Neuromodulation of intestinal inflammation
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Chapter 1

Neural networks in intestinal immunoregulation

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Abstract:

Key physiological functions of the intestine are governed by nerves and neurotransmitters. This complex control relies on two neuronal systems: an extrinsic innervation supplied by the two branches of the autonomic nervous system and an intrinsic innervation provided by the enteric nervous system. As a result of constant exposure to commensal and pathogenic microflora, the intestine developed a tightly regulated immune system. In this review, we cover the current knowledge on the interactions between the gut innervation and the intestinal immune system. The relations between extrinsic and intrinsic neuronal inputs are highlighted with regards to the intestinal immune response. Moreover, we discuss the latest findings on mechanisms underlying inflammatory neural reflexes and examine their relevance in the context of the intestinal inflammation.
Neural networks in intestinal immunoregulation

Introduction

Key functions of the gastrointestinal tract such as motility, secretion and vasoregulation are regulated by neural mechanisms provided by the autonomic nervous system (ANS). The extrinsic control of the intestine is provided by two branches: the sympathetic and parasympathetic branches. Sympathetic preganglionic fibers arise from cholinergic neurons located in the thoracolumbar intermediolateral nucleus of the spinal cord and synapse with noradrenergic postganglionic neurons in para- and prevertebral ganglia. Parasympathetic preganglionic fibers originate from motor neurons of the dorsal motor nucleus of the vagus (DMV) and make synaptic contact with postganglionic neurons within the intestine (i.e., the myenteric plexus) (Fig. 1A). The density of the vagal innervation displays a proximodistal gradient along the intestine with the highest density observed in the duodenum and the lowest density observed in the distal part of the ileum [1]. The large intestine differs from the rest of the gastrointestinal tract as it receives a parasympathetic innervation from two distinct sources. The vagus nerve innervates the proximal colon whereas preganglionic neurons located in parasympathetic nuclei of sacral vertebrae (S2 to 4) contact post-ganglionic neurons in pelvic ganglia. These postganglionic neurons then give rise to rectal nerves providing vagal input to the distal colon. Of note, in some species (e.g. mouse and rabbit), direct vagal innervation of the distal colon is also provided by nerves arising from sacral parasympathetic nuclei [2].

In addition to the extrinsic neuronal control, the gastrointestinal tract possesses an intrinsic nervous system, the Enteric Nervous System (ENS), able to regulate intestinal motility independently of the presence of the central nervous system (CNS) [3]. The ENS is composed of 2 plexuses: the myenteric plexus located between the longitudinal and circular muscle layers of the muscularis externa and the submucosal plexus.

A tightly regulated immune system has developed in the intestine in response to a constant exposure to pathogenic and commensal microbiota. This system allows microbe sampling and surveillance and a quick response to microbial threat. The CNS, supported by the proximity of its terminals to immune cells, plays a major role in the regulation of this level of host defense. These interactions between nervous and immune system allow the modulation of the inflammatory response and offer new therapeutic targets to tackle inflammatory disorders where a deregulation of the immune reaction constitutes the key element.
In this review, we address the impact of neuro-immune interactions in the context of the intestine. The relevance of neural reflexes is discussed with regards to the regulation of intestinal inflammatory disorders.

**Intestinal neuro-immune interactions**

*Sympathetic innervation of the gut and interactions with immune cells*

The sympathetic regulation of the immune response relies on the binding of noradrenaline (NA) with α (subtypes α₁ and α₂) and β (subtypes β₁, β₂, β₃) adrenergic receptors. With a half-life of 1 to 2 minutes and a capacity to diffuse on a long distance (up to 1 μm) [4], a wide range of cell types can be affected by NA release without need of direct contact with neuronal fibers [5]. Sympathetic efferent fibers densely innervate the intestine (Fig. 1B). Terminals are found in the serosa [6] and the mucosa and an important network of sympathetic fibers contacts neurons of the myenteric plexus [7] [8]. Networks of fibers are also found in the circular muscularis [7] while some fibers pass through the longitudinal muscle layer [9,10]. Evidence of contacts between sympathetic terminals and non-vascular intestinal myocytes is however lacking. This innervation of the different intestinal compartments by noradrenergic fibers may therefore affect many immune cell types and thereby influence the innate and adaptive immune response. A good example of sympathetic immune regulation via such contacts is found in the lymphoid system of the gut.

The intestinal mucosa exhibits a dense network of immune cells particularly in organized lymphoid structures such as Peyer’s patches (PPs). This component of the gut-associated lymphoid tissue (GALT), constituted by B and T-cell follicles and a subepithelial dome rich in dendritic cells (DCs), participates in the response to microbial threats. The presence of noradrenergic fibers was reported in PPs implying that the sympathetic nerve could play a role in the regulation of the immune response. Noradrenergic axonal fibers are found in close proximity to DCs [11], plasma cells and in T cell zones [12]. NA can affect DCs migration as well as their cytokine production (i.e., IL-12, IL-6 [13]) through the binding to adrenoceptors (β2 [13], α1 [14], α2 [15,16]). The potential of NA to dictate the cytokine profile secreted by DCs allows the sympathetic innervation to affect the skewing of naïve T cells and thereby indirectly influences the shaping of the adaptive immune response.
In vitro studies reported that NA can influence the skewing of naïve T cells expressing β-adrenoceptors and thereby their cytokine secretion (IFN-γ IL-12 for Th1 and IL-4 for Th2) (for a review see ref. [17]). B cell proliferation [18] and immunoglobulin secretion [19] are triggered by NA treatment through β2-adrenoceptors signaling. In vivo studies using 6-OHDA (a chemical sympathectomy) confirmed the effects of NA on T and B cells [20] and revealed the capacity of NA to modulate the migration and accumulation of lymphocytes in the GALT [21] during inflammation. Of note, the method used to deplete sympathetic inputs presents limitations as chemical sympathectomy not only depletes the noradrenergic input to the intestine but also affects the catecholamine content of immune cells [8]. Additional in vivo approaches are therefore required to determine the exact contribution of the intestinal sympathetic innervation in the modulation of the immune system.

The intestinal muscularis represents another compartment where neuro-immune interactions are observed. Recently, a direct contact between varicosities of sympathetic axons and resident intestinal macrophages was reported [22]. NA, through β-adrenoceptors signaling, can suppress TNF-α secretion [23] and phagocytosis of macrophages [24] thereby affecting their clearance capacity. Of note, sympathetic fibers synapsing with motor neurons of the enteric nervous system [25,26] could modulate immune functions via an indirect effect on enteric neurons.

Vagal innervation of the gut and interactions with immune cells

The vagus nerve modulates immune cells through the release of acetylcholine (ACh). In contrast to catecholamines, ACh exhibits a very short half-life of 1 to 2 milliseconds, due to the presence of ACh esterase which hydrolyzes ACh at a high rate. Close contact between cholinergic nerve terminals and cells expressing ACh receptors is therefore required for the cholinergic control of these cells.

Receptors for ACh are of two types: muscarinic receptors (mACHR) comprising of 5 subtypes (M1-M5) and nicotinic receptors (nACHR) constituted of homomeric or heteromeric combinations of 5 subunits (among 17 subunits). On a cellular level, ACh binding on one or the other type of receptors leads to significantly different mechanisms. Activation of muscarinic receptors, belonging to the G-protein-coupled receptors, leads to the activation of a cascade of secondary messengers only allowing a slow response
from the target cell [27]. On the contrary, nicotinic receptors belonging to the ligand-gated ion channel family allow a fast transmission of the cholinergic signal [28,29]. In immune cells however, these variations in transmission don’t imply differences in the onset or length of the cholinergic signal as both nicotinic and muscarinic receptors trigger long-lasting effects.

The vast majority of immune cells express one or both types of receptors. Bone-marrow-derived dendritic cells, as well as PBMC-derived [30] and peritoneal macrophages [31] express both mAChR (M1-M5) and nAChR (α2, α5, α6, α7, α10, β2). T and B cells also exhibit receptors for ACh (nAChR α2, α5, α9, α10, β1, β2, β4 and M1, M3, M4, M5 mAChR for T cells; α4, α7, α2 nAChR and M2 mAChR for B cells) [31]. Interestingly, the expression of these different subunits on both innate and adaptive immune cells varies with their maturation and development stage. Intracellular signaling triggered by the binding of ACh to these receptors has been shown to modify greatly the function of these cells. In vitro studies demonstrated the capacity of ACh to modulate both the innate and adaptive compartments of the immune system. α7nAChR agonists suppress TNF-α secretion by peritoneal macrophages exposed to LPS. Moreover, treatment of freshly isolated murine T cells with both agonists and antagonists of nAChR and mAChR interferes with their skewing towards Th1, Th2 and Th17 profiles [32]. Finally, a suppressive effect of nicotine on B cell activation was reported and is mediated by α2, α4 and α2 subunits of nAChR [33,34]. Importantly, no contact between vagal terminals and immune cells in the intestine has so far been reported. However, preganglionic efferent terminals make synaptic contact with neurons of the myenteric ganglia [35-37] indicating that neural cholinergic influence on immune cells is solely provided by myenteric neurons (Fig. 1B). Importantly, T and B cells were recently shown as a crucial source of ACh able to modulate the immune response [38],[39].

**The Enteric Nervous System and interactions with immune cells**

Enteric neurons display a wide variety of neurotransmitters and neuropeptides determining the chemical coding of the ENS (for a review see refs. [40,41]). Enteric neurotransmitters and neuropeptides are able to bind and influence a variety of immune cells.

Cholinergic enteric neurons are targeted by both branches of the ANS [35,42,43].
Evidence of direct interactions between ‘basket-like’ cholinergic endings and cholinergic myenteric neurons was recently reported [37]. This vagal innervation of cholinergic enteric neurons stimulates the production of ACh and may represent the neuronal circuitry by which the vagus nerve mediates its anti-inflammatory signal. Choline acetyltransferase (ChAT) positive fibers, most likely originating from the enteric neurons, are found in close proximity to resident intestinal macrophages [44]. Phillips and Powley recently brought evidence of direct interactions between cholinergic fibers (i.e., axons and dendrites) and macrophages in the gut muscularis [22]. This neuronal source of ACh can suppress macrophage activity through its binding to α7nAChR, and can thereby inhibit the release of pro-inflammatory cytokines [44]. On the contrary, sympathetic inputs to cholinergic enteric neurons inhibit ACh release through the binding of NA to α2 adrenoceptors [45,46]. This observation suggests that sympathetic inputs may counterbalance the cholinergic anti-inflammatory effect.

Of note, cholinergic enteric fibers are not restricted to the muscle layers. Some ChAT+ fibers are found in PPs, suggesting a possible cholinergic modulation of the immune reaction [47]. However, no neuronal contact with immune cells has been reported so far in PPs. Determining what immune cells are contacted by cholinergic fibers in PPs is of crucial importance to better understand the interplay between cholinergic and immune system in the intestinal inflammatory response to microbial threats.

Vasoactive intestinal peptide (VIP), a well-established immunomodulator (for review see ref [39]), is expressed by enteric neurons. Sympathetic and vagal efferent fibers make contact with VIP+ enteric neurons providing another neuronal mediator by which the brain can affect the gut immune response. In vitro and in vivo studies revealed the suppressive action of VIP on their activity as it inhibits their chemokine and cytokine production (e.g. MIP-1, MIP-2, KC and IL-12) during endotoxemia [4]. In addition, VIP treatment modulates DCs activity [48] in vitro and the expression of VPAC1 and VPAC2, the receptors for VIP, was observed on DCs isolated from PPs [49]. The modulatory effect of VIP on those DCs could potentially influence the IgA production by B cells, in addition to its direct effect already reported [50]. These results imply that VIP released by enteric neurons could interfere with both innate and adaptive immune systems.

In addition, both vagal [37] and sympathetic fibers [51] target nitricergic neurons in the ENS. Nitric oxide (NO) exerts both protective (e.g. T cell suppression [52]) and pro-
inflammatory effects (e.g. increase in IFN-γ production by natural killer cells [53]) and can greatly affect the inflammatory reaction (for review see ref [54]). This places NO as an additional neuromodulator of the innate immune system.

Serotonergic enteric neurons receive inputs from both autonomic branches [25] [55,56]. Serotonin triggers the release of pro-inflammatory cytokines (i.e., IL-1β, IL-8) by DCs in vitro [57]. Interestingly, in postoperative ileus (POI) serotonin activates cholinergic enteric neurons leading to the dampening of intestinal inflammation [58]. Thus, despite a lack of evidence of contact between serotoninergic fibers and immune cells, serotonin can indirectly modulate the innate immune response. Of note, enterochromaffin cells are a major source of gastrointestinal serotonin [59] suggesting that its effect on the immune system could also be mediated through a non-neuronal release.

Extrinsic sympathetic inputs also target secretomotor neurons immunoreactive for substance P (SP) [60]. Receptors for substance P (i.e., NK) are found on a large variety of immune cells (e.g. mast cells, NK cells, macrophages, lymphocytes). SP induces the secretion of pro-inflammatory cytokines by macrophages [61] and increases T cell proliferation [62]. Importantly, mast cells are found in the close vicinity of SP positive fibers in the intestine [63] and SP activates peritoneal mast cells leading to their degranulation [64]. The degranulation and consequent release of inflammatory mediators can in turn activate sensory nerves, a phenomenon involved in the pathophysiology of irritable bowel syndrome (IBS) [65]. Moreover, the use of an inhibitor of Syk [67], a kinase involved in the downstream signaling of SP receptors, showed its efficacy in dampening the inflammation in POI in mice. Although the SP-mast cells interaction is a key event in IBS, the neuronal source of this neurotransmitter (i.e., enteric neurons or vagal sensory afferents) remains unclear [68].

In addition, a dramatic alteration in the expression of enteric neurotransmitters was observed in animal models of IBS and in IBD patients [69]. This phenomenon, most probably driven by the ongoing inflammation [70] is likely to influence the modulation of the intestinal immune system. Treatment with VIP in a TNBS-colitis model decreased the severity of the inflammation [71] suggesting a beneficial role of this neuropeptide on the colonic inflammation. In experimental colitis [72] and Crohn’s disease (CD) patients [73], an increase in the number of VIP+ neurons was measured and correlated
to an elevated colonic VIP content. However, contradictory results were found on VIP expression in ulcerative colitis and CD patients [74] leaving under debate the role of VIP in the colonic inflammation.

Figure 1. Intestinal innervation and interactions between nervous and immune system in the intestine. (A) Both branches of the Autonomic Nervous System innervate the small and large intestine. The parasympathetic innervation of the distal colon differs between species: direct innervation from sacral nuclei is observed in some species while postganglionic neurons located in pelvic ganglia provide inputs to the colon in others. (B) Vagal inputs (in blue) solely innervate myenteric neurons. Sympathetic inputs (in red) make synaptic contact with enteric neurons and immune cells in both smooth muscle layers and Peyer’s patches (PPs). Enteric fibers (in green) also project to PPs and are found in the close vicinity of macrophages. CM: circular muscularis; LM: longitudinal muscularis; MP: myenteric plexus; SP: submucosal plexus

CNS control of the immune response: the inflammatory reflex

A fine tuning of the immune response is required to maintain the organism homeostasis. This refined control relies on interactions between the CNS and immune cells mediated through neural autonomic reflexes. The initiation of inflammatory neural reflexes is characterized by the activation of sensory nerve terminals by inflammatory mediators. This peripheral information is conveyed to the CNS where it triggers the activation of motor neurons which in turn provide signals to immune cells present in the target organ.
Sympathetic reflexes and involvement in intestinal inflammatory diseases

Sympathetic reflexes are involved in diverse intestinal pathophysologies. In POI, sympathetic reflexes play a role in both stages of this inflammatory disorder: the early neurogenic and the late inflammatory phase [75]. In the early phase, the activation of spinal mechano-sensory afferent fibers [76] by handling of the intestine triggers NA release by sympathetic motor fibers. The inhibitory effect of NA on intestinal smooth muscle cells leads to an impaired gastrointestinal motility. In the late inflammatory phase, the activation of this sympathetic reflex is maintained through the release of NO and the induction of Cox-2 secretion by infiltrated leukocytes [77-79] thereby resulting in a prolonged intestinal paralysis. This sympathetic feedback loop is proposed to participate in the disseminative nature of POI [80].

In other intestinal inflammatory disorders, activation of sympathetic reflexes showed to play an important role in modulating the intestinal immune system. In intestinal parasitic infection (e.g. Trichinella spiralis) [81] and experimental colitis models (TNBS [82] or DSS [83]) motor sympathetic fibers are affected as shown by a decreased NA release in the gut. Importantly, observations of intestinal sympathetic innervation in CD patients showed a lack of TH⁺ fibers in every intestinal layer [84]. This result however remains debatable as other studies showed an increased amount of TH⁺ fibers in the myenteric plexus of CD patients [73]. Additionally, the α2-adrenoceptor antagonist RX821002 inhibited the colonic mRNA level of pro-inflammatory cytokines (TNF-α and IL-1β) in TNBS colitis [85]. In the same model, chemical sympathectomy showed to ameliorate colonic inflammation [86]. These findings underscore the pro-inflammatory role of NA and α2-adrenoceptors signaling in colitis. Interestingly, exposure to TNBS also led to an increase in the number of sympathetic ‘basket-like structures’ in the dorsal root ganglia showing that colonic inflammation triggers sprouting of sympathetic fibers. These structures surround sensory neurons of the dorsal root ganglia, suggesting that sympathetic sprouting could influence the plasticity of these sensory neurons and be partly responsible for the visceral hypersensitivity in IBS and the chronic pain observed in colitis patients [87].

Vagal reflexes in intestinal inflammatory diseases

The ability of the immune system to activate the sensory arm of the vagus nerve is well
Neural networks in intestinal immunoregulation

established. In the intestine, close anatomical contacts between vagal sensory fibers and mucosal granular cells resembling granulocytes [88] and mucosal mast cells [89] have been reported. These cells can release inflammatory mediators such as IL-1β and prostaglandins which in turn activate vagal afferents fibers [90]. Only ten years ago, the motor part of the vagus nerve was discovered as a modulator of the immune response. Indeed, activation of the vagus nerve (by electrical stimulation) showed to suppress the pro-inflammatory cytokine release by macrophages in a rat model of endotoxemia [91]. This effect mediated by the α7nAChR was named ‘cholinergic anti-inflammatory pathway’ (CAIP) and brought to light the concept of a vagal inflammatory reflex [92].

In POI, vagus nerve stimulation (VNS) applied prior to the intestinal manipulation prevents the inhibition of the gastrointestinal motility by suppressing the activation of resident intestinal macrophages via α7nAChR. In the same line, endogenous activation of the vagal reflex by administration of enteral lipid-rich nutrition [94] dampens the pro-inflammatory cytokine secretion by resident macrophages therefore preventing ileus. Altogether, these observations suggest two distinct functions of the vagus nerve in the modulation of the immune response: a preventive role when the vagus nerve is activated (by VNS) prior the immune challenge or insult; a role in the restoration of homeostasis when the vagal reflex is activated after the inflammation is settled.

The cholinergic anti-inflammatory pathway was also demonstrated in experimental colitis. Subdiaphragmatic vagotomy in DSS-exposed mice enhanced pro-inflammatory cytokine levels (i.e., TNF-α, IL-6 and IL-1β) worsening colonic inflammation [95]. Although the α7nAChR plays a key role in mediating the cholinergic suppressive effect on macrophage activity, another cholinergic receptor, α5nAChR, was shown to participate in the vagal anti-inflammatory mechanism during colitis [96]. With regards to the colon innervation, further investigations are required to determine the exact involvement of direct vagal innervation and sacral parasympathetic inputs in the cholinergic anti-inflammatory pathway regulating colitis.

Spleen innervation: involvement in the vagal reflex controlling immune responses

The spleen and its innervation were recently identified as key players in mediating the
vagal anti-inflammatory effect in sepsis [97,98]. Interestingly, no evidence of a direct vagal innervation to the spleen has been shown to date [99,100]. The vagal control on the spleen was consequently proposed to rely on vagal innervation of splenic postganglionic sympathetic neurons expressing α7nAChR located in celiac ganglia [97,98].

This vagal control of NA release by splenic sympathetic fibers was recently shown to target a specific memory T cell population producing ACh (as they express ChAT) [38]. This non-neuronal source of ACh is proposed to suppress pro-inflammatory cytokines (i.e., TNF-α) secretion by macrophages. However, some controversy exists as recent studies demonstrate the absence of neuronal contact between vagal and splenic nerve [101], suggesting that the anti-inflammatory effect observed may be driven by a spinal sympathetic reflex rather than a vagal reflex.

Nevertheless, the discovery of these cholinergic T cells may suggest a role of these cells in other inflammatory disorders where cholinergic regulation of the immune system is involved. The presence of this cell population may indeed not be restricted to the spleen but could also be found in other lymphoid structures such as PPs or mesenteric lymph nodes which present a comparable distribution of noradrenergic fibers. It would therefore be of interest to consider the contribution of this ChAT+ T cell population in the cholinergic anti-inflammatory mechanisms regulating intestinal inflammatory diseases such as POI and IBD.

**Conclusion and outreach**

A large body of evidence points towards an integrated regulatory role of both extrinsic and intrinsic innervation in intestinal immunity. The importance of inflammatory reflexes in regulating acute and chronic intestinal inflammatory disorders is emerging and the ENS appears as a pivotal element linking sympathetic and more particularly vagal inputs to the immune system. Our understanding of the interactions between nervous and immune system in the context of the intestine has strongly increased during the past decade. However, the exact mechanisms underlying these neuro-immune interactions still remain partly unclear and numerous challenges need to be addressed to comprehend the functioning of this neuro-immune system. On the one hand, further anatomical evidence are required to determine precisely which neural
axis is able to affect the different components of the immune system in the intestine. On the other hand, the clear role of the nervous system in the pathophysiology of intestinal inflammatory disorders is not yet clarified in the current literature and further investigations are necessary to unravel the therapeutic relevance of this neural component. From a clinical point of view, the prevalence of intestinal inflammatory disorders such as IBD, IBS and POI and the limitations and cost of current treatments call for therapeutic alternatives. Regulating the immune system via the modulation of the nervous system may provide us with a powerful tool to resolve the intestinal inflammation underlying these disorders.
Chapter 1

References


Neural networks in intestinal immunoregulation


Chapter 1


Neural networks in intestinal immunoregulation


Chapter 1

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Neural networks in intestinal immunoregulation


