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REVIEW

Neuroimmune interactions in cardiovascular diseases

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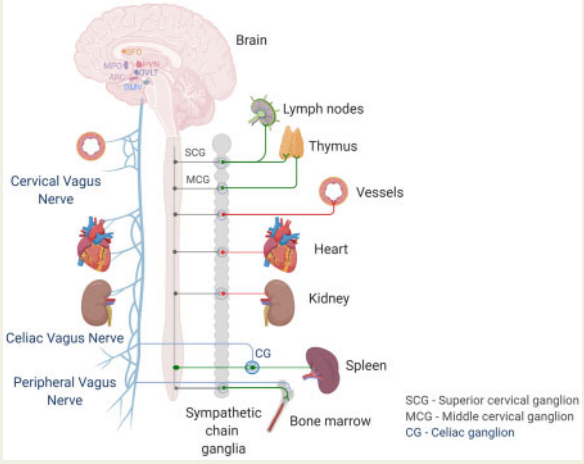
Abstract

Our body is continuously in contact with external stimuli that need a fine integration with the internal milieu in order to maintain the homoeostasis. Similarly, perturbations of the internal environment are responsible for the alterations of the physiological mechanisms regulating our main functions. The nervous system and the immune system represent the main interfaces between the internal and the external environment. In carrying out these functions, they share many similarities, being able to recognize, integrate, and organize responses to a wide variety of stimuli, with the final aim to re-establish the homoeostasis. The autonomic nervous system, which collectively refers to the ensemble of afferent and efferent neurons that wire the central nervous system with visceral effectors throughout the body, is the prototype system controlling the homoeostasis through reflex arches. On the other hand, immune cells continuously patrol our body against external enemies and internal perturbations, organizing acute responses and forming memory for future encounters. Interesting to notice, the integration of the two systems provides a further unique opportunity for fine tuning of our body's homoeostasis. In fact, the autonomic nervous system guides the development of lymphoid and myeloid organs, as well as the deployment of immune cells towards peripheral tissues where they can affect and control several physiological functions. In turn, every specific immune cell type can contribute to regulate neural circuits involved in cardiovascular function, metabolism, and inflammation. Here, we review current understanding of the cross-regulation between these systems in cardiovascular diseases.

Graphical Abstract



Keywords Nervous system • Autonomic nervous system • Immunity • Spleen • Bone marrow • Cardiovascular disease

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1. Introduction

The 20th century assisted to a change in the epidemiological scenario regarding the global burden of diseases. While the mortality and disability caused by communicable diseases significantly dropped, the non-communicable diseases raised their impact in a dramatic way. Among the non-communicable diseases, cardiovascular diseases (CVD) prevailed over all the other causes of mortality and morbidity worldwide.[1] Prevention strategies helped in developing effective approaches counteracting acute events and deaths from CVD.[2] However, in the last decade, basic and translational studies revealed several previously neglected pathophysiological mechanisms underlying CVD, which may not be adequately targeted by the current therapies. Hence the need of filling the gap between clinical epidemiological observations on classical risk factors for CVD and newly identified mechanisms sustaining and further aggravating CVD.

Over the last decade, there has been an intense investigation in the involvement of immune system in CVD, leading to the awareness that an altered immune response may represent a central underlying cause of CVD.[3–6] A predominant concept envisages that immune cells participate to physiological functions of the cardiovascular system and respond to perturbations of the homoeostasis. On this notice, the connection established between cells of the cardiovascular system and immune cells, at the steady-state and in pathological conditions, became the focus of mechanistic investigation, fuelling an impressive stream of basic and translational studies. The resulting growing body of evidence led to the notion that immunity is a master regulator of CVD. Until then, the only other known system capable to profoundly modulate cardiovascular function was identified in the nervous system through the enormous array of reflexes established by the autonomic nervous system.[7,8] The initial stages of our understanding of the autonomic nervous system control on cardiovascular function can be dated back to several decades ago.[9,10] For many years, the investigation in the pathophysiological roles of the sympathetic and the parasympathetic nervous systems in CVD dominated the scene, producing a huge amount of data clearly showing that autonomic neurohumoral imbalance can dramatically influence CVD morbidity and mortality.[11,12]

Interestingly, the nervous and immune systems share many similarities, being able to monitor and promptly respond to perturbances of the homoeostasis. The synergy and the cross-regulation established between the two systems accomplish a further level of control exerted on steady-state and pathophysiological conditions. The concept of neuroimmune communication was proposed as early as at the beginning of 1900 when preliminary observations highlighted the possibility that inflammatory mediators affected the nervous system and *vice versa* (extensively reviewed elsewhere).[13–15] In more recent periods, prominent studies revealed that lymphoid organs are innervated,[16–19] opening to studies aimed at exploring the functional consequences of neuronal activation on the immune system. On the other side, various cell types belonging to the immune system manifested their own ability to produce neurotransmitters, raising the possibility of a non-neuronal regulation of the local tissue milieu.[20]

The mounting evidence of a bilateral communication between the immune and nervous systems gave rise to a resurging interest in exploring the pathophysiological and molecular mechanisms responsible for neuroimmune crosstalk in various disease contexts. This review aims at providing a contextual background, highlight recent advances, and challenges in the field of CVD.

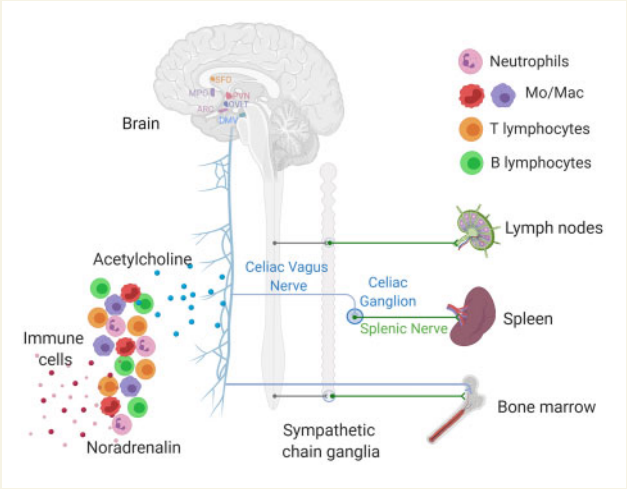

Figure 1 Anatomical basis of the neuroimmune communication. Neuroanatomical and humoral routes establish a bilateral connection between the central nervous system and the periphery. Organized in the two branches consisting of somatic and autonomic systems, the peripheral nervous system forms the interface ith the external and internal milieu, respectively. The neural control of immune system is based on hard-wired connections formed by afferent and efferent arms of the autonomic nervous system. Humoral and cell-based routes of neuroimmune communication are permitted by the expression of cholinergic and noradrenergic systems in immune cells. Created with BioRender.com.

2. Overview of the anatomical basis of the neuroimmune communication

The mutual interaction established between the nervous and immune systems is mainly based on neuroanatomical and humoral routes connecting the central nervous system (CNS) to the periphery and vice versa. The peripheral nervous system represents the part of the nervous system that is outside the brain and spinal cord, anatomically connecting the CNS to peripheral tissues. Mainly organized in the two branches consisting of somatic and autonomic systems, the peripheral nervous system allows interfacing with the external and internal milieu, respectively. Each system is further organized in two arms composed of sensory/afferent neurons carrying information from the periphery to the CNS, and motor/efferent neurons sending inputs outwards to effector tissues. Besides the physical networks established between peripheral tissues and CNS by sensory/afferent neurons, a humoral route does exist as well. Both possibilities will be detailed in the context of immune to nervous system communication (*Figure 1*). Then, the opposite way will be considered, i.e. how the nervous system communicates with the immune system.

2.1 CNS—the system of circumventricular organs

The humoral route of CNS activation by perturbations of peripheral tissues' homoeostasis requires that blood-borne metabolites, bacterial-derived substances, or host-derived cytokines are sensed by the brain. It is generally recognized that the transmission of such 'danger' or 'alarm' signals from the blood to the CNS occurs in those brain regions where

the blood–brain barrier (BBB) is less tight. In fact, the BBB is characterized by a highly regulated permeability, aimed at ensuring that neurons are protected from potentially dangerous substances. However, a structural adaptation of the endothelium in specific brain regions, called circumventricular organs (CVO), allows the detection of blood-borne substances, guaranteeing the brain a constant monitoring of hormones and metabolites in the periphery. The CVO are organized in two groups with different functions, mainly ascribable to sensory and secretory roles.

Among the sensory brain regions, the subfornical organ (SFO), the organum vasculosum lamina terminalis (OVLT) and the area postrema (AP) are the essential players of the neuroimmune communication.[20] Interestingly, prominent works of the last decade also highlighted their involvement in the modulation of cardiovascular function.[21–23] In fact, the axonal projections of the AP innervate the nucleus tractus solitarius (NTS) and the dorsal motor nucleus (DMN) of the vagus nerve, which is a master regulator of cardiovascular function.[24] The SFO is enriched of angiotensin II (Ang II) receptors, whereas the OVLT comprises osmoreceptors responsible for sensing extracellular solute concentrations (mainly sodium). By this way, the SFO and the OVLT, together with the median preoptic nucleus, elaborate the peripheral variations informing on the status of blood volume, pressure, and extracellular fluid osmolality.[24]

The axonal projections reaching the hindbrain structures of the NTS establish a further rostral connection to brain regions located in the hypothalamus and the amygdala and along the lamina terminalis.[20] In particular, the paraventricular nucleus (PVN) of the hypothalamus receives both ascending input from the hindbrain and descending projections from the SFO-OVLT. As such, the PVN acts as an integrative brain station processing information that is important for maintaining cardiovascular homoeostasis and body fluid. In turn, the PVN gives rise to sympathetic premotor neurons that project to the intermediolateral cell column of the spinal cord, either directly or indirectly via the rostral ventrolateral medulla (RVLM). Several observations suggested that various peripheral perturbations, including hypotension, hypoglycemia, hypoxia, hypercapnia, and bacterial infection, could activate the locus coeruleus via a nucleus of catecholaminergic/glutamatergic cells residing in the RVLM.[25,26] Interestingly, the locus coeruleus represents the largest cluster of noradrenergic neurons in the brain and its activity facilitates arousal and attention, and controls noradrenergic firing in various CNS areas as well as towards peripheral tissues.[25,27] The intermediolateral cell column comprises the preganglionic neurons of sympathetic axons exiting the CNS.

Usually, the above delineated neural pathways mediate responses to peripheral challenges associated with altered cardiovascular regulation and/or fluid balance. However, it has been recognized that the brain network that controls sympathetic nervous system activity may be also recruited by psychosocial stressors. Although less known and defined at a mechanistic level, the modulation of neural reflexes controlling cardiovascular function by psychosocial stressors is becoming an area of growing interest in the field of CVD.[28]

The brain regions of the CVO responsible for secretory functions are the pineal gland, median eminence, neurohypophysis, and the subcommissural organ, which are reviewed elsewhere.[29,30]

2.2 Role of the PNS in sensing perturbations of the peripheral inflammatory milieu

Afferent neurons of both the autonomic and the somatosensory peripheral nervous systems express receptors for inflammatory and microbial peptides, thus suggesting their involvement as hardwired connection signalling inflammatory and immune reactions from the periphery to the brain. Vagal and spinal neurons are the two main neural pathways accomplishing the above functions.

Belonging to the series of cranial nerves, the vagus nerve is the longest one throughout the body, providing innervation to a wide variety of tissues, comprising the heart, the gastrointestinal tract, and lungs. Made of about 70% of afferent axons, vagus nerve activation can be triggered by a variety of stimuli, ranging from micro-nutrients to mechanosensitive receptors in the respiratory and cardiovascular systems.[20] With cell bodies engulfed in the nodose and jugular ganglia, once activated in the periphery, the vagus nerve determines synaptic transmission in the NTS. In turn, the neural signal is integrated and transmitted along the DMN, where preganglionic neurons reside and give rise to the efferent neurons, responsible for reflex modulation of target tissues.

In the context of immune and inflammatory reactions, the vagus nerve directly responds to stimuli arising from inflammatory mediators like pathogen-associated molecular patterns[31–33] and danger-associated molecular patterns, like ATPs and cytokines.[20] A prototype activator of afferent vagus nerve is the endotoxin of Gram-negative bacteria, lipopolysaccharide (LPS), sharing with other pathogen-associated molecular patterns a molecular motif able to bind specific toll-like receptors (TLR).[20] Interestingly, the expression of TLRs is a feature shared by both afferent neurons of the peripheral nervous system and immune cells, proposing this molecular pathway as a candidate mediator of neuroimmune mutual interactions. Among the various TLRs, the selective receptor for LPS, TLR4, is the only one expressed by vagal afferent neurons.[34] The evidence that a subdiaphragmatic vagotomy hampered the NTS activation by peripheral LPS,[35] further supported the concept that afferent neurons of the vagus nerve serve as detectors of infection, for signalling the information to the brain, which in turn can integrate immunomodulating reflex functions. The exposure to infectious agents is also accompanied by the activation of proinflammatory cytokines from a plethora of immune cells. Hence, the resulting alteration of the local environment has a dual implication, being able to modulate local inflammation and, at same time, activating specific receptors on afferent fibres to function as alarmins for the brain.

The somatosensory peripheral nervous system encodes specific subsets of neurons whose cell bodies reside in dorsal root ganglia (DRG) and are responsive to various perturbations of the peripheral environment, like physical, thermal, or chemical stimuli. Afferent neurons passing through the spinal cord express receptors for molecular inflammatory mediators as well.[20] Although these neurons are primarily characterized by the ability to sense and transfer noxious information to the brain, at the same time they can also act as modulators of the local environment by releasing neuropeptides. One of the most relevant examples is the capability of peripheral terminals of spinal afferent neurons to release substance P and calcitonin gene-related peptide.[36–38]

2.3 Role of the PNS in the regulation of the immune system

One of the earliest evidence suggesting the existence of a mutual structural and functional interaction established between nervous and immune systems, was the histological observation that primary and secondary lymphoid organs are densely enriched in nerve fibres.[16–19] Almost 40 years ago, experiments exploiting labelling techniques of anterograde and retrograde neurons, mapped the hardwired connections of sympathetic and peptidergic innervation in primary, secondary, and

mucosa-associated lymphoid tissues.[16,18,19] This work shed light on the potential routes of neural regulation of immune responses.

2.3.1 Bone marrow

Like most of the organs, bone is densely innervated by autonomic nerves. Besides reaching the bone's skeletal structure, nerves also penetrate the bone marrow, entangling deep regions where the haematopoietic activity takes place, i.e. the haematopoietic stem cells (HSCs) niches, responsible for generation of all blood cell lineages. The dense innervation that reaches out bone and marrow is comprised of sympathetic, parasympathetic, and sensory fibres and provides the basis for the neural regulation of processes taking place in these anatomical locations: bone formation, haematopoiesis, and immune functions. Thus far, it has been generally recognized that parasympathetic nerves primarily regulate processes of bone remodelling.[39,40] On the other hand, the sympathetic innervation is involved in suppression of bone formation,[41] but also in fine tuning of the haematopoietic activity.[42]

In physiological conditions, when HSCs usually remain in a quiescent state, the circadian oscillations of noradrenaline regulate the expression of genes involved in the retention/release of HSCs from the bone marrow.[43,44] When various stimuli perturbate the homoeostasis, the sympathetic nerves entangling the bone marrow activate HSCs.[45] Although the most typically recognized condition of HSCs recruitment by the SNS is related to the development of haematological malignancies,[46] it has becoming clear that haematopoiesis is also crucially involved in CVD. Specifically, it has been demonstrated that HSCs mobilized as a consequence of stressful stimuli imposed on the cardiovascular system, migrate to the spleen for contributing to extramedullary monocytopoiesis, needed to supply the increased demand of immune cells to the myocardium.[47,48] Interestingly, it has also been shown that cholinergic signals originating in the brain regulate HSCs mobilization through glucocorticoid-related mediators.[49] Upon integrating the various signals, HSCs adjust their activity to regulate the balance between quiescence or production of mature blood cells, according to the body's necessity.

An interesting amount of recent basic science and epidemiological studies started to investigate the role of the clonal expansion of HSCs, a process now referred to as clonal haematopoiesis.[50–52] The presence of cells with clonal haematopoiesis potential in the peripheral blood was associated with nearly a doubling in the risk of coronary heart disease in humans and with accelerated atherosclerosis in mice.[51] Interestingly, it was found that the epigenetic regulator *Tet2* presents frequent mutations in blood cells of subjects displaying clonal haematopoiesis. At the mechanistic level, when *Tet2* was deficient in haematopoietic cells of murine models of heart failure, mice showed greater cardiac dysfunction associated with elevated IL-1β signalling.[52] Taken together, these data suggest that *Tet2*-mediated clonal haematopoiesis may determine an increased risk of developing heart failure and that, at the same time, those patients may better respond to strategies inhibiting the IL-1β–NLRP3 inflammasome pathway.[53]

2.3.2 Lymph nodes

The vasculature and lymphatic channels that enter lymph nodes in the medulla and then branch into small parenchymal vessels are innervated by noradrenergic fibres.[17,54] In addition, it has been described that the medullary and paracortical structures of the lymph nodes contain nerves non-associated with blood vessels.[55] Conversely, the nodular regions and the germinal centres are spared from noradrenergic fibres.[17]

Although the adrenergic innervation of lymph nodes was recognized for long time, only more recently it was found that noradrenergic neurons regulate immune cell function. By using experimental models of T cell-mediated inflammation, it has been demonstrated that neural signals, transmitted through β2-adrenergic signals in lymph nodes, restrict T cell egress, thereby modulating the ensuing tissue inflammation.[56] It was later shown that, as adrenergic nerves release noradrenaline in a circadian way, lymphocytes are subjected to diurnal recirculation in lymph nodes through a mechanism dependent on β2-adrenergic receptor.[57,58]

2.3.3 Spleen

While devoid of direct cholinergic innervation, the spleen is innervated primarily by noradrenergic efferent fibres branching from the superior mesenteric coeliac ganglion.[59,60] The fibres entering the spleen entangle the splenic artery, travelling along the vasculature, until reaching the white pulp. In fact, the vast majority of sympathetic neurons branching in the spleen is associated with the central artery irrorating the white pulp.[16,19] Noradrenergic innervation through axons of the sympathetic nervous system reaches the T-cell area, the marginal zone comprising macrophages and B cells, the site of the lymphocyte entry into the spleen.[16,18] Red pulp innervation is instead sparse and mainly composed of scattered fibres.[19]

The spleen carries out numerous functions, among which the regulation of adaptive immunity and antibody production are the most commonly known.[61] However, the spleen is also a monocyte reservoir that is recruited in response to tissue injury.[48,60,62–64] How the neural innervation of the spleen participates in the various immune-related processes by both autonomic (efferent) and sensory (afferent) fibres, is object of intense investigation in the field of CVD.

2.3.4 The cholinergic inflammatory reflex

One of the best-known functions of splenic neural innervation is the immune-modulation in the context of the so-called 'inflammatory reflex'.[65] The peripheral inflammatory and immune environment is perceived by sensory neurons that are located in proximity to immune cells and respond to perturbations to communicate signals to the brain. On this notice, the spleen plays a crucial role in the acute response to inflammatory/immunological stimuli, which include the bacterial endotoxin peptide LPS.[66] Although the induction of proinflammatory cytokines in response to inflammatory challenges like LPS has been conventionally considered as a response dependent on local and/or systemic immune response, in the last decade, we learned that this phenomenon is potently modulated by neural influence.[65,67]

A series of experiments conducted by electrical stimulation demonstrated that efferent fibres of the vagus nerve are able to control LPS-induced endotoxemia by dampening the release of proinflammatory cytokines, like TNF.[67] It was shown that, when LPS perturbs peripheral homoeostasis, vagus nerve afferent activity signals the danger to the brain, integrating the consequent response with efferent vagus nerve activity, to control peripheral cytokine levels and inflammation.[65] The effector arm of this neuronal circuit is represented by the splenic nerve, which exerts modulating functions on spleen-residing immune cells.[66] In more details, it was shown that the catecholaminergic nerve endings of the splenic nerve entangles immune cell areas in the proximity of lymphocytes, including a specific sub-population of T cells, which express choline acetyltransferase (ChAT), the enzyme responsible for acetylcholine biosynthesis.[66] Hence the electrical stimulation of the vagus nerve determines acetylcholine release by ChAT-expressing T cells through

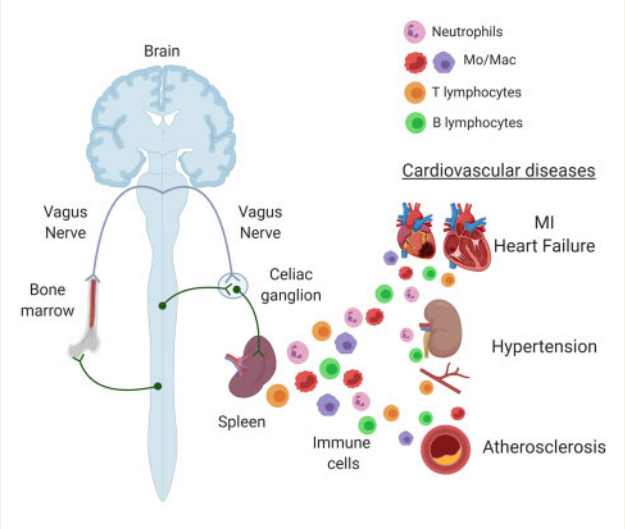

Figure 2 Neuroimmune interactions involved in cardiovascular disease. Cardiovascular investigations evidenced the mechanisms underlying the neuromodulation of immunity in various settings of cardiovascular diseases: hypertension and renal damage, myocardial infarction, heart failure, atherosclerosis, and metabolic disorders. The latest findings clearly demonstrate that the role of immunity in cardiovascular diseases is not limited to contributing to tissue damage, but is also a critical pathophysiological mechanism implicated in the aetiology, partly dependent on neural control.

catecholaminergic, β2-adrenergic receptor signalling.[66] The final effect of T cell-mediated neurotransmitter release in the spleen, upon vagus nerve stimulation, is lastly executed by a specific population of macrophages expressing the α7 nicotinic acetylcholine receptor (α7nAChR), which transduce anti-inflammatory intra-cellular signalling.[68] Hereafter, the neuronal reflex controlling splenic production of TNF and systemic inflammation was termed the 'cholinergic inflammatory pathway'.

3. Neuro-immune interactions involved in CVD

For many decades, cardiologists considered the 'fight or flight' responses orchestrated by the autonomic nervous system as the only reflexes regulating the cardiovascular system. In fact, the imbalance between the sympathetic and parasympathetic arms of the autonomic nervous system typically accompanies many CVD and interacts with the renin–angiotensin–aldosterone system, the other master regulator of cardiovascular function.[69] By overlooking the advances emerging from investigations in the mechanisms underlying the neuromodulation of immunity, the field of cardiovascular research continued to pursue for a long-time separate path of investigation. In fact, the role of immunity in CVD was mainly considered as a bystander effect of local tissue damage, before being recognized as a critical pathophysiological mechanism implicated in the aetiology (*Figure 2*).[3,70]

3.1 Hypertension

Very old observations suggested an involvement of immune system in the onset of hypertension.[71–73] However, until more recently, the activation of immune and inflammatory processes in hypertensive models and humans was mainly considered as a phenomenon related to the ensuing target organ damage. In 2007, a ground-breaking work elegantly demonstrated that lymphocytes are necessary to increase blood pressure in the widely used experimental model of hypertension induced by chronic infusion of Ang II.[74] Hereafter, many research laboratories started investigating the immunological basis of hypertension, with the rising perception that the current anti-hypertensive therapies still show inadequate or incomplete efficacy.[75,76]

One of the emerging questions was related to the possible mechanisms of interplay and integration of these novel concepts with the classical mechanisms regulating blood pressure. On this notice, the sympathetic nervous system circuits are among the archetypical mechanisms involved in the regulation of blood pressure levels.[8,11] The neurogenic regulation of key physiological parameters, including vascular tone and renal sodium excretion, is typically dominated by the sympathetic nervous system.[24,69,77]

The concept that neurogenic mechanisms of hypertension have the potential to control immune responses was also proposed by prior works highlighting the existence of immune-modulating functions of vasoactive agents like Ang II.[78] One of the first demonstrations that the brain controls peripheral inflammatory responses through the sympathetic nervous system came by works showing that the intra-cerebral ventricular infusion of Ang II drives the activation of immune response in periphery.[79] More mechanistic, it was shown that selective lesions of those brain regions with a leaky BBB, like the SFO, hamper the typical increase in blood pressure induced by the chronic infusion of Ang II through peripherally implanted osmotic minipumps.[80] In the absence of an intact SFO, the activation and infiltration of T lymphocytes in the vasculature was inhibited as well,[80] thus suggesting the existence of neural-mediated control of immune activation in hypertension.

Some years later, a mechanistic observation showed how the brain is connected to priming of immune responses in the spleen, during hypertensive challenges.[81] By using an approach of direct micro-neurographic recording of peripheral nerve activity, it was shown that, similarly to what observed in the renal district, Ang II increases the sympathetic outflow conveyed by the splenic nerve, enhancing the release of noradrenaline in the spleen.[63] The fact that the selective denervation of the neural drive on the spleen protected from blood pressure increase and immune activation upon Ang II, provided evidence of the critical role of this neural reflex in hypertension.[60,63] At the molecular level, the mediator of this neuroimmune pathway was also identified, whereby the release of noradrenaline in the spleen increases the expression of an angiogenic growth factor called Placental Growth Factor (PlGF),[60] paving also the way for new potential therapeutic strategies. Conversely to what is usually observed in the control of sympathetic outflow in hypertension, the preganglionic neuron that regulates the splenic nerve did not pass through the intermediolateral grey column of the spinal cord, where the massive bundles of sympathetic nerves controlling the cardiovascular system are hardwired to the brain.[24,25] It was in fact found that Ang II, but also other hypertensive challenges like DOCA-salt, recruits the splenic sympathetic nerve outflow through a vagus nerve connection established at the level of coeliac mesenteric ganglion.[63]

Interestingly, also one of the prototype neural pathways involved in the control of cardiovascular function, i.e. the sympathetic innervation entangling the kidney, withstood a new wage of investigation. In fact, although for many years the debate was centred on the efficacy of renal denervation, one of the most innovative strategies pursued for fighting hypertension,[82–84] recent evidence obtained experimental models of

hypertension has shown that renal denervation partly affects renal function through a previously unidentified modulation of immune responses in kidney.[85,86]

Altogether the above-described findings have clarified that hypertensive stimuli, like Ang II, do not increase blood pressure and recruit the immune response because of direct actions of the hormone on vasculature and immune cells. More interesting, the hypertensive stimuli, even when administered through peripheral routes, drive hypertension by activating those brain regions responsible for the recruitment of the neural pathways involved in regulation of immune function.

3.2 Renal disease

Blood pressure regulation is the result of a complex intertwining of various mechanisms, among which the kidney has always played a crucial role, in terms of handling of renal sodium, renin secretion, and the renal vasculature.[69] In addition, the interaction of renal mechanisms and the autonomic nervous system has been considered one of the mainstay regulators of cardiovascular function. Hence, perturbations in these regulatory mechanisms represent one of the most known factors leading to hypertension. On the other hand, chronic hypertension is associated with the development of renal failure as one of the most typical target organ damage ensuing as a consequence of long-standing conditions of high uncontrolled blood pressure.

Kidney innervation, in both the afferent and the efferent arms, forms one of the most well-known reflex system of the autonomic nervous system.[87] The increased renal sympathetic drive, typically observed in hypertension, represents the basis for the experimental and clinical approach of renal denervation for blood pressure-lowering strategies. Interestingly, a recent avenue of investigation highlighted an unprecedented role of the cholinergic inflammatory reflex in kidney disease,[88] thus paving the way to the dissection of molecular mechanisms underlying the neural control of immunity and inflammation in kidney disease.[89]

3.3 Heart failure

The autonomic nervous system is also a master regulator of cardiac function. In fact, the actions of parasympathetic and sympathetic branches of the autonomic nervous system tightly control myocardial contractility, conductance, and frequency, as well as vascular tone.[90,91] Much less is known about the neural control exerted by the autonomic nervous system on other non-myocytes cardiac cells. The increasing knowledge of the role exerted by the sympathetic nervous system on immune cells in various pathophysiological situations boosted the search of neuroimmune mechanisms underlying the typical relationship existing between chronic heart failure and over-activation of the sympathetic nervous system, which by itself considered one of the strongest predictors of negative outcome.[92]

The presence of inflammatory cells in the myocardium is typically observed upon acute ischaemic challenges and during the chronic evolution of heart failure.[3] For instance, acute ischaemia triggers myeloid production in the splenic reservoir by enhancing sympathetic signalling in the bone marrow.[3,47,48] In turn, this neuroimmune activation promotes the reduction of HSCs quiescence, stimulating the haematopoietic niche to replenish the spleen with monocytes egressed through CCR2 signalling.[42,48] A portion of the monocytes deployed from the spleen accrues in the ischaemic myocardium where it contributes to wound healing.[3,61]

The sympathetic nerve fibres departing from various ganglia, among which the superior cervical ganglia, the stellate ganglia, and upper thoracic ganglia, provide also direct innervation of the heart.[93] The efferent branches of these nerves entangle the myocardium, by projecting to cardiomyocytes and vasculature. However, it is also well known that immune cells populate the myocardium, both at the steady state and upon challenges, raising the question whether the nerve terminals exert neuroimmune functions. A recent work attempted at addressing this issue, by selectively denervating a major nervous station that gives rise to the innervation of the anterior myocardium, i.e. the superior cervical ganglia, in mice subsequently subjected to ischaemic challenge by ligation of the left anterior descending artery.[94] As expected, the ganglionectomy destroyed the sympathetic innervation in the left ventricular anterior wall but had no impact on the acute response to myocardial infarction.[94] However, the cardiac sympathetic denervation affected the chronic remodelling related to myocardial infarction by reducing the inflammatory infiltrate in the myocardium, resulting in a dampened cardiac dysfunction.[94]

Cardiac inflammation is not limited to acute myocardial infarction. Ensuing in conditions of chronic hypertension, renal failure, or other challenges that impose overload on the left ventricle, heart failure with preserved ejection fraction (HFpEF) is a condition characterized by expansion of cardiac macrophage numbers because of local proliferation and monocyte recruitment.[95,96] Similarly, in heart failure with reduced ejection fraction (HFrEF) the elevated circulating levels of pro-inflammatory cytokines and the increased number of cardiac macrophages revealed an important pathogenetic role for the immune system.[97] Even though a multitude of studies describes the involvement of immune pathways in the failing myocardium, the relationship established with the autonomic nervous system is still object of investigation.

3.4 Metabolic disorders and atherosclerosis

Uncovering how the neural control of immunity regulates the various cardiovascular risk factors impacting on the development of CVD may reveal new therapeutic targets. On this notice, it is becoming increasingly clear that many environmental modifiers deriving from lifestyle habits, as diet, exercise, stress, and sleep, affect chronic inflammatory diseases, including atherosclerosis.[28] In fact, epidemiological evidence and data obtained in experimental models indicate that high-fat/high-cholesterol diets and psychosocial stress exacerbate CVD, whereas healthy habits comprising regular exercise and adequate sleep provide some benefit.[28]

Obesity and type 2 diabetes affect the normal function of haematopoietic and immune cells and, at the same time, determine an imbalance of the autonomic nervous system. A recent study focused on the interactions established by the sympathetic nervous and the immune system in the context of diabetes and one of its main complications such as atherosclerosis.[64] It has been shown that catecholamines produced by leucocytes and sympathetic splenic nerve termini promote proliferation of haematopoietic cells, development of myeloid cells and recruitment to peripheral tissues.[64] The ablation of the splenic sympathetic innervation, obtained both by surgical and by pharmacological approaches, reduced the diabetes-induced splenic myelopoiesis and accumulation of inflammatory cells in the aorta, with an overall benefit on atherosclerosis progression and plaque formation.[64] Interesting to notice, the authors also found a significant correlation between the count of circulatory leucocytes and plasmatic levels of catecholamines in patients,[64] suggesting a translational relevance of the proposed interaction between sympathetic nervous and immune system in the context of diabetes and atherosclerosis.

Retention of macrophages enriched in lipid particles in the artery wall is a typical tract of the atherosclerotic plaque. Persistence of immune activation continuously fuels the plaques, leading to the progression of the

disease. Several studies have been performed to investigate the mechanisms underlying plaque formation and stability, but the potential interactions with neural regulation are just beginning to be identified. One of the earliest evidence associating a pure neural mechanism with immune activation in the arterial wall, was the discovery that netrin-1, a neuroimmune guidance cue, is produced in atheromatic macrophages in humans and mice, to inactivate their migration towards chemokines promoting their egress from plaques.[98] Hence, when netrin-1 was selectively deleted in macrophages, mice developed less atherosclerosis by promoting the emigration of macrophages from plaques. On the other hand, myeloid cells also accumulate in adipose tissue during obesity, inducing a state of chronic low-grade inflammation that is frequently associated with the development of insulin resistance.[99] An interesting subsequent work helped in elucidating that netrin-1 is also expressed in the fat tissue of obese humans and mice, suggesting the existence of a neural control of macrophages accrual in the adipose tissue as well.[100] In a way similar to that observed in the process of macrophages retention in the atherosclerotic plaque, it was shown that netrin-1 regulates the process of macrophages infiltration and retention in the adipose tissue in a model of high fat diet-induced obesity.[100]

On another notice, it has also been shown that neural signals can control the profile of activation of immune cells recruited during atherosclerosis. In the hypercholesterolemic *Ldlr*$^{-/-}$ mice, it was demonstrated that the absence of α7nAChR from bone marrow-derived cells aggravates the atherosclerotic process.[101] Interestingly, immune cells positive for the α7nAChR were found in human lesions.[101] Taken together, the above data suggested that cholinergic signals modulate atherosclerosis through α7nAChR expressed in immune cells, lastly inhibiting disease progression. Uncovering how the neural control of atherosclerosis is established may provide new therapeutic strategies.

The pressing priority of increasing the awareness of promotion of healthy lifestyles to reduce cardiovascular risk and consequent morbidity and mortality, raised the interest of scientific community in understanding the mechanisms through which lifestyle-related modifiers impact on disease onset and progression. An intriguing study clarified the molecular and cellular mechanisms underlying the well-known association existing between insufficient and/or disrupted sleep and increased incidence of CVD, highlighting a physiological role of the spleen in regulating haematopoiesis.[102] When sleep disturbances were induced by fragmentation, mice developed larger atherosclerotic lesions by producing more Ly6C high monocytes.[102] Interesting to notice, it was found that sleep-induced control of haematopoiesis is dependent on a neuroimmune molecular mechanism. In fact, the analysis of transcripts encoding proteins related to sleep regulation evidences a reduced expression of hypocretin in the hypothalamus of mice subjected to sleep fragmentation.[102] More interesting reduced hypothalamic hypocretin correlated with an increased leucocytosis that overall promoted monocytosis and accelerated atherosclerosis,[102] thus unravelling a neural control of the sleep-immune axis in atherosclerosis.

Epidemiological data frequently associate the advice of a regular physical activity together with other healthy habits. Known to potently influence the immune system and the risk of developing atherosclerosis, it is generally thought that all the benefits deriving from regular exercise are ascribable to improved metabolic balance that consequently lower cardiovascular risk by counteracting development of obesity. However, a recent mechanistic study demonstrated voluntary running reduces haematopoietic activity and protects from atherosclerosis in mice and in humans.[103] At the molecular level, voluntary running dampens leptin production in adipose tissue, promoting quiescence of the haematopoietic niche in the bone marrow.[103] Whether this effect underlies some interaction with neural mechanisms recruited by exercise remains to be elucidated.

4. Concluding remarks

Altogether the above discoveries provide compelling evidence that dysregulation of neuroimmune interactions is involved in the onset and progression of CVD. Despite the mounting body of data coming from animal studies and clinical observations, there remains a general scepticism in considering these aspects in the therapeutic approach at CVD. Drugs targeting the nervous system are comprised in the most used therapeutic strategies for various CVD. On the other hand, immunomodulating therapies for CVD are just at the beginning of their clinical investigation, especially after the CANTOS trial showed promising results in limiting cardiovascular mortality by using an IL-1β-specific monoclonal antibody.[104,105] The possibility to target dysregulated neuroimmune interactions is just dawning.

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