Neuro-immune interaction and the regulation of intestinal immune homeostasis

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Abstract

Many essential gastrointestinal functions, including motility, secretion and blood flow are regulated by the autonomic nervous system (ANS), both through intrinsic enteric neurons and extrinsic (sympathetic and parasympathetic) innervation. Recently identified neuro-immune mechanisms, in particular the interplay between enteric neurons and muscularis macrophages, are now considered to be essential for fine-tuning peristalsis. These findings shed new light on how intestinal immune cells can support enteric nervous function. In addition, both intrinsic and extrinsic neural mechanisms control intestinal immune homeostasis in different layers of the intestine, mainly by affecting macrophage activation through neurotransmitter release. In this mini-review, we discuss recent insights on immunomodulation by intrinsic enteric neurons and extrinsic innervation, with a particular focus on intestinal macrophages. In addition, we discuss the relevance of these novel mechanisms for intestinal immune homeostasis in physiological and pathological conditions, mainly focusing on motility disorders (gastroparesis and post-operative ileus) and inflammatory disorders (colitis).
The nervous system and immune system show an intricate interplay, both via hormones, neuropeptides and cytokines (immune-neuroendocrine axis) but also via direct modulation of peripheral immune cells by the autonomic nervous system (immune-autonomic nervous system axis). Recent evidence in the gastrointestinal (GI) tract introduces more complexity on this view, as immune responses in this tissue can be influenced by both intrinsic and extrinsic neural mechanisms. Intrinsic innervation of the gut is provided by the enteric nervous system (ENS), an interconnected network of neurons and glial cells that are grouped into ganglia located in two major plexuses: the myenteric plexus and the submucosal plexus (Figure 1). Enteric glial cells slightly outnumber enteric neurons, and together both cell types form an integrated circuitry that controls motility of the intestine, fluid exchange across the mucosal surface, blood flow and secretion of gut hormones (15). The majority of enteric neurons (ENs) receive extrinsic innervation by the autonomic nervous system composed of the sympathetic (SNS) and parasympathetic (PNS) system (Figure 1). Although it is already known for many decades that the ENS, together with extrinsic innervation, controls and integrates essential GI functions such as contractility and secretion, the interplay between the ENS and the intestinal immune system is an emerging field. Recent exciting studies have paved the way for future efforts to better understand how both intrinsic and extrinsic neural mechanisms contribute to intestinal immune homeostasis. In this mini-review, we discuss recent insights on the cellular and molecular mechanisms governing maintenance and modulation of the intestinal immune system and how these mechanisms contribute to preservation of intestinal immune homeostasis.

**Neuromodulation of intestinal immunity by intrinsic enteric neurons**

The ENS complements the sympathetic and parasympathetic nervous system as the third part of the autonomic nervous system. It consists of a network of millions of enteric neurons arranged in small ganglia that are interconnected by nerve fibers (15). Although the ENS receives ample input by the central nervous system by means of sympathetic and parasympathetic innervation (discussed below), it can operate autonomously. To enable the intrinsic control of complex gastrointestinal functions, including the regulation of smooth muscle contractility, blood flow and secretion, the ENS is equipped with a variety of neurons including sensory neurons, motor neurons and interneurons. Although approximately 70% of enteric neurons are cholinergic, other neurotransmitters and neuropeptides such as nitric oxide, VIP, GABA, ATP and serotonin have important functions in the ENS (14, 40). Enteric neurons (ENs) have recently been identified as important gatekeepers of immune homeostasis in the gastrointestinal tract, in particular ENs residing in the myenteric plexus. In addition to its role in the regulation of secretion and blood flow, the ENS plays a prominent role in the regulation of smooth muscle contractility, thereby regulating peristalsis. In addition to its cooperation with interstitial cells of Cajal (ICC), the ENS seems to
rely on resident immune cells, in particular muscularis macrophages (MMϕ), to efficiently execute this function. MMϕ were initially described in the early 90’s by Mikkelsen and colleagues and were defined as a macrophage subtype residing in the myenteric plexus associated with both ICC and ENs(29). Already in these early investigations, it was noted that MMϕ are a peculiar macrophage subtype with several primary cytoplasmic processes giving them a stellate appearance. Moreover, these cells showed very little evidence of lysosomal activity, suggesting that these macrophages are in an inactivated state. In a recent study by Gabanyi et al, this unique appearance of MMϕ was not only confirmed, but it was also noted that they have primarily static cell bodies with constant extension and retractions of their filopodial processes(16).

Although the precise function of MMϕ in gastrointestinal physiology is still poorly understood, they have been linked to the regulation of gastrointestinal motility in both pathological and physiological conditions. MMϕ were initially described to be essential for the onset of postoperative ileus, a major clinical problem characterized by prolonged dysfunction of bowel motility after surgical trauma of the abdominal cavity(5, 22). In early studies, it was found that intestinal manipulation led to rapid increase of pro-inflammatory mediators including inducible nitric oxide synthase (iNOS), interleukin-6 (IL-6), interleukin-1beta (IL1β) and tumor necrosis factor alpha (TNF-α) in both mice and man(21, 23). Inhibition of certain mediators such as iNOS, which is mainly produced by MMϕ after intestinal manipulation, led to improved spontaneous activity of smooth muscle cells after manipulation. In accordance with these observations suggesting that MMϕ are essential in the onset of POI, it was later shown that depletion of MMϕ by clodronate liposomes or in osteopetrotic mice lacking colony stimulating factor (CSF1op/op mice) led to markedly decreased expression of inflammatory markers, reduced influx of leukocytes and normalization of smooth muscle contractility(38). As these findings illustrate the role of macrophage-mediated inflammation in impaired smooth muscle contraction, restraining MMϕ activation seems to be of great therapeutic value for the treatment of POI. Interestingly, recent evidence from our group has convincingly shown that intrinsic neuronal mechanisms dampen macrophage responses in the myenteric plexus(27). In 2005, de Jonge et al showed that stimulation of the vagus nerve could ameliorate surgery-induced inflammation and POI by activating STAT3 in MMϕ(20).

Dampening of MMϕ by vagus nerve stimulation occurs through the release of acetylcholine which binds on the cholinergic alpha 7 receptor subunit present on MMϕ. Only recently, it was shown that activation of MMϕ is dampened by the vagus by an indirect mechanism. Indeed, vagal efferents in the myenteric plexus are seen in close contact to cholinergic myenteric neurons but never in contact with MMϕ(7). Accordingly, vagal stimulation likely affects local myenteric neuronal activity leading to reduced MMϕ activation through enhanced release of acetylcholine. Although in acute inflammatory conditions, such as
POI, neuromodulatory therapeutic opportunities mainly lie within dampening inflammatory activation of MMϕ, chronic motility disorders, such as diabetic gastroparesis, may benefit from additional approaches. Indeed, in a murine model of diabetic gastroparesis, the phenotypical switch from an iNOS$^+$ pro-inflammatory to a CD206$^+$, heme-oxygenase-1$^+$ (HO-1) anti-inflammatory macrophage phenotype, seems to be essential to protect these mice against the development of delayed gastric emptying through conservation of ICCs, upregulation of enteric nNOS expression and reduction of oxidative stress. Accordingly, in diabetic mice with low levels of anti-inflammatory MMϕ, induction of HO-1 completely normalized gastric emptying, whereas inhibition of HO-1 caused the development of gastroparesis(9, 10). In this respect, therapeutic strategies in chronic motility disorders should not only focus on blocking the release of inflammatory mediators by MMϕ but might also build on the phenotypical plasticity of MMϕ to shift them to an anti-inflammatory, tissue-supportive phenotype. To this end, more research is needed to pinpoint the tissue-specific cellular and molecular mechanisms governing macrophage plasticity in the muscularis externa.

As mentioned earlier, more profound insight in the interaction between MMϕ and enteric neurons might be key to better understand MMϕ function in health and disease. A recent report by Müller et al, convincingly showed that enteric neurons, by the release of macrophage colony stimulating factor (M-CSF or CSF-1) are important determinants of MMϕ homeostasis(30). MMϕ, often seen in close proximity to enteric neurons and nerve fibers, almost completely rely on neuron-derived CSF-1 for their maintenance (Figure 1). Although it is not clear why neurons provide the signals to support this macrophage pool, these observations support the idea of a neuro-supportive function of MMϕ. Secretion of BMP2 by MMϕ indeed modulates enteric neuron activity through interaction with BMPRII, ultimately affecting smooth muscle contractility in steady-state conditions. Although neuron-specific elimination of BMPRII was not performed in this study, the selective expression of the receptor and its downstream signaling cascade SMAD1/5/8 by enteric neurons supports this interaction. This finding suggests that modulation of MMϕ function by enteric neurons is aimed to establish a macrophage population that supports neighboring neurons. These observations are completely in line with the recent advances on the knowledge of tissue-resident macrophages. These immune cells are highly heterogeneous within different tissues and adopt context-dependent functions that meet the specific requirements of that particular tissue(36). As disturbances in neuron-macrophage interaction might eventually affect ENS homeostasis leading to GI dysfunction, research aimed at unraveling the specific cues mediating the neuro-supportive phenotype is warranted.

In conclusion, although MMϕ play an evident role in the onset of dysmotility by the release of pathogenic mediators in inflammatory conditions such as POI or gastroparesis, the neuro-supportive aspects of MMϕ
should not be underestimated and taken into account when designing therapeutic efforts to treat gastrointestinal dysfunction. Dampening of macrophage activation will hold great therapeutic promise in conditions such as POI. In contrast, chronic motility disorders such as gastroparesis might benefit more from therapeutic strategies aimed at reestablishing the neuro-supportive function of MMϕ. Acquiring more profound insight in the specific signals produced by enteric neurons to imprint these tissue-specific macrophage functions will be key efforts for the future. Knowledge on how intrinsic enteric neurons regulate intestinal immunity is currently limited to the muscularis externa, but it cannot be excluded that neuro-immune interaction in the submucosal plexus occurs through similar mechanisms as in the myenteric plexus. Given the strong diversity of immune cells in the lamina propria, defining the specific immune cell populations that are in contact with submucosal enteric neurons/fibers will be a first hallmark in understanding the role of intrinsic neuromodulation in this layer of the intestine.

**Immunomodulation by extrinsic innervation**

The gut is extrinsically innervated by the autonomic nervous system composed of the sympathetic (SNS) and parasympathetic (PNS) system. Although intrinsic enteric neural networks allow a substantial degree of autonomy over GI functions, extrinsic neural inputs provided by both SNS and PNS are essential to integrate, regulate, and modulate these functions(6). Whereas the sympathetic nervous system provides a principally inhibitory influence over GI muscle and mucosal secretion, the parasympathetic nervous system provides both excitatory and inhibitory control over gastric, intestinal and pancreatic functions, suggestive of a more complex homeostatic regulation. It is now well established that both the SNS and PNS and its neurotransmitters play a pivotal role in regulating inflammation in different tissues, including the intestine.

*Parasympathetic innervation*

The parasympathetic innervation to the GI tract is provided by the vagus nerve (VN). Parasympathetic innervation of the intestine is particularly dense at the level of the stomach and the upper GI tract whereas sparse innervation by the vagus is seen in the lower small intestine and proximal colon (1, 2). The vagus nerve contains approximately 90% sensory fibers, involved in both chemo- and mechanosensory functions, and 10% motor fibers, mainly involved in the regulation of contractility through interaction with enteric neurons(3, 4). It is now well established that the efferent branch of the vagus is also involved in fine-tuning macrophage-mediated inflammation in the intestinal muscularis externa in post-operative ileus most likely through activation of enteric neurons of the myenteric plexus (discussed above and reviewed in (11, 39)). Increasing evidence now also strongly supports the role of the vagus nerve in
modulating mucosal intestinal immune responses. The role of the vagal reflex in mucosal immunity was initially described in two models of colitis, i.e. dextran sulfate sodium (DSS) induced colitis and dinitrobenzene sulfonic acid (DNBS) induced colitis (18, 31). Surgical removal of the ventral and dorsal truncal branched of the subdiaphragmatic vagus (vagotomy) in mice significantly worsened colitis in both models as evidenced by increased disease activity, greater loss of colonic architecture and increased colonic inflammation. Notably, mice treated with nicotine or mice with lower numbers of circulating and tissue-resident macrophages (CSF1<sup>op/op</sup> mice) did not develop exacerbated colitis after vagotomy, suggesting that the vagus affects intestinal macrophage activation by cholinergic modulation (17). This concept matches the initial observations of the vagal reflex in modulating macrophage activation through activation of the α7 cholinergic nicotinic (α7nChr) subunit on resident macrophages in the spleen and the muscularis externa in a model of sepsis and POI respectively (27, 33). Recent data from our group support the role of mainly innate effector cells and less likely adaptive immune cells as target cells of the vagus nerve, as vagotomy only worsened colitis in a DSS model, but not in a T-cell transfer model, which completely relies on a disruption of T-cell homeostasis in the intestine and not on innate immune cells (19). In addition, mice that underwent vagotomy are unable to install oral tolerance to ovalbumin and show a reduction in OVA-specific regulatory T-cells in the intestinal lamina propria. As resident intestinal CX3CR1<sup>high</sup> intestinal macrophages and CD103<sup>+</sup> dendritic cells are key players in installing oral tolerance by inducing maturation and proliferation of regulatory T-cells (32), it seems likely that the vagus nerve is also involved in the maintenance of intestinal immune homeostasis in the lamina propria through modulation of innate immune cells such as macrophages and dendritic cells. Nevertheless, α7nChr deficiency does not affect the development of DSS colitis nor the induction of oral tolerance, indicating that the effect of the vagus nerve on mucosal immune homeostasis works via a different molecular mechanism compared to immune modulation in the muscularis externa and spleen. In conclusion, although evidence is increasing that parasympathetic innervation of the intestine controls mucosal immune homeostasis, future efforts should be aimed at acquiring deeper insight into the mechanisms of vagal modulation of mucosal immunity, including the interaction between sensory afferent vagal mechanisms and sympathetic efferent mechanisms (as discussed below). In addition, identification of different target cell types of neuromodulation including enteric glia or subsets of intestinal myeloid cells and the specific neurotransmitters or neuropeptides involved in this effect are prerequisite for further progress in this field of research.

**Sympathetic innervation**
In view of the pronounced role of parasympathetic innervation on mucosal immune homeostasis, it is important to mention that vagal innervation is strictly limited to the muscularis externa (7). In this respect, vagal mechanisms are likely affecting mucosal immune responses through indirect mechanisms. In contrast, efferent sympathetic fibers are found in all layers of the intestine from serosa to mucosa and are more likely to mediate direct effects on mucosal immune cells (12, 13). Efferent sympathetic pathways innervating the intestine project from the intermediolateral column of the spinal cord and reach sympathetic abdominal ganglia which activate postganglionic sympathetic noradrenergic nerve fibres entering the gut. Sympathetic innervation is most prominent in enteric ganglia of the myenteric and submucosal plexus (16, 25). In the myenteric plexus, macrophages are seen in close proximity to active adrenergic nerve fibers (16)(Figure 1). In the lamina propria, sympathetic fibers are seen in close proximity to different immune cells, mainly in gut-associated lymphoid tissue (GALT), supporting the involvement of sympathetic innervation in controlling intestinal immune cell function (26, 37). In line, sympathetic denervation exacerbates chronic colitis, both in DSS-induced colitis and in IL-10 knockout mice (28, 34) but on the other hand improves acute colitis, suggestive of a dual role of sympathetic innervation in modulating intestinal inflammation (34). Finally, sympathetic neurotransmitters effectively modulate both innate and adaptive immune responses (35). Until recently, the precise immune cells targeted by sympathetic fibers in the gut remained unidentified and formal proof of a direct intestinal immunomodulatory function of sympathetic innervation was lacking. A recent study by Mucida and colleagues initiated better understanding on how sympathetic innervation could affect intestinal immune homeostasis (16). In this study, CD11b+ MHCII+ F4/80+ macrophages residing in the lamina propria (LPMϕ) or muscularis externa (MMϕ) were shown to have a different gene expression profile. Whereas LPMϕ preferentially express pro-inflammatory genes involved in innate immune responses and oxidative burst, MMϕ showed enrichment of tissue-protective genes such as Mrc1, Cd163, and Il10. Remarkably, when mice were infected with a non-invasive mutant of Salmonella typhymurium, MMϕ further induced the tissue-protective gene expression signature, whereas LPMϕ showed few transcriptional changes. To elucidate how the intra-tissue specialization of both macrophage subsets is installed, the role of the different microenvironmental cues in the lamina propria and muscularis externa was investigated. Notably, one of the striking differences between LPMϕ and MMϕ was the enriched expression of β2 adrenergic receptor (Adrb2) along with the proximity to tyrosine hydroxylase+ adrenergic nerve fibers in MMϕ, supporting a role for sympathetic innervation in modulating this macrophage subset. Extrinsic sympathetic neurons in the superior mesenteric-celiac ganglia innervating the muscularis externa were shown to be activated upon oral administration of Salmonella typhymurium. Moreover, treatment of mice with butaxamine, a selective β2 adrenergic receptor blocker, or genetic ablation of Adrb2 significantly impaired expression of tissue-protective genes in MMϕ both in steady-state conditions and after exposure...
to Salmonella typhymurium. Accordingly, this report serves as the first direct evidence that sympathetic innervation directly modulates the phenotype of an intestinal macrophage subset residing in the muscularis externa. This report however does not provide an explanation how sympathetic innervation could affect mucosal immune responses. First, by using elegant genetic tools combined with state-of-the-art imaging technologies, dense sympathetic innervation was observed in the myenteric and submucosal plexus, but only sparse innervation was seen in the lamina propria. Second, LPMϕ express low levels of neurotransmitter receptors including Adrb2, and very few transcriptional changes were seen after activation of sympathetic nerve fibers by Salmonella typhymurium. These results suggest that LPMϕ are not modulated by sympathetic nerve fibers and argue for a different lamina propria immune cell population sensitive to sympathetic modulation. Given the strong sympathetic innervation of Peyer’s patches, it seems plausible that sympathetic modulation of mucosal immune responses occurs through neuromodulation of immune cells residing in the GALT(8, 24, 26, 37). In summary, increasing evidence supports the role of sympathetic innervation in modulating intestinal immune homeostasis. Although the precise cellular targets of sympathetic innervation in the lamina propria remain elusive, recent evidence has shown that MMϕ are the primary targets of sympathetic modulation in the muscularis externa.

Conclusion

Recent advances on intestinal neuro-immune interaction have evidently shown that both intrinsic and extrinsic neural mechanisms are involved in regulating intestinal immune homeostasis in physiological and pathological conditions. Muscularis macrophages are maintained and modulated by neuronal signals and the reciprocal interaction between these macrophages and enteric neurons is essential for the regulation of peristalsis in physiological and pathological conditions. Although it is becoming clear that mucosal immune homeostasis is also under control of neural signals, the precise molecular and cellular mechanisms are yet to be discovered. Given the potent effects of neuromodulation of intestinal immune homeostasis in models of ileus and colitis, more profound insights on the soluble mediators and specific immune cell populations involved will pave the way for future therapeutic efforts in these diseases.

References


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Figure 1. Overview on intrinsic and extrinsic neuro-immune mechanisms in the gastrointestinal tract

(A) (left panel) Anatomical overview of intrinsic innervation of the muscularis externa (ME), submucosal plexus (SMp) and mucosa and interaction of myenteric enteric neurons with muscularis macrophages. (upper right panel) Reciprocal interaction between Myenteric enteric neurons (MEN) and muscularis macrophages (MMφ) in the muscularis externa (ME) in physiological conditions. MEN provide colony stimulating factor-1 (CSF-1) to support maintenance of MMφ (a) that in return produce bone-morphogenic protein 2 (BMP2), involved in fine-tuning peristalsis, supposedly by modulating enteric neuron activity (b). (lower right panel) In surgery-induced dysmotility (postoperative ileus, POI), MEN can be activated through vagus nerve stimulation, leading to enhanced release of acetylcholine (Ach), decreasing inflammatory activation of MMφ by reducing secretion of tumor-necrosis factor alpha (TNFa) and interleukin-6.
(IL-6) (c). (B) (left panel) Anatomical overview of extrinsic innervation of the gastrointestinal tract. (upper right panel) Vagus nerve influences mucosal immune homeostasis as vagotomy worsens DSS colitis, but not T-cell transfer colitis and reduces the ability to develop oral tolerance. This coincides with reduction in FoxP3+ Treg cells, which might involve a disturbance in the macrophage-DC-Treg axis. (lower right panel). Sympathetic innervation induces anti-inflammatory gene expression of MMΦ.
Intrinsic Neuro-immune mechanisms

Steady-state

Disease (POI)

Extrinsic Neuro-immune mechanisms

Vagotomy:
- Worsens DSS colitis
- No effect on T-cell transfer colitis
- Reduced oral tolerance
  - ↓ FoxP3+ Treg

Mucosa

MEN

MMP

MEN

MMP

BMP2

CSF-1

ACh

↓ TNFa

↓ IL-6

↑ M2 gene expression
• ↑ Arg1
• ↑ Chi3l3

GALT

Mucosa

ME

MEN

SMN

DC

Treg

Mφ

Vagus nerve

Sympathetic nerve

Vagotomy:
- Worsens DSS colitis
- No effect on T-cell transfer colitis
- Reduced oral tolerance
  - ↓ FoxP3+ Treg

Mucosa

MEN

MMP

MEN

MMP

BMP2

CSF-1

ACh

↓ TNFa

↓ IL-6

↑ M2 gene expression
• ↑ Arg1
• ↑ Chi3l3

GALT

Mucosa

ME

MEN

SMN

DC

Treg

Mφ

Vagus nerve

Sympathetic nerve