceive and send information from the periphery to the brain and are able to directly act, via neuropeptide secretion, on the immune cells expressing receptors for neurotransmitters.[20] Neurons originating in the celiac ganglion and innervating the spleen also release neuropeptide Y that dampen local splenic inflammatory response after LPS stimulation and are able to restore immune balance in autoimmune diseases.[21] Although the spleen is not directly innervated by cholinergic fibers, acetylcholine was found in tissue homogenates, and recent work demonstrated that it is locally produced by FRCs[22] and immune cells[23] and is able to modulate lipid metabolism and immune cell differentiation and activation. In particular, it was shown that, upon splenic noradrenergic discharge, a specific subset of CD4 T cells, expressing the enzyme ChAT (choline acetyltransferase), release acetylcholine, which in turn modulates the activity of macrophages.[23] Acetylcholine is also produced by FRCs in the spleen of systemic

lupus erythematosus–prone mice where acting on B cells expressing both muscarinic and nicotinic receptors, promote autoreactive B-cell response by enhancing lipid metabolism and B-cell differentiation.[22] On another note, our studies demonstrated that hypertensive stimuli recruit a cholinergic-sympathetic modulation of the spleen through a different axis.[16,18] At the anatomic level, the connection between the 2 arms of the ANS, the noradrenergic and the cholinergic fibers, is established in the celiac ganglion.[16,18,24] Microneurography of peripheral nerves—a technique enabling to directly measure the electrophysiological activity of the ANS[25]—allowed to demonstrate that hypertensive stimuli, like Ang II (angiotensin II) chronic infusion or deoxycorticosterone acetate–salt administration, enhance splenic nerve discharge and prime splenic immune responses relevant to lymphocyte infiltration in target vasculature.[16–18] Interruption of the sympathetic outflow to the spleen, obtained by a selective denervation of the left celiac ganglion, inhibited blood pressure increase in response to Ang II[16] and deoxycorticosterone acetate–salt.[17] The celiac ganglia are nerve bundles providing innervation to the spleen but also to other splanchnic organs. However, when we performed a selective denervation of the splenic nerves by thermoablation, we obtained results overlapping those obtained with the apical removal of the celiac ganglion. This observation was suggestive of a specific role of the celiac ganglion in controlling the splenic innervation, in the context of blood pressure regulation.[16] The effect of celiac ganglionectomy has been successively confirmed also in genetically hypertensive mice[26] further indicating a critical role of this neural reflex in hypertension. In translational perspective, a recent application of bioelectronic medicine tools showed that it is indeed possible modulating the immune response by eliciting a splenic neural reflex through the efferent celiac vagus nerve,[18] opening the field to novel therapeutic strategies for cardiovascular diseases.

NEURAL CIRCUITS REGULATING IMMUNE SYSTEM

The brain perceives information on peripheral inflammatory alterations via sensory inputs from immune organs, like bone marrow and spleen, and through signals released by the peripheral inflamed tissue itself. Once alerted, the brain responds to stimuli challenging tissue homeostasis through specific reflex and hormonal mediated responses acting as the central hub of multiorgan cross talk. The ANS connects the brain and the peripheral organs through a combination of afferent and efferent fibers. Neural reflexes are typically formed by afferent neurons, often conveying sensory information once activated by a peripheral stimulus, and efferent neurons that generate a reflex response. Notably, it has been consistently demonstrated that ANS-induced modulation of the immune response is a crucial effector arm of the above-cited neuroimmune regulatory circuit, in many disease contexts.[27] Mounting work showed that cardiovascular disease is not an exception.[19]

It has been known from decades that the brain is one of the main regulators of blood pressure homeostasis and, at the same time, potently influences peripheral immune responses at the steady state and during disease.[28,29] A dysregulation of ANS and immune response has been frequently observed in cardiovascular diseases, including hypertension. Recent investigations prospected that ANS and immune system cooperate in this pathophysiological context. Anatomic bases might partly explain this relationship. Peripheral sympathetic fibers associate with lymphocytes, macrophages, and dendritic cells in lymphoid and peripheral organs, where they mediate the interaction between brain and local immune cells. When activated by peripheral challenges like hypertension, the ANS stimulates the immune response by releasing neurohormonal factors: (1) in lymphoid organs, where they modulate the release of inflammatory cells in the circulation and the recruitment and infiltration in peripheral target tissues and (2) in peripheral organs, where they shape the response of tissue-resident and migrating immune cells from lymphoid organs. However, immune cells residing in lymphoid organs are not the only participants in the immune response activated during disease establishment and progression. In fact, key roles are also played by tissue-resident immune cells in peripheral organs like the vasculature, kidneys, and heart. As an example, it has been shown that effector-memory T lymphocytes located in the kidney contribute to hypertension.[30] Additionally, it has been shown that the depletion of circulating myeloid cells and tissue-resident macrophages hampered the increase of blood pressure induced by Ang II.[31] This evidence also highlights that a cross talk between recruited and tissue-resident immune cells is involved in blood pressure increase and end-organ injury. Moreover, cytokine receptors are expressed by neurons, indicating that cytokines and other immune/inflammatory mediators might simultaneously modulate immune and neuronal functions. Sensory neurons transmit action potentials to the central nervous system in response to changes in the organ physiology, metabolic environment, and mechanical alteration imposed on cardiovascular system by hypertensive stress. Steinberg et al[32] demonstrated that the axons of afferent cervical vagus nerve, whose cell bodies are contained in the nodose ganglion, propagate action potential in response to administration of the proinflammatory cytokines TNF and IL (interleukin)-1β in mice. They showed that those neurons express receptors for TNF and IL-1β and the neuronal activities are abolished in mice lacking TNF receptors 1 and 2 and IL-1β receptor, indicating that the nervous system responds to inflammatory stimuli.[32]

Sympathetic nerves also contribute to the pathogenesis and maintenance of hypertension through effects on other organs like kidneys. Despite the availability of several classes of drugs to treat hypertension, some patients are drug resistant, requiring the search for new therapeutic approaches. A more recently emerged strategy, as promising antihypertensive therapy, is the catheter-based renal nerve ablation.[33,34] The development of this approach is based on the long-lasting evidence that the sympathetic innervation of the kidney has a crucial role in blood pressure regulation. In fact, one of the most broadly studied cardiovascular reflex systems is represented by the afferent and efferent renal innervations. It has been demonstrated that renal denervation, resulting from the ablation of both renal efferent and afferent nerves, decreases renal perfusion pressure and attenuates experimental hypertension.[35,36]

Sympathetic nervous system (SNS) regulation of blood pressure has been attributed to modulation of key physiological parameters, and treatment with Ang II or deoxycorticosterone acetate–salt in mouse experimental model causes end-organ damage characterized by vascular and renal dysfunction. However, a more recent view of tissue damage in cardiovascular diseases is the discovery that the ANS modulates the immune response in lymphoid organs. Once activated by increased sympathetic outflow, T cells egress and infiltrate the vasculature and kidneys where they release cytokines like IFN-γ (interferon gamma) and IL-17A that stimulate vascular production of reactive oxygen species and matrix metalloproteinases that promote vasoconstriction, vascular remodeling and causing an alteration of the mechanisms regulating renal function.[37–39] In particular, IFN-γ and IL-17A contribute to organ damage and blood pressure increase by inducing the activation of nephron sodium transporter and a significant decrease in the rate of excretion of sodium.[37] The ganglion block mitigates the immune response activated after Ang II or deoxycorticosterone acetate–salt treatment resulting in the reduction of vascular and kidney dysfunction (Table).

On a different note, renal diseases are also typically characterized by immune infiltration,[40] and renal denervation was effective in mitigating renal inflammation as well. A recent study compared the effect of surgical renal denervation and celiac ganglionectomy on blood pressure regulation in a genetically hypertensive mouse model.[26] They showed that the ablation of either renal nerves or splanchnic nerves chronically lowered blood pressure by 10 mm Hg throughout the 24-hour day-night cycle without affecting heart rate. These results indicate that both renal and splanchnic denervation could be used as an applicable non–drug-based therapy to treat hypertension.[26] Overall, the results of clinical trials on renal denervation remain controversial supporting the hypothesis that other mechanisms contribute to sympathetic overactivation in hypertension. As described above, selective denervation obtained by thermoablation has clinical potential for patients for whom the renal denervation has failed.[16] These results strongly suggest splenic denervation may be a prospective tool to be further explored for the treatment of resistant hypertension in humans.

Table.

Objective	Outcomes	References
Evaluate the involvement of T lymphocytes in blood pressure regulation and related target organ damage	The absence of mature lymphocytes in Rag1$^{-/-}$ mice, lacking the enzyme responsible for the formation of the T-cell receptor required for the generation of mature B and T cells, protects from blood pressure increase indicating that lymphocyte activation is a mechanism necessary for onset of hypertension.	Guzik et al[51]
	Increased sympathetic outflow induced by hypertension activates T cells that infiltrate target organs, like vasculature and kidneys, where the release of the proinflammatory cytokines IFN-γ and IL-17A stimulates vascular production of reactive oxygen species and matrix metalloproteinases, consequently leading to vascular and renal dysfunction.	Madhur et al,[39] Trott et al,[37] and Kamat et al[38]
	Ang II hypertension induces T-lymphocyte migration toward vessels establishing a vascular-immune interface. Activated CD8 T lymphocytes, but not CD4, significantly increased the myogenic tone of naive resistance arteries, suggesting that they have effector functions able to modulate vascular contractility.	Carnevale et al[56]
Evaluate the ANS activation and T-cell mobilization from immune reservoirs to target organs	Hypertension induces SNS activation to prime splenic immune response mediated by a neuroimmune growth factor, named PlGF.	Carnevale et al[15]
	Ang II and DOCA-salt enhance the splenic sympathetic nerve activity and blood pressure in mice. Denervation of the splenic artery or removal of the left celiac ganglion reduces blood pressure.	Carnevale et al[16]
	DOCA-salt activates the splenic neuroimmune pathway mediated by PlGF, to activate T-cell migration from the spleen and infiltration in kidneys, causing blood pressure elevation and end-organ damage.	Perrotta et al[17]
	Bioelectronic stimulation of the celiac vagus nerve activates a α-adrenergic signaling in the spleen that releases PlGF to activate T cells and promote their mobilization.	Carnevale et al[18]

Ang II indicates angiotensin II; ANS, autonomic nervous system; DOCA-salt, deoxycorticosterone acetate–salt; IFN-γ, interferon gamma; IL, interleukin; PlGF, placental growth factor; and SNS, sympathetic nervous system.

NEUROHORMONAL REGULATION OF BLOOD PRESSURE AND IMMUNE ACTIVATION

Ang II is the principal hormone influencing blood pressure and known to exert both systemic and central

actions, through neural networks that can differentially contribute to the development of hypertension. Emerging evidence supports a nonredundant role played by Ang II in the brain, during the onset of hypertension.[41] Notably, AT1a (Ang II type 1a) receptors are highly expressed in the circumventricular organs.[42,43] The subfornical organ is, for example, one of the circumventricular organs, enriched of AT1a receptors and characterized by fenestrated capillaries with a leaky blood-brain barrier. This particular anatomic conformation makes neurons and microglial cells in direct contact with circulating Ang II.[44,45] In fact, the expression of AT1a receptors in the subfornical organ is necessary for the onset of hypertension.[46] The circumventricular organs are structurally and functionally connected: subfornical organ sends axonal projections to the paraventricular nucleus of hypothalamus that is an important station for the control of sympathetic drive hypertension.[42,47] Indeed, the overexpression of AT1a receptors caused an increased sympathetic tone, promoting water intake and enhancing pressor responses to Ang II.[48,49]

Similar to the AT1 receptor, it has been demonstrated that also aldosterone receptors expressed in the brain are involved in blood pressure regulation. In an article published by Xue and colleagues they showed that both the AT1 receptor and mineralocorticoid receptor expressed in the brain are necessary for blood pressure increase. The intracerebroventricular infusion of mineralocorticoid receptor antagonists or AT1 receptor antagonist was effective in reducing blood pressure in both models of hypertension, suggesting that the functional integrity of both brain signaling pathways is necessary for the establishment of hypertension.

SPLENIC NEUROIMMUNE CROSS TALK RECRUITED BY HYPERTENSION

The finding that T-lymphocyte activation is a key process involved in central and peripheral mechanisms recruited by Ang II–induced hypertension[50] and the evidence that the absence of mature lymphocytes in mice lacking the *Rag1* gene protects from blood pressure increase[51] demonstrated that immune dysfunction is a crucial pathophysiological alteration of hypertension.[51] Nonetheless, it has been found that T lymphocytes are differently involved in hypertension, in a sex-dependent manner, suggesting that these cells interact with other factors contributing to hypertension.[52,53] Surprisingly, in the last years of research, other articles showed that *Rag1*$^{-/-}$ mice lacking the enzyme responsible for the formation of the T-cell receptor required for the generation of mature B and T cells maintained the B- and T-cell deficiency but lost their resistance to blood pressure increase in response to Ang II administration.[54,55] While these new observations indicate that other studies are necessary to unravel the causes of the loss of the protective phenotype, they do not undermine the general concept that the immune system is crucial for hypertension onset and progression, as demonstrated by a myriad of studies.[19]

The constant observation that activated lymphocytes migrate toward target vasculature led to the hypothesis that the establishment of a vascular-immune interface represents a key step in the alterations that determine blood pressure dysregulation. To look into the cellular and molecular mechanisms underlying the establishment of vascular-immune interfaces at peripheral organs, it has been developed in an in vivo 3-dimensional organ co-culture system that chronically allows culturing immune cells and resistance arteries the same time, hence reproducing the cell-cell interactions that occur in vivo.[56] The particularly innovative aspect of this system is determined by the fact that resistance arteries are kept pressurized and constantly subjected to inner and outer flow, hence mimicking the in vivo condition of a functional artery. By this way, it has been demonstrated that while CD4 T lymphocytes isolated from hypertensive mice do not modify vascular function of cocultured naive resistance arteries, hypertensive CD8 T lymphocytes significantly increased the myogenic tone of vessels, hence suggesting that they have key functions in the increased peripheral resistance typically associated with hypertension[56] (Table).

However, CD4 T cells might have other roles in modulating vascular function. As an example, the previously defined ChAT CD4 T lymphocytes, activated in the spleen as a consequence of noradrenaline release, impact on endothelial function by synthetizing acetylcholine, which has vasorelaxant functions responsible for endothelial-dependent arterial relaxation.[57]

In our previous work, we demonstrated that immune system is activated by a nervous drive and is mediated by the angiogenic growth factor named PlGF (placental growth factor). The disruption of sympathetic innervation of the spleen proved effective in hampering priming of immune system and blood pressure increase.[15–17] With a putative role of multitasking cytokine, PlGF can be produced by various cell types in different pathophysiological contexts. Yet, the cellular and molecular mechanisms underlying how hypertension converts a neural stimulus into PlGF-mediated immune activation is still undisclosed. Our data showed that both hypertensive stimuli and bioelectronic stimulation of the celiac vagus nerve increase PlGF release in the marginal zone of the spleen where it mediates the interaction between ANS and immune response.[15–18] Particularly, we showed that the noradrenergic signaling that induces PlGF release in the spleen is mediated by α-adrenergic receptors. Both α- and β-adrenergic receptors are expressed by splenic cells (Immunological Genome Project database, 2020), but only the blockade of α-adrenergic receptors, selectively

in the spleen, hampered PlGF upregulation and immune system activation, thus suggesting the dependence of PlGF activation on α-adrenergic signaling[18] (Table).

A recently published single-cell transcriptomic analysis of FRCs revealed the existence of diverse fibroblast cell niches in mouse spleen and defines their roles in orchestrating the immune responses. This work examined splenic fibroblastic stromal cell heterogeneity by single-cell RNA sequencing in mice at basal conditions, by clustering total spleen CD45-CD31-Ter119-stromal cells from mice (Figure [A]). Interesting to note, when we reanalyzed the publicly available data[4] (http://muellerlab.mdhs.unimelb.edu.au/frc_scrnaseq/), we found the expression of *Pgf* gene in the various fibroblastic stromal cell types, with T-cell zone reticular cell clusters in the white pulp having the highest expression of *Pgf* compared with other FRCs (Figure [B and C]).

These observations indicated that PlGF could be produced by various cell types like FRC subtypes that, besides performing structural functions, might also mediate the neuroimmune cross talk established by a variety of pathophysiological stimuli. Future studies will be necessary to unravel the complexity of neuroimmune synapses established in the spleen and to verify the role of that cross talk in the context of hypertension and cardiovascular diseases.

INVOLVEMENT OF THE SPLENIC/SPLANCHNIC DISTRICT IN HUMAN HYPERTENSION

Few data are available regarding the role of the spleen and splenic sympathetic innervation in humans in

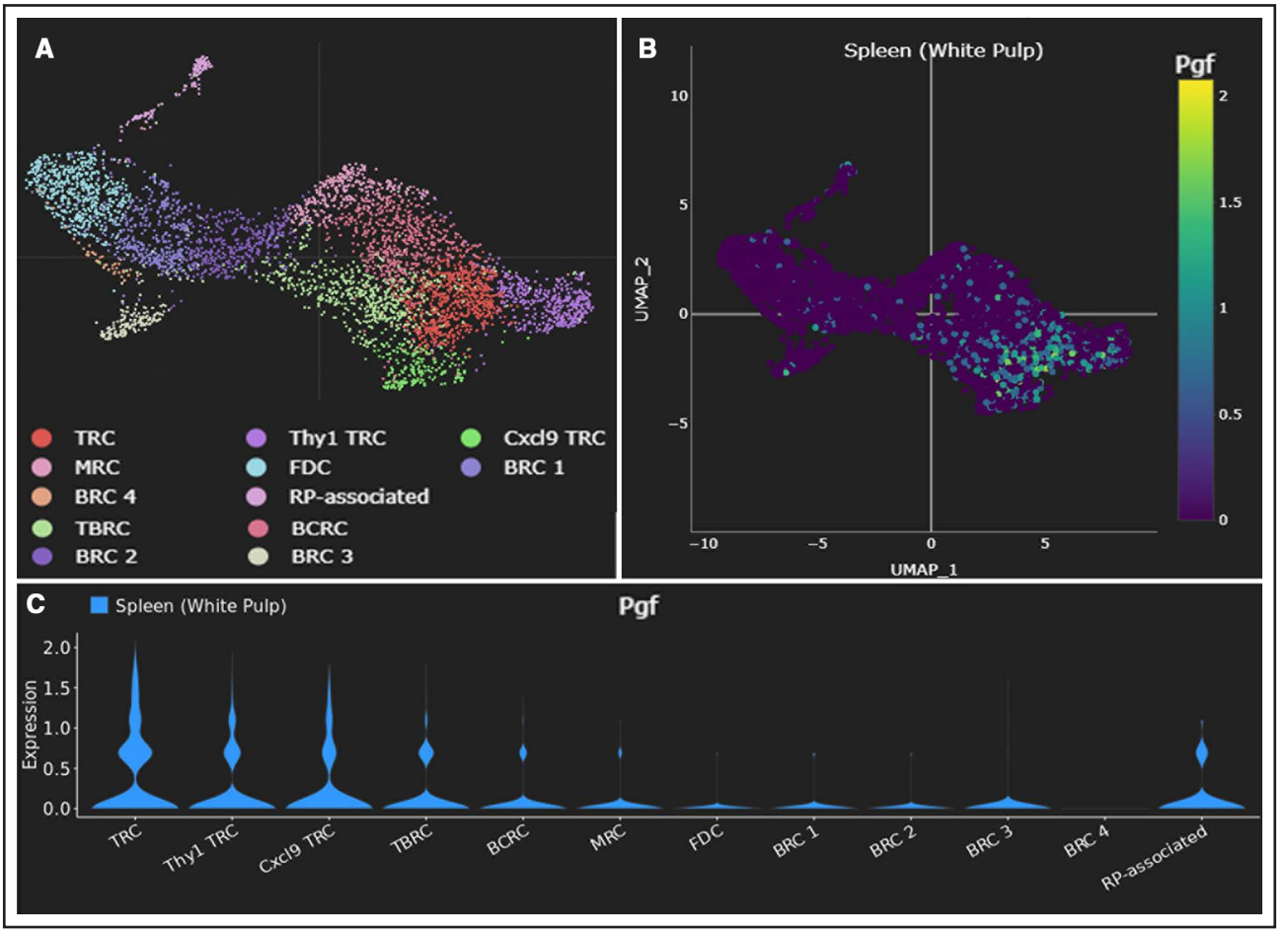

Figure. scRNA sequencing analysis at homeostasis of *Pgf* gene expression in fibroblast reticular cells (FRCs) of the white pulp of the spleen.

A, FRC distribution in the white pulp of the spleen; representation of *Pgf* expression analysis in FRCs by (**B**) UMAP plot and (**C**) violin plot. Bridging channel reticular cells (BCRCs) acting as an entry point for leukocytes in the white pulp. BRC 1 indicates B-cell zone reticular cell cluster 1; BRC 2, B-cell zone reticular cell cluster 2; BRC 3, B-cell zone reticular cell cluster 3; BRC 4, B-cell zone reticular cell cluster 4; Cxcl9 TRC, T-cell zone reticular cells expressing Cxcl9; FDC, follicular dendritic cell; MRC, marginal reticular cell; RP-associated, red pulp–associated reticular cell; TBRC, T-B reticular cell; Thy1 TRC, T-cell zone reticular cells expressing the surface marker Thy1 (CD90); and TRC, T-cell zone reticular cell. Figure and data were obtained by the publicly available online database (http://muellerlab.mdhs.unimelb.edu.au/frc_scrnaseq/) from the Mueller Laboratory with permission, as published in the study by Alexandre et al.[4]

relation to the risk of developing hypertension and cardiovascular diseases. On the other hand, a potential risk of developing pulmonary hypertension has been related to spleen removal in patients. Pulmonary hypertension is characterized by progressive pulmonary artery remodeling, which leads to increased right ventricular pressure overload and consequent right heart failure and premature death. Patients splenectomized for hemolytic disorders manifest hypercoagulability that associates with pulmonary hypertension. These patients showed increased incidence of pulmonary thromboembolism due to the absence of thrombus resolution causing the obstruction of the pulmonary arteries and subsequent pulmonary hypertension.[58] These considerations raise a concern on the aspects that should be considered when approaching at the splenic district. However, while splenectomy in mice was a useful model to demonstrate the role of this immune organ in the context of hypertension and cardiovascular disease, other approaches that preserve that integrity of this immune reservoir should be pursued for translation to the clinical setting. On this topic, many years ago, it has been shown that surgical celiac ganglionectomy attenuates blood pressure in essential hypertensive patients with no negative side effects of the surgical procedure on the global health of the patients.[59] Abdominal pain has also been treated with celiac plexus or splanchnic nerve block by surgical or pharmaceutical treatment in patients.[60] Based on these approaches, a case report showed that a patient with resistant hypertension who was unresponsive to antihypertensive agents was subjected to celiac plexus block by botulinum toxin administration obtaining a decrease in systolic blood pressure from 170 to 180 mm Hg to 150 mm Hg up to 4 months after treatment.[61] Among these, the selective denervation or a targeted neuromodulation of the spleen could be effective and, at the same time, without the deleterious consequences of organ removal. Also, pharmacological approaches aimed at directly modulating the signaling pathway mediating the role of the splenic neural drive, namely PlGF or downstream targets, could be a valuable strategy.

OTHER NEUROIMMUNE INTERACTIONS INVOLVED IN HYPERTENSION: THE CROSS TALK BETWEEN SNS AND BONE MARROW IMMUNE CELLS

Sympathetic efferent fibers also innervate primary immune organs, providing other routes of connection between the brain and the immune system. Sympathetic fibers in the bone marrow are localized in proximity of hematopoietic and lymphopoietic cells where, by releasing the neurotransmitter norepinephrine, they modulate hematopoiesis and immune cell migration.[62,63] Hematopoietic cells circulate in the bloodstream under steady-state conditions, and the SNS contributes to the regulation of circulating levels of different immune cells. The release from the bone marrow is rhythmically regulated in a circadian manner through diurnal variations in noradrenaline secretion, mediated by the effect of sympathetic nervous fibers[64] acting on β-adrenergic receptors expressed by hematopoietic cells.[63,64] SNS-mediated release of inflammatory cells in the circulation and recruitment to peripheral tissues occurs during the highest activity period both in mice and in humans.[64,65] Loss of circadian rhythmicity of the bone marrow cell release is strictly associated with elevated sympathetic drive to the bone marrow and altered adrenergic receptor signaling.[66]

The circadian blood pressure rhythm is characterized by night blood pressure levels 10% to 20% lower than those measured during the day. The alteration in blood pressure rhythm can produce different phenotypes: dipper hypertension, defined as a reduction in systolic and diastolic blood pressure of 10% to 20% from the daytime blood pressure; nondipper hypertension, a reduction in systolic and diastolic blood pressure of <10% from daytime to nocturnal blood pressure; reverse-dipper hypertension characterized by nocturnal blood pressure increase higher than daytime.[67] The alteration of the circadian rhythmicity influences the SNS activity and immune cell activation. It has been shown that systemic inflammation is higher in the reverse-dipper hypertension in newly diagnosed patients than in the dipper and nondipper groups.[68] Indeed, reverse-dipper hypertension is associated with increased mortality for cardiovascular disfunction, chronic kidney disease, and increased sympathetic nerve activity,[69] confirming the strict relationship between circadian blood pressure regulation and neuroimmune response.

Much evidence shows that the nervous system interacts with the immune system in the pathophysiology of cardiovascular diseases[70] and an impaired sympathetic nerve activity supplying the bone marrow release of inflammatory cells.[66] Ahmari et al explored the molecular cross talk between the nervous system and the immune system in the bone marrow, mediated by β1 and β2 adrenergic receptors and responsible for modulating immune homeostasis and regulating blood pressure. In brief, they transplanted bone marrow cells isolated from mice lacking β1 and β2 adrenergic receptors into wild-type irradiated mice and found decreased blood pressure and less circulating inflammatory T cells, monocytes, and neutrophils.[71]

On the other hand, an exaggerated immune system activation that occurs during cardiovascular disease is able to affect the brain. Bone marrow is a significant source of inflammatory cells in hypertension, and the infiltration of T cells and monocytes-macrophages in the paraventricular nucleus of the hypothalamus has been associated with neuroinflammatory responses

and ANS dysfunction during cardiovascular disease.[72] In the experimental model of Ang II–induced hypertension, it has been shown that the elevation of sympathetic drive in the bone marrow initiates systemic inflammatory responses in concurrence with elevation of blood pressure that in turn causes immune cell infiltration in the brain and microglia-dependent neuroinflammation.[72] These data evidence that an imbalance in the interactions established between immune system and ANS may exacerbate both inflammatory responses, neuroinflammation, and ANS dysfunction in cardiovascular diseases like hypertension.

ANS-GUT CIRCUIT MODULATES IMMUNE RESPONSE IN CARDIOVASCULAR DISEASES

In the framework of the intricate neuroimmune interactions established in different organs participating in the pathogenesis and progression of hypertension, it has more recently highlighted the role of the gut microbiota. Known to establish symbiotic relationship with the host, the vast spectrum of microbial metabolites can influence host physiology. Disturbance in the composition and function of microbiota is now recognized to play fundamental roles in various diseases, and the aberration of the compositions or functions of the gut microbes is strongly associated with hypertension.[73,74] However, it is still unclear whether dysbiosis plays a causative or supportive role or is simply a bystander of its progression due to morphological or functional alteration of the gastrointestinal or immune organs.

Gut microbiome and metabolites are involved in the regulation of blood pressure. A recent work showed that in humans, the variety of gut microbiome, microbial metabolites, and differentially prevalent bacteria taxa are associated with blood pressure variability. More specifically, *Alistipesfinegoldii* and *Lactobacillus* are associated with lower blood pressure variability and *Clostridium* and *Prevotella* with higher blood pressure variability.[75] Indeed, microbial colonization can influence blood pressure sensitivity to Ang II and related organ damage. The kidneys are characterized by a high sensitivity to microbial influence. It has been shown that in germ-free animals, the blood pressure response to Ang II was attenuated.[76] On the contrary, a more recent paper showed that germ-free mice exhibited high excretion of albumin with the urine, renal fibrosis, and inflammation compared with microbial colonized mice. Microbial metabolites alter total serum metabolome, and the worsening of renal damage in hypertensive, germ-free mice is due to naive T cells more readily polarized toward the proinflammatory Th17 phenotype induced by changes in the metabolic profile.[77] However, the field of microbiome–immune system interaction in the context of cardiovascular diseases is still a growing area of research that necessitates further studies for deeper understanding.

Besides elevated blood pressure, hypertension in human patients is also associated with heterogeneous multiorgan disorders, dysregulated proinflammatory response, and sympathetic overactivation,[78] indicating the existence of a gut-brain and gut-immune axis in both homeostatic and pathological conditions. An experimental model of hypertension in mice showed a reduction of tight junction protein expression and increased gut permeability during the prehypertensive phase. The alteration of gut morphology has been associated with inflammatory cell infiltration in the intestinal wall and increased sympathetic drive.[79]

SNS innervates the layers of the gastrointestinal tract through a rich network of vagal connections extended from the brain and nodose ganglia to prevertebral sympathetic ganglia. Noradrenergic fibers originating in the celiac-mesenteric ganglia, inferior mesenteric ganglia, and pelvic ganglia affect gastrointestinal functions.[80] In turn, the gastrointestinal tract influences the central nervous system via extensive vagal afferent nerves. Disturbances in vagal efferent modulation and increased sympathetic nerve activity have been correlated with increased inflammatory response both systemically and in the gut in hypertension.[66] Nervous and immune systems have mutual relationship: while immune cells can influence neuronal function by releasing different cytokines acting as immunologic mediators, neural pathways can regulate peripheral immunity via an inflammatory reflex mechanism,[81,82] and, in turn, gut microbes could influence the mutual interaction between the enteric nervous system and resident macrophages.[81,83] This evidence demonstrates that the gut microbiota is a key mediator of the gut-brain-immune cross talk, and the alteration of microbiota composition and function influences the heterogeneity of hypertension phenotypes by influencing the immune and nervous responses during the onset and progression of hypertensive disease.

Overall, only the gut microbiome does not participate in the onset of hypertension and related immune response; it has been reported in literature that other microbiomes are also involved. One example is the oral microbiome and his contribution in the establishment of inflammatory pathology like gingivitis and periodontitis. Recent observational studies and meta-analysis showed that there is a causal link between periodontitis and hypertension since it is frequently diagnosed in patients with hypertension.[84,85] In addition, patients with hypertension treated with drugs for periodontal therapy showed decreased levels of blood pressure and a reduction of circulating proinflammatory immune cells and cytokines associated with hypertension.[85] Overall, further studies are needed to strengthen the relationship between hypertension and oral microbiome–related inflammatory diseases.

CONCLUSIONS

The brain is a crucial station for the control of physiological homeostasis, and the ANS established a precise connection through neurons between the brain and peripheral organs. It has been known that the brain is a crucial regulator of blood pressure and peripheral immune responses during the disease establishment. Sensory neurons, which connect the periphery to the brain, are able to recognize peripheral immune alteration by the expression of cytokine receptors and inform the brain that establishes a reflex response to restore the physiological homeostasis. The ANS also contributes to the pathogenesis and progression of cardiovascular diseases: a dysregulation of ANS activity in peripheral lymphoid organs like the spleen induces an immune activation characterized by the egression of inflammatory cells in the circulation and the recruitment and infiltration in peripheral target tissue of hypertension where it could also modulate tissue-resident immune cells. Many molecular pathways participate in the regulation of vascular functions and immune response involved in blood pressure regulation.[15,50,51,86] Here, we reported that various cell types, like FRCs in the spleen, are able to respond to physiological stimuli by producing and releasing cytokines and soluble molecules like PlGF, involved in blood pressure modulation. However, there are still unidentified mechanisms that sustain blood pressure increase. How soluble molecules could shape immune responses in lymphoid and nonlymphoid organs needs further investigation, and future studies will be necessary to investigate the cross talk between SNS and splenic immune cells to unravel the neuroimmune mechanisms established in hypertensive disease.

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Disclosures

None.

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