Neuromodulation of intestinal inflammation
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Conclusions and discussion
Until recently, the central nervous system and the immune system were thought to constitute two independent entities. The discovery that the central nervous system could exert a tight and crucial control over inflammation through the release of neuropeptides [1] demonstrated the existence of interactions between these two physiological systems thereby opening a whole new field of research. Since then, the field of neuroimmunology has grown exponentially as these interactions have proven to be bi-directional, i.e., the central nervous system was shown to also perceive inflammation occurring at the periphery through the sensing of inflammatory mediators such as IL-1β and TNF-α [2]. In particular, the autonomic nervous system has shown to be largely involved in immuno-modulation. The vagus nerve was recently shown to exert a crucial anti-inflammatory effect in sepsis as vagus nerve stimulation applied prior to induction of sepsis in rodents decreased the production of pro-inflammatory cytokines thereby leading to improved survival [3]. From this observation the concept emerged of a vagal anti-inflammatory reflex defined as a sensing of the inflammation by vagal afferents leading to the activation of sensory vagal neurons in the NTS in turn leading to the activation of motor vagal neurons located in the DMV [4,5]. Vagal motor efferents subsequently release neurotransmitters such as ACh able to bind to nicotinic receptors present on macrophages and leading to a dampening of the production of pro-inflammatory cytokines such as TNF-α.

Cell-cell communication originating from the nervous system classically occurs via the release of various neurotransmitters by presynaptic terminals into synaptic connections and binding of these neurotransmitters on postsynaptic cells bearing the corresponding receptors for these neurotransmitters. Neurotransmitters released by the vagus nerve consist of a range of molecules with ACh representing the predominant one. Since cholinergic neurotransmitters and in particular ACh only have a limited range of action, close anatomical proximity between vagal nerve endings and immune cells is a prerequisite for the vagus nerve to be able to exert its modulatory action on the immune system. In the last decade, the vagus nerve has been shown to exert a crucial immunomodulatory role in intestinal inflammation. Despite thorough studies on the distribution of vagal nerve endings in the gastrointestinal tract, there still exists a debate on whether vagal nerve endings can be found in the close vicinity of immune cells. It therefore remains unclear if the vagus nerve acts directly on immune cells or whether preganglionic vagal nerve endings solely interact with postganglionic neurons of the enteric nervous system which in turn release neurotransmitters modulating immune
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...cells. Hence, Chapter 2 focused on bringing further knowledge on the distribution of vagal efferents in the gastrointestinal system. Using injection of the anterograde tracer biotin dextran amine in the DMV, we confirmed that preganglionic vagal efferent fibers could only be found in the myenteric plexus and synapsed with post-ganglionic myenteric neurons [6]. We failed to detect cholinergic preganglionic efferent fibers in the close vicinity of immune cells. In contrast, nerve endings of enteric postganglionic neurons, mostly positive for ChAT (the enzyme responsible for the synthesis of Ach), were found in the close proximity of F4/80+ macrophages. Of interest, vagal nerve fibers also contacted myenteric neurons positive for nNOS and VIP. In line, VIP receptors (i.e., VPAC) were found on F4/80+ macrophages located in the close proximity of enteric postganglionic neurons suggesting that VIP may be also be involved in the vagal modulation of intestinal inflammation. Altogether our results demonstrate that the vagus nerve indirectly modulates the resident muscular macrophages in the intestine through interactions with the enteric nervous system.

The existence of a vagal regulation of inflammation was recently brought to light in postoperative ileus (POI). POI is characterized by a generalized paralysis of the gastrointestinal tract observed after abdominal surgery. It occurs as a consequence of a local inflammatory response in the intestinal muscularis triggered by handling of the intestine by the surgeon [7-10]. As seen in sepsis, vagus nerve stimulation applied prior to abdominal surgery was shown to lead to the dampening of the production pro-inflammatory cytokines by macrophages leading to a dampening of the inflammation and ameliorating the ileus [11]. Evidence of the existence of an endogenous closed neural circuitry in response to intestinal manipulation with, on the one hand, activation of sensory vagal fibers as a consequence of intestinal inflammation and subsequent activation of motor vagal efferents was however still lacking. By mapping the activation of vagal neurons located in the NTS and motor vagal neurons located in the DMV, we could demonstrate that intestinal manipulation leads to the activation of sensory and motor vagal neurons innervating the intestine 24h after surgery, an activation that was abolished by selective vagal denervation of the intestine (Chapter 4). Furthermore, activation of those vagal neurons was not observed in mice only undergoing laparotomy demonstrating that this neuronal reflex is not due to anesthesia or opening of the abdominal cavity but is specific to the manipulation of the intestine. This neuronal activation was long-lasting as still observed 24h after the manipulation of the intestine, at a time when inflammation is well established. It is therefore likely...
that the intestinal inflammation rather than mechanical activation of the vagus nerve is the cause underlying this neuronal activation. Moreover, selective vagal denervation of the intestine increased the expression of pro-inflammatory cytokines IL-1β and IL-6 in the intestinal muscularis 24h after intestinal manipulation therefore showing that the endogenous vago-vagal reflex observed during postoperative ileus indeed exerts an anti-inflammatory effect (Chapter 5).

The severity and duration of POI has previously been shown to correlate with the severity of the surgical trauma [12]. Importantly, we showed in Chapter 6 that a higher intensity of intestinal handling in a mouse model of postoperative ileus leads to higher degree of tissue damage associated with the systemic release of pro-inflammatory cytokines and activation of neurons located in the area postrema. It was previously reported that circulating pro-inflammatory cytokines can activate sympathetic inhibitory networks that were shown to be involved in the early neurologic phase of the ileus and participated in the inhibition of the gastrointestinal motility [13-15]. Furthermore, the area postrema is devoid of a blood-brain barrier allowing its direct contact with circulating blood and detection of systemic mediators such as circulating cytokines [16]. The activation of the area postrema in addition to the activation of the NTS and DMV usually observed after gentle intestinal manipulation, could enhance the activation of inhibitory enteric neural networks that could participate in the more severe ileus observed after intense intestinal manipulation. These results therefore underline the fact that different types of inflammation (i.e., local versus systemic) can lead to a differential activation of the autonomic nervous system that will ultimately affect the ongoing inflammation.

The vagal inflammatory reflex was recently shown to be more complex than first proposed. Indeed, splenectomy performed prior to vagus nerve stimulation showed to abolish its anti-inflammatory effect in models of endotoxemia identifying the spleen as a crucial player in the anti-inflammatory effect of the vagus nerve [17,18]. Further studies however demonstrated that removal of splenic sympathetic innervation was sufficient to prevent the anti-inflammatory effect triggered by vagus nerve stimulation [19]. Since evidence of vagal direct innervation of the spleen is still under debate [20,21], the cholinergic anti-inflammatory pathway is now thought to consist and rely on the vagal control of splenic sympathetic nerve fibers with synaptic connections between vagal preganglionic neurons and sympathetic postganglionic neurons occurring in celiac ganglia [22,23]. The subsequent release of noradrenaline by the splenic nerve
in turn acts on ChAT positive T cells able to produce and release ACh that can bind on cholinergic receptors present on innate immune cells. Importantly however, evidence of the existence of synaptic connections between the vagus nerve and the splenic nerve in celiac ganglia is still lacking. We (Chapter 2) and others indeed failed to report such connections suggesting that other neural connections may be responsible for the inflammation-induced activation of splenic sympathetic inputs [24,25].

In POI, we reported that even though the spleen responds to the intestinal manipulation by a dramatic decrease in the number of splenocytes, a phenomenon that is partly regulated by the splenic nerve, the spleen does not participate in the intestinal inflammatory response occurring after intestinal manipulation (Chapter 3). This strongly suggests that the activation of the endogenous vagal reflex observed after intestinal manipulation (Chapter 4) is independent of the splenic innervation. This result was further confirmed in the study performed in Chapter 5 where we demonstrated that contrary to selective vagal denervation of the intestine, sympathetic denervation of the spleen prior to intestinal manipulation had no influence on the manipulation-induced inflammation. Similarly, splenic denervation prior to vagus nerve stimulation did not abolish its anti-inflammatory effect [26]. Altogether, the results of these studies demonstrate that in POI and unlike sepsis, the vagus nerve exerts an anti-inflammatory effect independent of the spleen and solely by targeting the inflamed area, i.e., the intestine.

The existence of a vagal anti-inflammatory neuromodulation was also demonstrated in another intestinal inflammatory disorder, i.e., Inflammatory Bowel Disease (IBD) consisting of Crohn’s disease and Ulcerative Colitis. Both diseases are remitting and relapsing chronic intestinal inflammatory disorders. Alterations of the autonomic nervous system are observed in IBD patients and up to 35% present with a dysbalance in the autonomic nervous system [27,28]. In rodents, vagus nerve stimulation dampens colonic inflammation in models of colitis underlining the importance of neural control of inflammation in this disease [29,30]. Interestingly, a recent study provided evidence that, as in sepsis, this vagal anti-inflammatory effect did not directly target the inflamed intestine. Instead, vagally-mediated sympathetic inputs was shown to be responsible for this vagal anti-inflammatory effect [31]. The results of the study we performed in Chapter 7 bring further evidence of the importance of the splenic innervation in the dampening of colonic inflammation. Indeed, we showed that splenic denervation
performed prior to induction of colitis dampened the production of pro-inflammatory cytokines. We however failed to report activation of vagal motor neurons by colonic inflammation. These data thus suggest that not vagal but other neural networks are activated during colitis and lead to the activation of the splenic nerve. This observation is in accordance with our study of the distribution of vagal fibers in the gastrointestinal tract where we failed to report synaptic connections between the vagus nerve and the splenic nerve (Chapter 2). Since the splenic nerve is under the control of sympathetic nervous system via the greater and lesser splanchnic nerves, it is tempting to hypothesize that the activation of said sympathetic inputs may be responsible for the activation of the anti-inflammatory effect of the splenic nerve during colitis. This hypothesis is in accordance with a recent study demonstrating a sympathetic rather than a vagal control of inflammation in sepsis [24]. Importantly, selective vagal denervation of the proximal colon showed a trend, however non-significant, towards increased secretion of pro-inflammatory cytokines in colitic animals. We can therefore not exclude the participation of direct vagal innervation of the proximal colon in the immunomodulation of colitis. Further studies are required to determine the extent of the participation of these vagal inputs in the regulation of colonic inflammation as well as the exact neural networks involved.

Altogether the results described here clearly underline the fact that the immunomodulatory effect of the autonomic nervous system is different in relation to the type and location of inflammation. In POI, where the inflammation is confined to the intestinal muscularis, we observed activation of a vagal reflex consisting in a closed circuit between the inflamed organ and the brainstem, independently of the splenic innervation. In contrast, in colitis, where the integrity of the intestinal barrier is compromised and where the inflammation becomes systemic, the spleen becomes a more prominent target for neuromodulation of the immune response. Altogether our results demonstrate that the modulation of the immune system occurs both on local and systemic level, most likely depending on the nature of the immune response triggered.
Perspectives and application
New technical developments to study neural networks

Despite numerous studies, the exact neural networks interacting with each other to exert a neuromodulatory effect on the immune system remain unclear and a matter of debate. This lack of clarity is largely due to the limitations of the techniques available to determine the distribution of nerve endings, their interactions with neuronal and immune cells and the neurotransmitters involved. In the past recent years however, new techniques have been developed allowing a more detailed insight in neural activities. These methods, including thermogenetics and optogenetics, use light to selectively control and monitor the activity of specific neurons [32]. By using these techniques one can for example determine the relevance of a set of neurons in the dampening of inflammation as well as the neurotransmitters involved. Such technical improvement will undoubtedly bring further understanding in the mechanisms underlying the anti-inflammatory neuromodulation in the coming years.

Clinical application of the vagal anti-inflammatory neuromodulation

Neuro-immune interactions have opened the way to new therapeutic strategies to modulate inflammation. Activation of the vagus nerve in particular has proven successful in several inflammatory disorders. Several approaches have been investigated to activate vagal inputs whose neurotransmitters could ultimately decrease the pro-inflammatory activity of immune cells. In postoperative ileus, gum chewing has been hypothesized has a potential safe and inexpensive mean to achieve activation of the vagus nerve that could ultimately lead to a dampening of the inflammation and ameliorate ileus. Meta-analysis studies have recently shown a beneficial effect of gum chewing on postoperative ileus [33] but whether this effect is actually due to activation of the vagus nerve remains and requires further investigation. Moreover, stimulation electrodes positioned around the cervical vagus nerve and stimulated by a subcutaneous pacemaker have proven to improve neurological disorders such as epilepsy [34]. In IBD, triggered by preclinical data in colitis experimental models, electrical stimulation of the vagus nerve is currently evaluated as a potential new strategy to obtain remission in patients. Both animal and clinical studies are currently being performed and the coming years will be decisive in determining whether activation of the vagus nerve represents an alternative to conventional anti-inflammatory treatments in IBD.
In conclusion, the results reported in this thesis bring further insight in the neuro-immune interactions existing in the gastrointestinal tract as well as in the mechanisms underlying the anti-inflammatory neuromodulation in the intestinal inflammatory disorders such as POI and colitis. Our results furthermore provide an important fundamental understanding necessary to further consider and use this anti-inflammatory neuromodulation as a new therapeutic strategy in the clinic.
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References


