Neuro-immunity in intestinal disease: in vivo studies of postoperative ileus and colitis
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Chapter 1

Neuro-immune regulation of epithelial barrier function: focus on postoperative ileus
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Neuro-immune regulation of epithelial barrier function: focus on postoperative ileus

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PATHOPHYSIOLOGY OF POSTOPERATIVE ILEUS

Clinical aspects and definition
Postoperative ileus (POI) refers to the disruption of the normal coordinated propulsive motor activity of the gastrointestinal tract, resulting in constipation and the intolerance of oral intake (1). Nausea, vomiting, abdominal pain and distension, and postoperative fatigue further contribute to the morbidity and prolonged hospitalization of patients (1). The transient inhibition of gastrointestinal motility involves the entire GI tract and not all segments are equally affected; small intestinal motility is on average disturbed for approximately 24hrs, gastric motility between 24 – 48 hrs whereas colonic motility is impaired between 48-72 hrs (reviewed in (2)), implying that colonic motility is the main determinant of clinical recovery. The definition of POI is currently under debate mainly because the clinical endpoints used to determine the duration of POI are subjective and unspecific. Some surgeons consider the inability to tolerate food and absence of bowel sounds during the first few postoperative days as a normal phenomenon, and only consider “prolonged” or “pathologic paralytic ileus”, which lasts more than 3 days after surgery, as clinically relevant (3). Others propose to prolong this period to more than 6 days (4). In a recent review by Boeckxstaens and de Jonge, ‘normal’ postoperative ileus is defined as ‘time until first flatus or stool + adequate oral intake during 24h with the duration of 2–4 days. Conversely, ‘prolonged’ or ‘paralytic ileus’ is the consequence of a complication during surgery that occurs in 10–25% of the cases with a duration of more than 6 days (1). It is important to mention that the data presented in this thesis were generated by studying the ‘normal’ course of postoperative ileus as defined by Boeckxstaens and De Jonge.

Pathophysiology and treatment
POI comprises a first and a second phase with different mechanisms involved. The early phase (1–3 hours after surgery) is mainly the consequence of activation of sympathetic afferent inhibitory reflexes by the incision (somatic fibers) and the manipulation of the intestines (visceral fibers) (5). However, activation of these reflexes during abdominal surgery will cease once the abdomen is closed (1). The second phase starts 3–4 hours after surgery and results from an inflammatory reaction in the muscularis externa and is responsible for the sustained hypomotility of the GI tract that characterises postoperative ileus. This second phase is clinically most relevant and results in the high costs related to POI, around US$1.47 billion in the USA (1). The available treatment for POI targets the first phase of POI like the use of prokinetics that block sympathetic activity or directly stimulate colonic motility (6). Also, selective opioid antagonists that prevent IM induced gut hypomotility but do not affect pain reduction are used in the treatment of POI (1). Non pharmacological strategies to shorten the period of postoperative ileus include laparoscopic surgery and peri-operative measures including early feeding and mobilisation, optimised analgesia and restricted fluid management (1). The use of a combination of techniques as part of the concept of multimodal postoperative rehabilitation (fast-track surgery), has been proven to reduce the duration of POI with 24–48 hours (7). However, blocking the inflammatory response following IM may provide most benefit in the treatment of POI. In the last two decades, studies in rodent models of POI have given great insight in the mechanisms behind the inflammatory cascade that is initiated during abdominal surgery and thus provide new targets for prophylaxis of postoperative ileus.
Inflammatory mechanisms: animal models

In the late nineties, studies performed in rodent models demonstrated that gentle manipulation of the small bowel induces an inflammatory reaction in the muscularis externa of the small bowel (8). This inflammation is initiated by activation of resident leukocytes including mast cells (9) and macrophages, upregulation of the adhesion molecule ICAM-1 and subsequent influx of polymorphonuclear neutrophils (PMNs), monocytes, and mast cells (8;10;11) into the muscularis externa of the small intestine (see Fig. 1). As a consequence, muscle function is affected during intestinal manipulation as spontaneous and in vitro stimulated contractions of muscle strips are suppressed during this inflammation (8;10;11). This is mainly due to the increased expression of iNOS and COX-2 by resident macrophages in inflamed areas (12;13). The subsequent release of the mediators NO (14) and prostanoids (15) inhibit the contractility of intestinal smooth muscle locally. Pre-treatment with a mix of antibodies against the adhesion molecule ICAM-1, and the integrin molecules CD11a and CD18 (that together form Lymphocyte function-associated antigen 1 (LFA-1)) prevented the influx of leucocytes and also preserved normal neuromuscular function of muscle strips (11). It was later demonstrated that inflammatory mechanisms not only directly affect muscular function, but also trigger an inhibitory adrenergic pathway resulting in a delay in gastric emptying (16;17). In these experiments, the gastric muscularis was not inflamed indicating that neural pathways are responsible for gastric dysfunction and prolonged ileus after the small intestine muscle contractility has recovered (16). Importantly, treatment with anti-LFA-1 and anti-ICAM (18) antibodies or an antisense ICAM-1 oligonucleotide (16), reduced the influx of leukocytes but also prevented activation of the inhibitory neural pathway (16) and subsequent gastroparesis (18). Pro-inflammatory cascades are also activated in areas distant from the manipulated site where muscle contractility is inhibited locally; this is referred to as the “field-effect” (19). This clinically important aspect of POI was recently shown to involve CCR9+T-cells that are activated at the site of manipulation and migrate to unmanipulated areas (20). There, IFN-γ release from the CCR9+T-cells activate local resident muscularis macrophages and suppress motility distant from manipulated areas (20). In the last two decades, many inflammatory mediators that contribute to the pathogenesis of postoperative ileus have been identified. The mediators that are upregulated in the muscularis layer of the small intestine include the adhesion molecule ICAM-1 and integrin LFA-1, several cytokines including IL-6, MCP-1 (21;22) iNOS and NO, COX2 and PGE2, and activation pro-inflammatory MAP-kinase pathways. Also the anti-inflammatory mediators HO-1 and IL-10 (22;23) are upregulated during intestinal manipulation. In Fig. 1 a schematic overview is shown of the inflammatory events that occur after abdominal surgery.

Figure 1: Schematic representation of the timing of the inflammatory events triggered by abdominal surgery.

COX-2, cyclooxygenase 2
Inflammatory mechanisms: human data
In conjunction to the observed inflammatory response to intestinal manipulation in rodent models, inflammation induced by handling of the intestine is also demonstrated in human tissue. Production of cytokines TNF, IL-6 and IL-10 was shown in the peritoneal fluid as well as systemically in patients that underwent gastrointestinal surgery (24). Also in muscle strips prepared from human small intestine specimens taken during abdominal surgery, mRNA of IL-6, IL-1β, TNF, iNOS and COX-2 was increased in a time dependent manner (25) and ex vivo muscle contractility was enhanced by application of iNOS and COX-2 inhibitors (25). Resident muscular macrophages in these muscle strips expressed LFA-1, IL-6 and IL-6-induced transcription factor Stat-3 during bowel surgery (25). These results were confirmed by a subsequent study showing that mRNA of ICAM-1 and iNOS as well as leucocyte influx was increased by intestinal handling in jejunal muscle specimens collected during biliary reconstructive surgery (26). This study also included groups undergoing abdominal hysterectomy that involves manipulation of the bowel, and transvaginal or laparoscopic hysterectomy, where the bowel is left untouched. The cytokines IL-6 and IL-8 and mast cell mediator tryptase, measured in peritoneal fluid, were increased during abdominal hysterectomy but not during transvaginal hysterectomy. In addition, leukocyte spect-imaging techniques showed an increase of leucocytes only when the bowel was touched (26). Importantly, these results indicate that also in patients, intestinal handling is the main cause for induction of inflammation in the muscularis externa of the small bowel.

KEY ROLE FOR MACROPHAGES AND MAST CELLS
The main cell types that initiate the inflammatory cascade following intestinal manipulation are mast cells and macrophages that reside in the muscularis externa of the small bowel. How these cells are triggered and how they further activate the inflammatory cascade after intestinal manipulation is one of topics investigated in this thesis. In the following section some background is given on these cell types.

Muscularis macrophages
The muscularis macrophage (-like) cells are organized into a layer or “network” at the level of the myenteric plexus and at the serosal side of the intestinal tract (27). This phagocyte population in the muscularis externa has an interesting nature and most likely consists of different subsets of phagocytes including macrophage-like cells expressing F4/80 (27) and dendritic cell-like cells expressing most common DC markers such as CD11c and DEC205 (28). However in the mouse bowel wall, MHCII+ cells outnumber F4/80+ cells, indicating that a large number of these resident muscularis macrophages function as antigen presenting cells (APCs). Though double stainings for these markers have not been performed, so the MHC-II population may consist of an F4/80+ and F4/80- group (29). Also, these cells express the LPS-binding receptor CD14 (28) and stain positive for macrophage scavenger receptor CD163 (30), that has been shown to possess bacteria binding and sensing capacities (31). In a functional study, F4/80 positive phagocytes that were isolated from the murine muscularis externa and cultured for 3–4 days showed Ca2+ influxes and superoxide production upon stimulation with inflammatory mediators adenosine triphosphate (ATP), platelet-activating factor (paf) and bacterial lipopolysaccharide (LPS) (32). Both Ca2+ release and subsequent superoxide production play a role in immune responses thereby further indicating that muscularis macrophages may play a role in host defence.
As mentioned before, macrophages (8) that reside in the muscularis externa of the GI tract are
activated upon intestinal handling and (8). However, only few studies have been conducted to assess a causal role for muscularis macrophages in postoperative ileus. These studies were performed in two rodent models of macrophage depletion. In the rat model, macrophages were pharmacologically depleted and inactivated by administration of chlodronate liposomes; only mature macrophages are able to phagocyte the chlodronate liposomes in an effective (toxic) quantity. In the second model, mice carrying a mutation in the colony stimulating factor-1 gene, completely lack intestinal muscularis muscularis macrophages and have a diminished number of macrophages in the mucosa (33). Pharmacological or genetic depletion of resident macrophages resulted in a decrease of inflammatory mediators and diminished the recruitment of leucocytes into the muscularis after intestinal manipulation. Moreover, macrophage depletion led to a recovered in vitro jejunal circular muscle function and gastrointestinal transit after surgical manipulation (34;35). In IM induced ileus, it is clear that the phagocyte network consisting of dendritic- and macrophage-like cells is crucial in the IM induced inflammatory response that leads to POI.

**Mast cells**

In the body, mast cells reside in tissues that form a barrier with the external environment including skin, respiratory tract, intestinal tract and also near blood vessels (36). In rodents, mast cells can be classified into two subtypes, the mucosal mast cells (MMC), and connective tissue mast cells (CTMC). These types of mast cells are characterized by differences in phenotype, biochemistry, size, function and their responsiveness to drugs (36). In the gut, mucosal mast cells are mainly located in the small intestinal lamina propria, whereas CTMC are located within the serosa and mesentery. Like in rodents, also human mast cells are classified based upon their protease content. The MCT (Mast cells containing tryptase) is similar to the MMC, and the MCTC (Mast cells containing tryptase and chymase) is similar to the CTMC (36). However in human, mast cells are more closely related to monocytes and macrophages, whereas murine mast cells seem to be more similar to human basophils (37).

The importance of mast cells in the inflammatory cascade triggered by intestinal manipulation, was demonstrated in experiments using mast cell stabilizers ketotifen and doxantrazole. Both compounds reduced the inflammatory response and delayed gastric emptying 24 h after abdominal surgery. Conversely, incubation of intestinal loops in solution containing the mast cell activator 48/80 induces an inflammatory response and POI (9). KitW/WV mutant mice that lack mast cells fail to develop an intestinal infiltrate following intestinal manipulation and reconstitution with wildtype mast cells restores the capacity of mutant animals to recruit leucocytes to the intestine after surgery (9).

When mast cell activation was determined in human POI, even very gentle inspection of the intestines at the beginning of the abdominal procedure increased the level of peritoneal mast cell derived tryptase (26). In contrast, in patients undergoing a laparoscopic or a vaginal hysterectomy the increase in tryptase was minimal. Clinically, the time until discharge and first bowel movement was significantly lower after vaginal hysterectomy as compared to abdominal hysterectomy (26). This important finding was followed by a pilot study in patients to investigate the effects of mast cell stabilizers in the course of POI. Administration of the mast cell stabilizer ketotifen was effective in reduction of gastric emptying time and abdominal cramps (38). Altogether, the results from animal and human studies show that mast cells are activated by intestinal handling and play a role in the in the pathogenesis of POI. Importantly, mast cells are important mediators of intestinal barrier function and their activation results in loss of epithelial barrier integrity and subsequent disease progression in animal models of chronic stress (39;40), allergic
inflammation (41), parasitic infection (42) and endoxemia (43). In addition, during abdominal surgery the intestinal barrier is disturbed and may involve mast cell activation.

**Barrier dysfunction during abdominal surgery**

The occurrence of barrier dysfunction following abdominal surgery may also contribute to the pathogenesis of gastro intestinal stasis and post-operative- or septic ileus. Although gastric and colonic dysmotility are regarded as important contributors to ileus, small bowel motility is an important factor in regulation of the enteric bacterial population. A delayed transit time results in bacterial overgrowth, especially in the small bowel, and predisposes to bacterial translocation (44). Bacteria from the intestinal lumen might activate intestinal resident macrophages, given the observation the pretreatment with antibiotics prior to the intestinal manipulation reduced inflammatory responses. The ileus-associated bacterial translocation reflects disturbances in intestinal barrier function and does not only refer to the transepithelial passage of viable bacteria, but also (endo)toxins or antigens from the intestinal lumen. This is illustrated by the observation that in abdominal surgery, barrier dysfunction has been associated with increased postoperative septic morbidity in surgical patients undergoing laparotomy. Moreover, in experimental model for post-operative ileus the occurrence of ileus and the reduction of small intestinal smooth muscle contractility was not observed after surgery in TLR4 deficient mice (45). The occurrence of septic morbidity and even multiple organ failure in serious conditions such as surgery, trauma, ischemia reperfusion injury might be the result of a breakdown of the intestinal barrier and subsequent bacterial translocation. In a recent study including 927 patients over 13 years showed that surgery induced bacterial translocation and was associated with increased postoperative septic morbidity (46). However, the evidence for the so-called ‘gut origin of sepsis’ is at least in humans, controversial (47). The development of septic morbidity is multifactorial and in certain patients measures taken to prevent septic morbidity such as selective gut decontamination, and the use of pre- or probiotics have not been successful.

**REGULATION OF EPITHELIAL BARRIER BY IMMUNE MEDIATORS**

Mast cell activation and more in general, inflammation, is closely associated with intestinal barrier dysfunction and contributes to disease perpetuation and progression. In the following section background is given on the composition of the epithelial barrier and how inflammation affects barrier function. The causes of intestinal barrier dysfunction can be quite diverse and range from intestinal infection to allergic food components, malnutrition, toxic chemicals, NSAIDs and mechanical trauma. In addition, during recent years we have gained a lot of knowledge on the possible role of psychological-stress related impairment in barrier function in irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).

**Epithelial cell function and barrier regulation**

The intestinal epithelial barrier (IEB) stays in close contact with the environment and is composed of a monolayer of specialised intestinal epithelial cells (IEC). The IEB has developed specific mechanisms to prevent access of luminal contents into the lamina propria, which includes the restriction of paracellular transport and the maintenance of the architecture of the epithelial barrier. This function is brought about by the apical junctional complex, which is composed of the TJ, or zonula occludens (ZO) and the subjacent adherens junction, or zonula adherens. Members of the ZO family are proteins that form a bridge between these membrane proteins and actin filaments, which are connected to the peri-junctional ring, a component of
the cellular cytoskeleton (48;49). The desmosomes, or macula adherens, are located along the lateral membranes beneath the adherens junction. Intracellular junctions including adherens junctions, desmosomes, gap junctions and TJs tightly regulate paracellular transport in conjunction. Whereas TJs seal the paracellular pathway, the adherens junctions and desmosomes provide the strong bonds necessary to maintain cellular proximity and allow TJ assembly. Adherens junctions are also critical for epithelial polarization and differentiation, mucosal morphogenesis, and tumor suppression, processes that occur through a variety of interactions with other proteins, including actin and β-catenin. TJs are the most apical components of these intercellular junctions. The main functions of TJs are to prevent diffusion of membrane proteins and lipids between basolateral and apical membranes so that cell polarity is preserved (fence function) and importantly, function as a selective barrier to paracellular transport (barrier function). TJs complexes are composed of a network of proteins that are coupled to actin filaments of the cytoskeleton (49). ZO-1, Occludin (62-82kDa), several members of the claudin family (20-27kDa) and junctional adhesion molecule (JAM) 1 (36-41kDa) are proteins that make up the membrane part of TJs (50-53) (Fig. 2).

**Inflammatory mediators affecting epithelial TJ integrity**

Increased concentrations of pro-inflammatory cytokines are present in the intestine in active phase of inflammatory conditions such as IBD but also in conditions such as hemorrhagic shock or sepsis. In vitro studies in intestinal cell lines have demonstrated that pro-inflammatory cytokines decrease the barrier function of intestinal epithelial monolayers and induce reorganisation of several TJ-associated proteins, including ZO-1, JAM-1, occludin and claudin-1, and -4 (54;55). Examples of such cytokines that cause TJ rearrangements are TNF, IFN-γ, IL-8 and IL-1β (56-58). These cytokines influence the IEB primarily by acting on the epithelial expression and activation of myosin light chain kinase (MLCK) through PKC-activation. Upon activation, MLCK phosphorylates myosin light chain (MLC) which in turn causes contraction of the perijunctional ring, a component of the cellular cytoskeleton, so that permeability of TJs increased (59-61). Another example of cytokine-mediated barrier changes is TNF-induced barrier defects that are associated with MLCK activation, and IL-13 dependent increase in claudin-2 expression (62). As pointed out by the latter (62), cytokine releases and immune responses resulting from enhanced paracellular transport further augment MLCK-related TJ rearrangement. IL-1β increases the intestinal permeability by the induction of MLCK gene transcription resulting in an increased MLCK protein activity, probably mediated by a rapid activation of transcription factor NF-κB (63). IL-1β-mediates increased intestinal permeability via increased paracellular transport of luminal antigens (57). Also TNF-mediated increased intestinal permeability leads to an NF-κB dependent down-regulation of ZO-1 proteins and alteration in junctional localisation (64). In turn, the anti-inflammatory cytokines IL-10, TGF-β and IL-17 protect from loss of TJ proteins (57). The role of IL-6 in modulation of the epithelial barrier is controversial, and may depend on the specific cell type or model system used (57) IL-6, as well as IL-13 (65) and TNF affect epithelial permeability and cell turnover through activation of pro-apoptotic pathways (66) and possibly the activation of PI-3kinase dependent signalling pathways (57). Altogether, cytokine mediated barrier dysfunction is brought about via modulation of TJs through distinct mechanisms and intracellular signalling pathways. Data indicate that MLCK and ZO-1 might be effector molecules in this process.
Mast cell mediators
Initially studied in the context of allergic diseases, we now know that mast cells are highly versatile cells that not only have a sentinel role in host defence but also play a central role in intestinal disorders like IBD and IBS. Mast cells can be activated by a variety of stimuli and the type of stimulus determines their mediator release profile and subsequent consequences for neighbouring cells. In reference to the long list of cytokines that are be involved in modulation of barrier function (i.e. IL-10, IL-6, IL-13, TGF-β and TNF) it is important to notice that all of these can be expressed by mast cells (67). Most of them are de novo synthesized upon mast cell activation but an important exception to this is TNF. Results obtained by Bischoff et al. not only showed that this pro-inflammatory cytokine is constitutively expressed by these cells but also indicated that approximately 60% of TNF positive cells in the gut are in fact mast cells (68). As mentioned before, TNF induced barrier dysfunction depends on MLCK-mediated modulation of TJs (66). In addition, however, it was also shown that TNF potentiates histamine-induced ion secretion in enterocyte cell lines and isolated distal colon (69). Thus, although histamine was originally one of the classic mast cell mediators involved in itch, vasodilation and vascular permeability,
here it was shown that its synergistic action with TNF induces enhanced chloride secretion across the intestinal epithelium. This is highly relevant because it may lead to excessive water secretion and subsequent diarrhoea as observed in e.g. IBD and IBS. Next to histamine, another preformed mediator relevant to barrier dysfunction is tryptase that controls paracellular permeability through PAR-2. Tryptase-mediated cleavage of the N-terminal extracellular domain of this G-protein coupled receptor not only induces the redistribution of TJ proteins via extracellular signal-related kinases (ERK1/2) (70) but also via Ca2+/calmodulin mediated activation of MLCK (71). Being far from complete, this small overview clearly shows that mast cells and their mediators are major players in direct modulation of intestinal barrier function.

**NEUROIMMUNE REGULATION OF EPITHELIAL BARRIER FUNCTION**

**The enteric nervous system (ENS)**

Intestinal phagocytes and mast cells are part of the gut extensive immune system with a number of immune cells equal to those in the remainder of the body. Equally impressive is that the GI tract neuronal network, the enteric nervous system (ENS) contains as many neurons as the spinal cord. The ENS comprises parasympathetic and sympathetic systems, as well as non-adrenergic non-cholinergic systems that and can operate without the participation of the CNS. Importantly, in order to regulate gut function, the CNS interacts with the GI tract largely through the ENS in a bidirectional fashion via the so-called ‘brain-gut’ axis (see Fig. 2). The ENS is organized in several plexuses throughout the intestinal wall: the myenteric and submucosal plexuses, and the mucosal plexus, that contains nerve endings that are in close contact with mucosal immune cells and enterocytes. The ENS contains sensory neurons, interneurons and motor neurons, which primarily control peristalsis, local changes in blood flow, and secretion of water and electrolytes (72). Also, the ENS is involved in regulation of intestinal barrier function (73). An important component of the ENS is the population of enteric glial cells (EGC), that form a large and widespread network at all levels of the GI tract (74) and serve as intermediaries in the enteric neurotransmission and information processing. They show morphologic and functional similarity to brain astrocytes and control several aspects of gut function, including motility, microvascular circulation, epithelial secretion of fluid, ions, bioactive peptides and recently have been identified as important regulators of the intestinal barrier (75).

**Neuropeptides**

More than 30 different neurotransmitters exist in the ENS, with most neurons expressing multiple transmitters. Neuropeptides are considered key mediators in the communication between neurons (in particular sensory neurons) and effector cells (smooth muscle, glands and immune cells) (76) and exhibit a variety of functions in the gastro-intestinal tract. Neuropeptides are involved in secretion of salivary, gastric fluids and intestinal fluids and electrolytes. Besides the action on motor function of the gut, neuropeptides also function as co-transmitters of enteric cholinergic neurons, increase enteric neuron excitability, and consequently induce the release of enteric neurotransmitters, including acetylcholine (77). Neuropeptides are increasingly recognized as potent modulators of the immune response, which is underscored by the fact that, in addition to (afferent) neurons, several immune cells produce neuropeptides. In table 1 a selection of G protein coupled neuropeptides and their role in intestinal disease is depicted.
<table>
<thead>
<tr>
<th>Neuropeptide</th>
<th>Peptide Family</th>
<th>Peptide source</th>
<th>Receptor Type</th>
<th>Effects of receptor activation in intestinal disease</th>
</tr>
</thead>
</table>
| Opioids                      | Opioids        | Recruited immune cells Nerves throughout the GI tract Enteric nerve plexus CNS | mu-, kappa- and delta-opioid receptors | Induction of bowel dysfunction in patients (78).  
• mu-opioid receptor agonists: Decrease intestinal inflammation in TNBS induced and CD45RB+ transfer models of colitis (79)  
• kappa-opioid receptor agonists: Inhibit GI transit in mice (80)  
• delta-opioid receptor agonists: Reduce ileus by reduction of inhibitory reflexes and visceral nociception in rats (81) |
| Corticotropin-releasing hormone | CRH            | Recruited immune cells Epithelial and enterochromaffin cells Enteric nerve plexus CNS | CRHR1 CRHR2                           | Proinflammatory effects in development of TNBS colitis in mice (82)  
Proinflammatory effects in toxin A induced intestinal inflammation in mice (83;84)  
Induction of gastric stasis via peripheral and central pathways in rats (85;86) |
| Ghrelin, Cortistatin         | Motilin        | Recruited immune cells Enteric nerve plexus Gastric EECs CNS Recruited immune cells CNS | GHS-R GHS-R SST1-5 MrgX2              | Reduction of inflammation in TNBS induced colitis in mice (87)  
Acceleration of gastric emptying in POI in rats (88) and septic gastric ileus in mice (89).  
Amelioration of TNBS induced colitis in mice (90) |
| Vasoactive intestinal peptide | VIP PACAP Glucagon Secretin | Recruited immune cells Enteric nerve plexus Lamina propria nerves CNS | VPAC1 VPAC2                           | Amelioration of TNBS induced colitis by shifting T-cell responses from Th1 to Th2 (91;92).  
Inhibition of gastrointestinal transit in POI in rats (93) |
| Substance P                  | Tachykinins    | Recruited immune cells, lamina propria macrophages, colonic glia Motorneurons of intestinal muscularis Lamina propria nerves CNS | NK-1R NK-2R NK-3R                     | Antagonists ameliorate disease in a rat model of TNBS induced colitis (94;95)  
NK-1R-/- mice protected from inflammatory diarrhoea in C. difficile toxin A (96) |
| Neuropeptide Y               | Pancreatic polypeptide | CNS Mononuclear blood leukocytes T-cells APCs | Y1-6R                                 | Enhanced T cell cytokine release (97;99)  
Reduced APC cytokine release and function (100;101) |

Table 1: Distribution of neuropeptides and effects in intestinal disease.

The vagus nerve in barrier regulation
The ENS receives input directly from the brain by the vagus nerve, the largest nerve in the body. Classically, the vagus nerve controls heart rate, hormone secretion, gastrointestinal (GI) peristalsis and digestion, and in the last decade has also been put forward as a regulator of the immune system (102). Activation of vagal activity to modulate barrier function has been achieved via pharmacological and nutritional (103–105) techniques. For instance, in a model of hemorrhagic shock, cholecystokinin (CCK)–dependent stimulation of vagal activity by high fat nutrition has been shown to maintain intestinal barrier integrity. Translocation of bacteria, permeability to horse radish peroxidase (HRP), disturbed expression of ZO-1 (104) and enterocyte damage (103) in high-lipid-treated animals was significantly reduced when compared with those in low-lipid–treated and fasted controls. The protective role of vagal nerve stimulation has been described in several other studies. Vagal efferent activity has previously been shown to decrease histological gut injury and intestinal inflammation in a model of colitis (106;107). Alternatively, in addition to fat feeding–mediated vagal improvement in barrier function in a shock model, peripheral as well as intracerebroventricular injection with ghrelin ameliorated the disrupted barrier function in rat models of intestinal ischemia–reperfusion (108) and sepsis (109). In both studies, vagotomy prevented the effect of ghrelin, demonstrating that these effects...
depended on an intact vagus nerve.

It is interesting to speculate on the working mechanism behind the vagal potential to affect barrier function. Vagal activity may lead to peripheral release of its principal neurotransmitter ACh, but the vagal nerve particularly projects to other post-ganglionic enteric neurons, in particular VIP (110) (studied in the duodenum) and 5-HT (111). In fact, some of the functional effects of vagus nerve stimulation are counteracted by VIP antisera (112). The release of VIP by enteric neurones is shown to inhibit intestinal epithelial cell proliferation and to maintain epithelial barrier integrity, and the effect of VIP on epithelial permeability is concomitant with a neural induced increase in ZO-1 mRNA and protein expression in IEC (113). Besides VIP, ACh increases paracellular permeability in the healthy gut (114) setting the basis for a fine ‘tuning’ of the barrier permeability by the ENS, either with or without the intermediate action of enteric glia (Fig. 2). Interestingly, preliminary data from our own laboratory indicate that ACh reduces cytokine-induced epithelial activation and loss of tight junction integrity in epithelial cell lines (S. Dhawan, unpublished observations 2010).

The vagus nerve in intestinal immune regulation

In the gut, cholinergic fibres are located in close apposition to antigen presenting cells APCs (115) and would thus be the ideal site for neuro immune modulation by the vagus nerve (Fig 2). It has been shown that activation of the vagus nerve negatively regulates macrophage immune responses via the peripheral release of acetylcholine (ACh) (116;117). Activation of the so-called ‘cholinergic anti-inflammatory pathway’ has been shown to ameliorate disease in various models of inflammation, including sepsis (116), ischemia reperfusion (116) haemorrhage (105). In particular, the nicotinic acetylcholine receptor α7 (nAChRα7) is proposed as the receptor subtype that potentiates the anti-inflammatory actions of ACh on immune cells and is expressed by macrophages (115;116;118;119), but also by monocytes, dendritic cells and mast cells (120;121). In the gut, vagus nerve activity can have a beneficial effect on the course of intestinal inflammation as shown in a mouse model for POI (122). In addition, administration of selective nAChRα7 agonists ameliorated inflammation and disease parameters in postoperative ileus (122). Also in mouse models of colitis, enhanced parasympathetic output is involved in the negative regulation of intestinal inflammation via efferent activity of the vagus nerve (106;107).

Innate immune cells express a broad range of nAChR receptors (nAChRs), muscarinic ACh receptors (123), and peptidergic receptors (124), and are therefore target cells for regulation by neuronal derived mediators. In this way, the ENS may mediate regulation of gut immune cell activity including cytokine production and phagocytic properties, thereby also indirectly affecting intestinal barrier function (Fig. 2).

Taken together, novel therapeutic approaches to be designed the next years to specifically target and intervene in disruption of neuroimmune communication in the gastrointestinal tract would likely to benefit in treatment of disease. The advantages of the use of neuron derived chemical messengers are that they are short lived and act local. Importantly, in addition to the potential to ameliorate IEB, neuronal messengers also affect inflammatory processes, motility, mucus production and water/electrolyte secretion, thereby further contributing to disease amelioration.
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