Neuromodulation of intestinal inflammation
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General introduction
The gastrointestinal tract plays an essential role in the digestion of food, assimilation of nutrients and elimination of waste. As in other physiological systems, its key functions are governed by neural mechanisms but the innervation of the gastrointestinal tract however appears to be very unique. Indeed, as compared to other organs which are solely regulated by extrinsic parasympathetic and sympathetic fibers of the autonomic nervous system (ANS), the gastrointestinal tract is the only organ that possesses its own intrinsic nervous system or enteric nervous system (ENS) [1,2]. The ENS exerts an intrinsic neural control of the gastrointestinal tract, comprises of two plexuses, namely the myenteric and submucosal plexus and is able to regulate intestinal motility independently of the central nervous system (CNS) [3].

As a consequence of its digestive role, the intestine is constantly exposed to food proteins, commensals and pathogenic microbiota. In order to maintain the integrity of the organism by responding in an appropriate manner to threats while tolerating food proteins and commensals, a tightly regulated intestinal immune system has developed and a fine-tuned regulation of the immune response is required. The CNS, thanks to the proximity of its terminals to immune cells, plays a major role in this refined control of the immune system. Indeed, interactions between nervous and immune systems allow the modulation of the inflammatory response and offer new therapeutic targets to tackle pathophysologies where a deregulation of the immune reaction constitutes the key element. Current data on the innervation of the intestine and its involvement in the modulation of the immune system in the gastrointestinal tract are reviewed in Chapter 1.

In particular, the vagus nerve was recently shown to be an essential regulator of the immune response. The ability of the immune system to activate the sensory arm of the vagus nerve is well established as vagal afferents have been shown to respond to pro-inflammatory stimuli. In the intestine, close anatomical contacts between vagal sensory fibers and mucosal granular cells resembling granulocytes [4] and mucosal mast cells [5] have been reported. These cells release inflammatory mediators such as IL-1β and prostaglandins which can in turn activate vagal afferents fibers [6]. The motor part of the vagus nerve, on the other hand, was only discovered as a modulator of the immune response ten years ago. Activation of the vagus nerve (by electrical stimulation) showed to suppress the release of the pro-inflammatory cytokine TNF-α by macrophages in a rat model of endotoxemia [7]. This effect mediated by the binding of acetylcholine...
(ACh) to receptors located on immune cells was named ‘cholinergic anti-inflammatory pathway’ (CAIP) and led to the emergence of the concept of vagal inflammatory reflex [8]. This concept relies on the existence of close proximity of vagal fibers and immune cells. However, despite thorough study of the anatomical localization of nerve terminals [9,10], anatomical evidence of vagal neuro-immune interactions still remains a matter of debate. In Chapter 2, we aimed to bring further knowledge on the interactions between vagal nerve terminals and innate immune cells in the intestine. By using the high-definition dextran amine labeling anterograde tracer, we established that the vagus nerve does not directly make contact with resident macrophages in the intestine but contacts myenteric neurons whose nerve terminals are found in the close vicinity of those macrophages.

In the past decade, the vagal anti-inflammatory reflex triggered by VNS was shown to rely on the presence of the spleen in models of sepsis [11,12] and colitis [13]. However, the exact neural networks targeting the spleen involved in the inflammatory reflex still remain controversial as some studies provided evidence of a direct vagal innervation of the spleen [14] while others showed that nerve bundles innervating the spleen are solely sympathetic [15-17]. Finally, it was proposed that the vagal control on the spleen relied on vagal innervation of splenic postganglionic sympathetic neurons located in celiac ganglia and expressing the alpha 7 nicotinic acetylcholine receptor (α7nAChR) [11,12]. Vagal endings are indeed found in celiac ganglia [18], but anatomical evidence of synaptic contact between preganglionic vagal neurons and postganglionic sympathetic splenic neurons in those ganglia supporting this concept was however lacking. Therefore in Chapter 2, we investigated whether there existed a direct or indirect vagal innervation of the spleen.

One of the inflammatory disorders where the CAIP was shown to play a crucial role is postoperative ileus (POI) [19]. POI occurs in virtually all patients undergoing abdominal surgery and is characterized by a transient impairment of the gastrointestinal motility consequently to the manipulation of the intestine by the surgeon [20]. Its pathophysiology relies on an inflammatory reaction taking place in the gut muscularis with the activation of resident macrophages as well as the recruitment of leukocytes that will release pro-inflammatory cytokines which in turn activate neural inhibitory signals leading the paralysis of the gastrointestinal tract [21-24]. The infiltration of leukocytes to the gut wall represents a crucial event in the development of POI [25] but
little was known to date on the origin of these immune cells. Therefore in Chapter 3 we investigated whether the spleen, a major secondary lymphoid organ known to act as a cell reservoir in several local and systemic inflammatory disorders [26,27], responded to the local intestinal inflammation and was involved in the inflammatory reaction underlying POI.

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 binding of ACh to α7nAChR [28], independently of the spleen innervation [29]. However, the existence and activation of a vagal reflex consequently to the inflammation under endogenous conditions had not been documented so far. In Chapter 4 and 5, we investigated whether an endogenous hard-wired neural circuitry was activated and led to a dampening of the inflammation in POI. To this end, we made use of the neuronal activation marker c-Fos to determine whether manipulation of the intestine triggered activation of sensory neurons leading to the integrated activation of motor vagal neurons (Chapter 4). The results of this study allowed us to demonstrate that the intestinal inflammation triggered consequently to the intestinal manipulation led to the activation of vagal sensory neurons present in the nucleus tractus solitarius (NTS) as well as the dorsal motor nucleus of the vagus (DMV). In Chapter 5, we further investigated the involvement of this endogenous vagal reflex in the pathophysiology of POI. We hypothesized that, as observed for the anti-inflammatory effect of VNS, this endogenous vago-vagal reflex exerted an anti-inflammatory effect on manipulation-induced intestinal inflammation. To address this question, we selectively lesioned direct vagal inputs to the intestine and assessed the influence of the lack of those vagal inputs on the severity of the inflammation and ileus. Furthermore, to determine whether the splenic nerve played a role in the endogenous vago-vagal reflex, we lesioned the splenic nerve prior to intestinal manipulation and assessed whether the lack of splenic nerve impacted on the severity of POI.

The severity of POI varies considerably between patients. Accumulating evidence pointed towards a relationship between the intensity of the surgical trauma inflicted during manipulation of the intestine and the severity of the subsequent POI as increased surgical trauma was associated with systemic release of cytokines [20,22,30]. In the brain, specific areas such as the area postrema are devoid of blood-brain barrier and thus exposed to the systemic circulation. Interestingly, the presence of cytokines in
the systemic circulation was shown to trigger neuronal activation in the area postrema [31]. In Chapter 6, we hypothesized that the level of tissue damage induced during manipulation of the intestine was associated with enhanced brain activation and directly linked to the severity of POI.

Regulation of gastrointestinal inflammation by neural networks is not limited to the sole POI. Inflammatory Bowel Disease (IBD) is a chronic intestinal inflammatory disorder comprising two major forms, Crohn’s Disease and Ulcerative Colitis (UC). A vagal anti-inflammatory role of the vagus nerve and its principal neurotransmitter ACh was demonstrated in a model of experimental colitis [32-34]. Subdiaphragmatic vagotomy in mice exposed to dextran sodium sulfate (DSS), an experimental model for UC, enhanced pro-inflammatory cytokine levels (i.e., TNF-α, IL-6 and IL-1β) hence worsening colonic inflammation [33]. In the same model, central activation of the vagus nerve showed to dampen the colonic inflammation, an effect relying on the integrity of the splenic nerve [13]. The approach used in these studies however relied on the general activation of the vagus nerve at a very high anatomical level and therefore did not allow to precisely determine which neural networks are involved in the anti-inflammatory effect. In Chapter 7 we chose to use selective lesion of vagal inputs innervating the proximal colon, as well as lesion of the splenic nerve to unravel the exact involvement of these two neural pathways in the modulation of colonic inflammation in a DSS-induced model of colitis.
References


