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Research article

CENTRAL NERVOUS REGULATION OF SPLEEN FUNCTION: NEW INSIGHTS FROM ANIMAL STUDIES

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ABSTRACT : The spleen is located in the upper left quadrant of the abdomen. It has two main functions that is acting as part of the immune system and as a filter. The spleen has a thin connective tissue capsule from which short septa extend inwards. These septa are, in turn, connected to a complex reticulin framework.There are two distinct components of the spleen, the red pulp and the white pulp. The red pulp consists of large numbers of sinuses and sinusoids filled with blood and is responsible for the filtration function of the spleen. The splenic venomotor fibres join the left phrenic nerve in the mid-cervical region. Coursing with it as non-medullated fibres, they eventually perforate the diaphragm, where for a time they accompany the inferior phrenic artery. Deviating towards the celiac ganglion, they next join company with the splenic vein, and are eventually distributed to localised parts of the vein. This review article evaluates the conventional knowledge and points to new insights into neural regulation of spleen.

Key Words : Spleen, Suprachiasmatic nucleus, pineal gland, T cells, immune.

INTRODUCTION

Splenic immune function is modulated by sympathetic innervation, which in turn is controlled by inputs from supraspinal regions. The white pulp of spleen consists of aggregates of lymphoid tissue and is responsible for the immunological function of the spleen. There is a complex system of blood vessels within the red pulp arranged to facilitate removal of old or damaged red blood cells from the circulation. A small proportion of the splenic blood flow passes through more rapidly without undergoing this process of filtration.

The white pulp contains T cells, B cells and accessory cells. There are many similarities with lymph node structure. The purpose of the white pulp is to mount an immunological response to antigens within the blood. The white pulp is present in the form of a periarteriolar lymphoid sheath. This sheath contains B cell follicles and T cells. At the edge of the T zone is a region known as the marginal zone where larger lymphocytes and antigen presenting dendritic cells are located. Centrally, splenic innervation arises from sympathetic preganglionic neurons (SPNs) located in the thoracic and rostral lumbar spinal cord. These SPNs are controlled by a specific supraspinal circuitry, which is ultimately responsible for the regulation of neural–immune interactions in the spleen. Dr. Rosas-Ballinas began following the winding path of the vagus nerve to establish the route it follows to reach the spleen (Rosas-Ballina *et al* 2011). He was trying, without much luck, to find fibers of the vagus nerve in this organ. And then he went a little further posterior to the splenic nerve, the nerve that innervates the spleen. Their results indicate that the vagus nerve inherently communicates with the splenic nerve to suppress TNF production by macrophages in the spleen.

According to the prevailing paradigm, the autonomic nervous system is anatomically and functionally divided in sympathetic and parasympathetic branches, which act in opposition to regulate organ function. The present review characterizes the synaptology of neurons that potentially modulate splenic activity and new emerging concepts about neural regulation of spleen function.

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Autonomic nervous regulation of spleen

Despite the demonstrated influence of the splenic nerve on immune responses, little is known about the identity of central nervous system (CNS) cell groups that innervate splenic SPNs. Sympathetic innervation of the spleen appears to play an important role in the modulation of immune function, since changes in splenic nerve activity alter a variety of cellular and humoral immune responses in this organ (Wan *et al* 1994). Innervation of the spleen by sympathetic noradrenergic fibers has been extensively characterized (Bellinger *et al* 1999). These fibers, originating in the celiac-mesenteric and sympathetic chain ganglia (Nance & Burns, 1989; Cleland & Tait 1927), are distributed among specific compartments of the white pulp and establish direct contact with immunocompetent cells, which, in turn, express functional adrenergic receptors.

One anatomical link between spinal cord, coeliomesenteric ganglia, and spleen is represented by noradrenergic neurons of autonomous nervous system. Using tyrosine hydroxylase (TH) immuno-histochemistry, noradrenergic cell bodies of coeliomesenteric ganglia, were identifiable. As shown after double-labelling experiment most of these neurons presented scrapie prion protein (PrPsc) deposits on their membrane (Bencsik *et al* 2001). Noradrenergic processes from these cell bodies innervate the spleen; endings were located within the trabeculae, around blood vessels, within the white pulp, in the mantle zone near immune cells, in the outer zone and between germinal centers and mantle zone.

In 1989, the first neuroanatomical study on the source of innervation to the spleen was published in the scientific journal 'Brain, Behavior and Immunity". In addition to the retrograde tracer WGA-HRP, the fluorescent retrograde tracer fluorogold was used. Utilizing small injections of tracers into the rat spleen, application of a diffusion barrier to the spleen post-injection, and surgical sectioning of the splenic nerve, it was found that prevertebral sympathetic ganglia associated with the celiac-mesenteric plexus provided a major sympathetic input to the spleen. In addition, many retrogradely labeled neurons were identified bilaterally in the thoracic sympathetic chain. Denervation of the spleen verified the specificity of the labeling and established that the splenic nerve is the final common pathway for neural input to the spleen. Importantly, and similar to the thymus, no evidence was found for a sensory input to the spleen from either the vagus nerve or dorsal root ganglia. It was concluded that neural input to the spleen was exclusively sympathetic, with no evidence for a sensory or vagal nerve supply. This contention was supported by studies conducted by Bellinger *et al* who in 1993 suggested that there was lack of cholinergic innervation in the spleen. Further evidence for the absence of any vagal or parasympathetic input to the spleen was subsequently published in Brain Behavior and Immunity which demonstrated the absence of choline acetyltransferase (ChAT) in the spleen, which is a more specific marker of cholinergic nerve fibers than AChE. Similarly, immunohistochemical studies for vesicular acetylcholine transporter, a highly specific marker for cholinergic neurons and fibers, indicated their complete absence in lymphoid tissue (Cano *et al* 2001). Finally, a transneuronal study of the innervation of the spleen with psuedorabies virus (PRV) has verfied this conclusion and suggested that sympathetic preganglionic neurons that innervate the spleen arise from the T1-T12 region of the thoracic spinal cord. Longer survival times identified sympathetic premotor brain nuclei projecting either directly or indirectly to the spinal sympathetic preganglionic neurons, and again consisted of many of the same nuclei in the brainstem, pons and hypothalamus that are activated by immune stimuli. Thus, neuroanatomical and neurochemical evidence demonstrates that neural innervation of the spleen is entirely sympathetic in origin, and indicates further that there is no evidence for parasympathetic or sensory input to the spleen (Katafuchi *et al* 1993).

However, recently pulished data reveal cholinergic nerve involvement in the spleen. The division between the parasympathetic and sympathetic nervous systems is not clear cut," said Dr. Rosas-Ballina, explaining that the vagus nerve (the major parasympathetic nerve) acts through the splenic nerve to modulate immune function. Neural circuits regulate cytokine production to prevent potentially damaging inflammation. A prototypical vagus nerve circuit, the inflammatory reflex, inhibits tumor necrosis factor–α production in spleen by a mechanism requiring acetylcholine signaling through the α7 nicotinic acetylcholine receptor expressed on cytokine-producing macrophages. Nerve fibers in spleen are deficient in the enzymatic machinery necessary for acetylcholine production; therefore, how does this neural circuit terminate in cholinergic signaling.

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Ballina *et al* identified an acetylcholine-producing, memory phenotype T cell population in mice that is integral to the inflammatory reflex. These acetylcholine-producing T cells are required for inhibition of cytokine production by vagus nerve stimulation. Thus, action potentials originating in the vagus nerve regulate T cells, which in turn produce the neurotransmitter, acetylcholine, required to control innate immune responses. Another evidence comes from studies in the fish. The mechanisms of splenic control in the Antarctic fish, Pagothenia borchgrevinki, were investigated using isolated spleen and mesenteric artery strips in vitro and perfused spleen preparations *in situ*. Isolated spleen strip preparations contracted in response to carbachol, a response that was antagonized by atropine. The response to acetylcholine was markedly enhanced by the specific cholinesterase inhibitor BW-284c51. Catecholamine effects were somewhat irregular, and maximal contraction force with epinephrine and norepinephrine was 41% and 56%, respectively, of the carbachol response. The results suggest a mainly, if not solely, cholinergic autonomic control of the borch spleen, and a major function of the cholinergic innervation in the control of hematocrit in this species (Meredith *et al* 1993)

Again, any sensory neuropeptide-positive fibers identified in the spleen are not involved in providing sensory feedback from this immune organ. Some brain regions involved in the control of splenic immune function have been identified in brain lesion and stimulation studies (Irwin *et al* 1994, Starkey *et al* 1995) however, it is unclear how these regions are interconnected and whether other brain areas are also involved. To understand the neural regulation of splenic immune function, it is important to determine the CNS circuits involved in the control of SPNs giving rise to splenic innervation.

Role of Pineal Gland in control of splenic function :

A key function of the pineal gland is to transform information about environmental lighting into biological rhythms, which has led to its designation in some species as a "third eye." In mammals, light information reaches the pineal gland via a circuitous route. The mammalian pineal gland consists of the large, cone-shaped, superficial pineal connected by a stalk to the deep pineal that is intimately associated with the habenula from which it may derive partial innervation. Light-dark information detected by the retina is relayed by the retinohypothalamic pathway to the suprachiasmatic nucleus (SCN) of the hypothalamus, which has been established as the principal biological clock in mammals. Cells from the SCN project to the paraventricular hypothalamic nucleus. Of all the target tissues of melatonin, the SCN has attracted the most attention because of its central role in circadian rhythms (Lewy *et al* 1992). Neuronal firing of SCN neurons recorded *in vitro* exhibits a circadian rhythm that peaks during the light phase and is minimal during the dark phase of the circadian cycle. Melatonin can inhibit SCN firing *in vitro* in organ culture experiments. The circadian peak in SCN neuronal activity can be phase-shifted by melatonin in a dose- and time-dependent manner *in vitro*. Melatonin can entrain mammalian circadian rhythms and attenuate the phase-delaying effects of light pulses applied during subjective night (Dubocovich *et al* 1996). Molecular cloning of melatonin receptors has greatly enhanced our insight into melatonin-SCN interactions and melatonin receptors have been characterized (Pertsov, 2006). The strategic level means that the normal development and function of both parts of this network are reciprocally dependent. In chicken embryo, pinealectomised very early during incubation, a retarded development of the primary lymphoid glands and a decreased immune response accompanied by the significant changes in the biogenic amines concentration in the spleen and brain have been demonstrated (Schafer *et al* 1998).

Hypothalamic control of splenic function

Hypothalamus may regulate the functions of the spleen through the release of neurotransmitters and 2nd messengers.Stimulation or ablation of central hypothalamic nuclei is correlated with changes in electrical activity of the splenic nerve. The cytotoxicity of NK cells associated with these treatments seems to be driven by the medial part of the preoptic nucleus of the hypothalamus (MPO). Bilateral lesion to the MPO results in suppression of NK cytotoxicity, an effect completely blocked by prior splenic denervation and independent of circulating corticosterone. MPO lesions raise splenic nerve activity, whereas electrical stimulation of the MPO inhibits signals, including fever, anorexia, sleep and nocioception (Hori *et al* 1998). All the effects of manipulating MPO activity on NK cytotoxicity rely on an intact splenic nerve and can be mimicked by stimulation or section of this nerve.

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Peptidergic innervation of spleen

Neuro-peptide-like immunoreactivity has been identified in the spleen and immunoreactive profiles showing NPY-like, Met-enkephalin-like, cholecystokinin-8 (CCK)-like, and neurotensin-like immunoreactivity are mainly associated with the central artery of the white pulp and its smaller branches, rarely entering the parenchyma (Felten *et al*, 1985). Also, VIP-positive nerves accompany large arteries and central arterioles ending in the white pulp. What is the functional importance of central ANG II-splenic SND-splenic cytokine gene expression interactions needs to be explored. Although, the current results do not address this question, the pathophysiology of heart failure suggests an interesting possibility.The renin-angiotensin system is altered in heart failure. Intra-cerebroventricular injection of losartan decreases levels of resting renal SND in rats with chronic heart failure, and chronic central AT_1 receptor blockade normalizes the enhanced sympathoexcitation, reduced sympatho-inhibition, and desensitized baroreflex responses observed in rats with congestive heart failure after myocardial infarction (Ganta *et al* 2005, Ader *et al* 1990).

Arcuate Nucleus and Suprachiasmatic nucleus crosstalk in splenic neural regulation; new insights into splenic function regulation

The arcuate nucleus (ARC) is crucial for the maintenance of energy homeostasis as an integrator of long- and short-term hunger and satiety signals. The expression of receptors for metabolic hormones, such as insulin, leptin, and ghrelin, allows ARC to sense information from the periphery and signal it to the central nervous system. The ventromedial ARC (vmARC) mainly comprises orexigenic neuropeptide agouti-related peptide and neuropeptide Y neurons, which are sensitive to circulating signals. That there are innervations from vmARC to SCN is a novel finding as shown in Figure 1. However, previous electrophysiological studies on ARC indicated this connection. Other proof for the functional interaction between vmARC and SCN comes from a number of studies indicating that physiological and behavioral circadian rhythms are also affected by ARC malfunction (Yi *et al* 2006). The fact that ARC lesions by neonatal mono-sodium glutamate treatment, which mainly destroys NPY cells, is able to compromise the circadian activity while the SCN remains intact , could be interpreted, together with the present data, to mean that the innervations from vmARC to the SCN may relay peripheral hormonal information to the SCN and may thus affect the circadian activity of the SCN (Meister *et al* 1989, Mistlberger & Antle, 1999). At the same time, input from the SCN to the ARC may time the activity of the ARC, as shown by rhythmic Fos expression in the ARC and the rhythmic activity of dopaminergic ARC neurons negatively driven by vasoactive intestinal peptide from the SCN (Horvath, 1997). Our present data provide the anatomical and functional basis for the hypothesis that both humoral and neuronal metabolic information may be relayed back to the SCN via the AMC. Herein the AMC area is responsible for the first order integration of circulating signals.

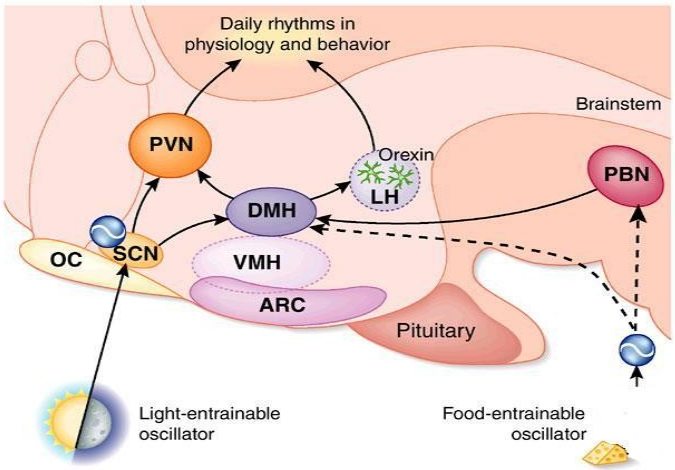

Figure 1: Brain regions controlling spleen function

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Consequently, the GHRP-6 information relayed by the vmARC to the SCN, as indicated by Fos diminishment in the SCN, is another functional support for the anatomical connection between the AMC and SCN, and suggests that pharmacological effects of ghrelin may inhibit the activity of the SCN during the light period (Saeb-Parsy & Dyball, 2003). Moreover, the AMC area has reciprocal connections with visceral sensory integration sites such as the PB and NTS, as was shown in part previously. This indicates that the vmARC and seME may integrate hormonal information with signals from first order visceral sensory centers. Logically, the outcome of this interaction should be signaled to many sites, including the SCN, and is necessary for the multilevel central control of visceral activities. N-acetyltransferase and melatonin rhythms are regulated by a suprachiasmatic nucleus (SCN) clock and light, in the form of adrenergic innervation of the pineal gland (Jamali & Tramu, 1999, Gerhold *et al* 2002, Moore & Lenn, 1972, McArthur *et al* 1991). Cells from the SCN project to the paraventricular hypothalamic nucleus. Fibers from this nucleus descend to synapse in the intermediolateral column of the spinal cord. Preganglionic sympathatic neurons from this region then project to the superior cervical ganglia from which postganglionic neurons ascend along the internal carotid artery to enter the pineal gland (Skwarlo-Sonta, 2002). A central dopaminergic regulation of the spleen has to be established. Whether central dopaminergic modulation through the arcuate nucleus which may modulate the blood pressure control can also affect splenic function needs to be evaluated (Sim & Hsu, 1990, Mok *et al* 1990]. Thus the crosstalk between the vmARC and SCN may regulate the function of the spleen and this seems to be a not yet elucidated pathway. The influence of melatonin on spleen is partly mediated by these structures of the brain.

CONCLUSION

In conclusion it can be stated that the spleen is a crucial secondary lymphoid organ for circulating infectious agents that is densely innervated by sympathetic nerve fibres and regulated by central neuroinnervation and peptidergic mechanims. Sympathetic nerve endings contact immune cells and macrophages. Neurotransmitters are released into the vicinity of nerve terminals and bind to specific postsynaptic receptors on the surface of these cells. Local bi-directionality exists through cytokines and neurotransmitters from immune cells that modulate the release of sympathetic neruotransmitters from nerve terminals. This complex 'dialog' depends on microenvironmental factors such as infectious agents, and this 'conversation'is needed to balance the function of spleen.

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