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EDITORIAL

Interplay between inflammation, immune system and neuronal pathways: Effect on gastrointestinal motility

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Abstract

Sepsis is a systemic inflammatory response representing the leading cause of death in critically ill patients, mostly due to multiple organ failure. The gastrointestinal tract plays a pivotal role in the pathogenesis of sepsis-induced multiple organ failure through intestinal barrier dysfunction, bacterial translocation and ileus. In this review we address the role of the gastrointestinal tract, the mediators, cell types and transduction pathways involved, based on experimental data obtained from models of inflammation-induced ileus and (preliminary) clinical data. The complex interplay within the gastrointestinal wall between mast cells, residential macrophages and glial cells on the one hand, and neurons and smooth muscle cells on the other hand, involves intracellular signaling pathways, Toll-like receptors and a plethora of neuroactive substances such as nitric oxide, prostaglandins, cytokines, chemokines, growth factors, tryptases and hormones. Multidirectional signaling between the different components in the gastrointestinal wall, the spinal cord and central nervous system impacts inflammation and its consequences. We propose that novel therapeutic strategies should target inflammation on the one hand and gastrointestinal motility, gastrointestinal sensitivity and even pain signaling on the other hand, for instance by impeding afferent neuronal signaling, by activation of the vagal anti-inflammatory pathway or by the use of pharmacological agents such as ghrelin and ghrelin agonists or drugs interfering with the endocannabinoid system.

Key words: Sepsis; Ileus; Nitric oxide; Prostaglandins; Oxidative stress; Residential macrophages; Mast cells; Neurons; Afferent; Neuroimmunomodulation; Inflammation

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DEFINITION AND CLINICAL RELEVANCE OF SEPSIS

Sepsis originates from the Greek word sepsios meaning "rotten" or "putrid". Sepsis is defined as a systemic inflammatory response syndrome secondary to infection. It represents a leading cause of death in critically ill patients, mainly due to the development of organ dysfunction and tissue hypoperfusion[1-3]. The incidence of severe sepsis is still increasing and ranges from 11%-15% in intensive care unit (ICU) patients: 11.8 patients per 100 ICU admissions in an Australian and New Zealand population[4]; 14.6% in a French ICU population[5], 11% of all ICU admissions in the US[6] and 12% of ICU patients in Spain[7]. Although

the overall mortality rate among patients with sepsis is declining - related to general improvements in acute and intensive hospital care rather than specific sepsis-related therapy - the number of sepsis-related deaths still increases and ranges between 25%-60%[2,4-6,8]. The development of organ dysfunction is a major determinant of mortality and is influenced by the co-existence of chronic comorbidity[8].

According to the International Guidelines of Severe Sepsis 2008[3], the management of severe sepsis complicated with hypoperfusion and organ failure is based on initial resuscitation (first 6 h) with, as main goals, maintaining the mean arterial pressure above 65 mmHg, a CVP of 8-12 mmHg, a urinary output above 0.5 mL/kg per hour and a central venous oxygen saturation above 70%. Secondly, the source and type of infection needs to be established by obtaining appropriate cultures before antibiotic therapy is initiated. The use of fluid therapy, vasopressors (norepinephrine and dopamine as initial choice) and dobutamine as inotropic therapy is recommended[3]. The use of corticosteroids remains controversial in the management of sepsis and is advised only in refractory sepsis, as is the use of recombinant human activated protein C which should be reserved for patients with organ dysfunction and a high clinical risk of death[2,9].

During sepsis a complex interaction takes place between the infecting microorganism, the host immune response, inflammatory and coagulation responses[9]. Different mechanisms such as the innate immune system, the coagulation pathways, endothelial dysfunction, mitochondrial dysfunction and apoptosis are described and associated with severe sepsis[9]. A cross-talk takes place between different immune cells including macrophages, dendritic cells and CD4+ T cells, leading to either a proinflammatory or anti-inflammatory cytokine reaction[10]. Patients can thus present with either an exaggerated proinflammatory systemic inflammatory response (often described in the early phase) or rather a state of immunosuppression and even anergy in a later phase[10,11]. Generally accepted is the theory that cells of the innate immune system recognize microorganisms and initiate responses through pattern recognition receptors (PRRs), pathogen-associated molecular patterns (PAMPs) and Toll-like receptors (TLRs). The latter will result in activation of intracellular signal transduction pathways such as the activation of nuclear factor (NF)-κB and caspase-1[9,11]. On the other hand, an excess production of reactive oxygen and nitrogen species is described resulting in oxidative and nitrosative stress. Other pathogenic mechanisms leading to sepsis-related organ dysfunction are exacerbated coagulation, impaired anticoagulation and decreased fibrin removal, together with endothelial disturbances, mitochondrial dysfunction and apoptosis[9].

ROLE OF THE GASTROINTESTINAL TRACT IN SEPSIS

Hassoun *et al*[12] described in the early 2000s how the gastrointestinal tract might play a pivotal role in the pathogenesis of post-injury multiple organ failure. Gut hypoperfusion is an important inciting event in the pathogenesis of organ failure, whereas the reperfused gut is an early source of proinflammatory mediators. Ischemia and reperfusion in the gastrointestinal tract will activate a cascade of stress-sensitive protein kinases (MAPK, ERK, p38, JNKs) that converge on transcription factors regulating the expression of proinflammatory genes[12]. Important mechanisms playing a role in gastrointestinal dysfunction as a result of post-injury multiple organ failure are increased intestinal permeability, bacterial translocation and paralytic ileus[13]. Bacterial translocation is defined as the passage of both viable and non-viable microbes and microbial products such as endotoxins across the mucosal barrier[12], whereas ileus is defined as an inhibition of propulsive intestinal motility[14]. These mechanisms play an important role in the maintenance of multiple organ failure and secondary infections[12]. Frequently, the source of bacteria can be traced to the endogenous flora of the gastrointestinal tract[15]. Ileus predisposes to luminal accumulation and bacterial colonisation of the stomach and small intestine and therefore promotes bacterial translocation and pneumonia by aspiration of gastric contents. Ileus therefore plays a pivotal role in the occurrence and maintenance of infections in multiple organ failure[12]. The guidelines on the management of severe sepsis of 2008 concluded that prophylactic use of selective digestive tract decontamination in severe sepsis patients would be targeted towards preventing secondary ventilator-associated pneumonia[3]. There are, however, insufficient data available from severe sepsis patients to support global use of selective digestive tract decontamination.

The gastrointestinal tract therefore has a dual role in sepsis, being a target organ and a pathogenic player. It is now understood that the gut is not only a source of bacteria and endotoxins, but also a source of pro-inflammatory mediators and a cytokine-generating organ. These inflammatory mediators reach the circulation *via* the intestinal lymph[16,17]. Bacteria and endotoxins crossing the mucosal barrier further potentiate the gut inflammatory response, even when the bacteria and their products are trapped within the gastrointestinal wall or intestinal lymph nodes, not reaching the systemic circulation[16]. Studying the impact of experimentally-induced sepsis on gastrointestinal motility and its immunological modulation therefore merits further attention.

ANIMAL MODELS OF SEPSIS

Several animal models of sepsis exist, all with their advantages and disadvantages (Table 1)[18-20]. One of the main criticisms of the animal models is that the demonstrated benefits of therapeutic agents in animals are rarely translated into successful clinical trials, indicating the difficulty of mimicking the complex interaction between current illness, sepsis and supportive therapy in an animal model. The lack of supportive therapeutic interventions in animal models represents therefore an important caveat in the use of animal models. Also, the

Table 1 Overview of septic animal models displaying advantages and disadvantages (adapted from[18-20])

Endotoxin model
Advantages
Endotoxins play a significant role in the pathogenesis of sepsis
Simple model
Using sublethal doses, providing active resuscitation, using continuous infusion and the use of intraperitoneal injection are four measures reproducing more accurately the human situation
Lipopolysaccharides is stable (compared to the use of bacteria), therefore the model is more accurate and reproducible compared to the bacterial infection models
Disadvantages
Exaggerated release of host cytokines
Most of the time only Gram-negative sepsis
Single toxin does not mimic human sepsis
Therapies shown to be effective in animal models, failed in clinical trials
Rats are very resistant compared to humans
Lack of an infectious focus
Bacterial infection model
Advantages
Endotoxins play a significant role in the pathogenesis of sepsis
Reduction of the dose, increasing the infusion time, giving active resuscitation can prolong survival and render the model more comparable to the human situation
Disadvantages
Uncommon clinical occurrence
High doses of bacteria are needed
Significant interlaboratory variability
Survival is short
Serum cytokine responses are transient and exaggerated
Peritonitis model: cecal ligation puncture model
Advantages
Resemblance to clinical situation
Peritoneal contamination with a mixed flora
The cytokine response is comparable to human situation
Severity can be adjusted by increasing the needle puncture size or the number of punctures, delaying mortality over several days
Disadvantages
The model needs a surgical procedure that by itself may induce ileus
Difficult to control the magnitude of septic challenge
Variability within the cecal ligation puncture model

timing of most animal models is not comparable to the human situation as most animal models represent acute syndromes unlike sepsis in humans (hours to days in animal models *vs* days to weeks in humans)[20].

Animal models of sepsis are generally divided into 3 categories: endotoxin models, bacterial infection models and peritonitis models[18-20]. The major advantages and disadvantages of these models are described in Table 1. In the endotoxin model lipopolysaccharides (LPS) of bacteria are injected, while in the bacterial infection models the bacteria themselves are injected. Different peritonitis models are described, such as cecal ligation and puncture (CLP), implantation of a fibrin clot suspended with bacteria in the abdominal cavity or implantation of a colonic stent. These peritonitis models have as a major advantage the presence of a local infection focus and some authors consider the CLP model as the gold standard for sepsis research[20]. However, it is important to understand that this procedure requires a major surgical procedure which might have no effect in sepsis survival studies but strongly interferes with gastrointestinal motility because of the induction of postoperative ileus.

In the endotoxin model, LPS is injected intravenously or intraperitoneally. The choice of the animal (mouse, rat, guinea pig), and the strain and gender of the animal are all confounding parameters in these models, as well as the strain of bacteria or endotoxins used, the dose and the administration route. Studying gastrointestinal motility and its immunological modulation by administering a single intraperitoneal injection of endotoxin at a sublethal concentration represents an adequate model for experimental sepsis[13,21]. It has been shown previously in different animal species that a single dose of LPS alters gastrointestinal motility. By 1963, Turner *et al*[22] had already shown that endotoxins reduce water and food intake and gastric emptying in mice. We investigated the effect of a single intraperitoneal injection of LPS of *Escherichia coli* and showed a significant delay in gastric emptying and small intestinal transit[23-26]. In rats, endotoxins delay gastric emptying, increase small intestinal transit[27-31], and reduce jejunal spontaneous circular muscle activity[32,33]. In dogs, endotoxins delay gastric emptying and abolish intestinal migrating motor complexes[34-37]. In horses, a low dose of endotoxin was reported to disrupt the motility pattern and to decrease the cecal and colonic contractile activity[38,39]. Therefore, endotoxins are definitely able to induce gastrointestinal ileus, which we will now refer to as sepsis- or endotoxin-induced ileus.

On the other hand, ileus is often studied in a surgically-induced postoperative model. It is generally accepted that postoperative ileus is triggered by two different phases: an early neurogenic and a late inflammatory phase[40]. During the initial neuronal phase, inhibitory effects on motility are related to the activation of an inhibitory reflex pathway involving adrenergic, nitrergic and VIP-ergic neurons[41,42]. In the second phase, the activation of an inflammatory cascade plays a crucial role and is triggered by the handling of the intestines activating the cross-talk between the immune system, the autonomic nervous system and the muscle effector apparatus of the gastrointestinal wall[40].

Both the endotoxin-induced model and the postoperative ileus model accentuate the important role of inflammation in the development and maintenance of gastrointestinal ileus. It is therefore our opinion that the endotoxin-induced ileus model and the postoperative ileus model are both relevant in the study of inflammation-induced motility disturbances.

PATHOGENESIS OF INFLAMMATORY-MEDIATED ILEUS

Initial research focussed on the mediators that could be involved in the inflammation-induced impairment of gastrointestinal motility by a direct action on the intestinal smooth muscle cells. Later on, the focus was broadened in an attempt to clarify not only the mediators involved but also the cell types and the transduction pathways. The

main goals were to identify possible target molecules enabling the development of novel drugs for clinical use.

Mediators involved in the pathogenesis of inflammatory-mediated ileus

Nitric oxide (NO) was one of the first molecules postulated to play an important role in the pathogenesis of LPS-induced motility disturbances in rats and mice mainly mediated *via* the inducible isoform of NO synthase (iNOS)[23,27,29,30,32,43]. Several groups showed that blockade of NOS reverses the endotoxin-induced changes in gastrointestinal motility in different animal species by the use of selective or non-selective NOS blockers and iNOS knock-out mice[23,27,29,30,32]. Our group proved that this effect of NO, derived from iNOS, is mediated at least partially by activation of guanylyl cyclase in a murine endotoxic model[23,44]. Furthermore, we also found evidence of a role for NO-mediated oxidative stress mechanisms, indirectly *via* the use of the solvent DMSO which also has radical scavenging properties, and directly by the use of antioxidant molecules[23,24,45]. Treatment of mice with the antioxidant pegylated superoxide dismutase reversed the endotoxin-induced delay in gastric emptying and improved the delay in intestinal transit. This was associated with a decrease of iNOS-positive residential macrophages and a decrease of immunohistochemical staining for nitrotyrosine and 4-hydroxy-2-nonenal, markers for oxidative and nitrosative stress, in the gastric and ileal mucosa of LPS-treated mice[24]. In agreement with these results, we found that the antioxidant melatonin reversed the endotoxin-induced motility disturbances in mice through a reduction of intestinal lipid peroxidation, MAPK activation, NF-κB activation, iNOS transcription and expression, and nitrite production[45].

In addition, NO produced from neuronal NOS may be involved in sepsis-induced ileus. Quintana *et al*[46,47] showed synthesis of NO in postganglionic myenteric neurons during the early phase of endotoxemia (30 min after injection of LPS) in rats, as well as an increase in nNOS mRNA in the dorsal vagal complex of the brainstem 2 h after administration of LPS.

Prostaglandins are also postulated to play an important role in the pathogenesis of inflammatory-mediated ileus[13,21,48]. In rat studies on postoperative ileus, a role for prostaglandins was proven by the presence of COX2 mRNA and protein in residential macrophages, recruited monocytes and in a subpopulation of myenteric neurons[49]. This study also demonstrated an amelioration of the gastrointestinal motor function by treatment with the COX2 inhibitor, DFU[49]. Other animal studies, also from our own group, showed a differential effect of different COX inhibitors on postoperative motility induced by laparotomy alone or laparotomy with bowel manipulation, suggesting a possible involvement of both COX1 and COX2 isoforms and different sites of action in different stages of postoperative ileus[50,51]. A recent clinical trial comparing the effect of diclofenac (standard non-steroidal anti-inflammatory drug) and celecoxib (COX2 selective inhibitor) in patients after abdominal surgery also showed a differential effect of both drugs. Celecoxib significantly reduced the development of paralytic ileus, whereas both drugs did not result in a more rapid restoration of the gastrointestinal function compared to placebo[52]. To our knowledge, no studies of different COX inhibitors on sepsis- or endotoxin-induced ileus are available.

Moreover, prostaglandins are postulated to modulate afferent nerve signaling from the gut to the spinal cord and higher brain centers, indicating that they play a role not only in motility disturbances but also in sensitivity disturbances and pain signaling pathways. These effects are described both in the postoperative ileus model and in the endotoxin-induced ileus model[50,53].

Cell types involved in the pathogenesis of inflammatory-mediated ileus

The initial search for the location of iNOS production in the endotoxin-induced ileus model pointed to an important role for residential macrophages[24,32]. We clearly showed the presence of iNOS in residential muscular macrophages in the stomach and ileum of LPS-treated mice[24]. Besides residential muscular macrophages, the gastrointestinal tract contains a dense population of mucosal macrophages which play a crucial role in tissue homeostasis on the one hand and in the initiation, propagation and resolution of inflammation on the other hand. Mucosal macrophages are conditioned towards an anti-inflammatory role under normal circumstances and switch towards a pro-inflammatory modus during inflammation[54-56]. The relationship and the interaction between muscular and mucosal residential macrophages in the gastrointestinal tract have not been studied so far. In the field of ileus, research is focussed largely on the muscular population. Several groups have hypothesized that LPS initiates an inflammatory cascade consisting of the activation of the normally quiescent network of residential muscularis macrophages, resulting in the production of a plethora of inflammatory cytokines, chemokines and other substances such as nitric oxide and prostaglandins[13,23,24,32,33,43,48]. This inflammatory milieu results in the recruitment of circulating leukocytes and consequently in the further release of leukocyte-derived substances such as nitric oxide and prostaglandins capable of altering gastrointestinal motility[23,48] and activating inhibitory neurogenic reflex pathways[57]. The presence of iNOS is not only demonstrated in residential macrophages but also in the recruited leukocytes, thereby augmenting the inhibitory effects on gastrointestinal motility[21,58]. Monocyte-chemoattractant protein-1 (MCP-1), derived from the residential macrophages, is a key molecule in the recruitment of additional monocytes during endotoxemia, leading to an enhanced secretion of kinetically active substances that may alter gastrointestinal motility[59,60]. In a polymicrobial model of sepsis, such as the CLP model, this complex inflammatory response is induced within the intestinal muscularis with recruitment of leukocytes and mediators that inhibit intestinal muscle activity[48]. The activation of residential muscular macrophages within the gastrointestinal wall also plays a crucial role in the late inflammatory phase

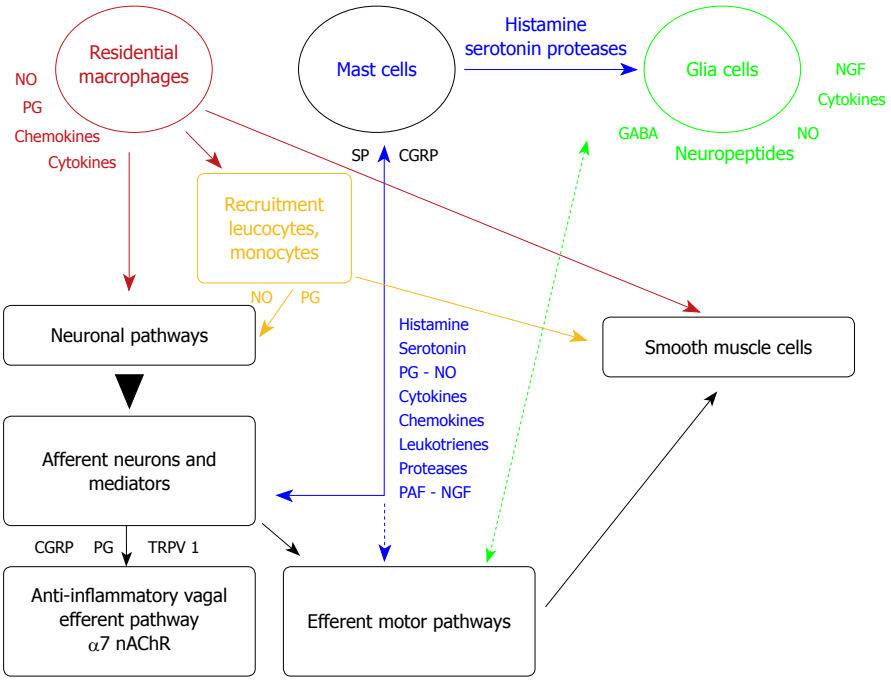

Figure 1 Hypothetical scheme of the complex interplay between, on the one hand residential macrophages (red), mast cells (blue), glial cells (green) and the recruitment of inflammatory cells (yellow), and on the other hand the activation of neuronal reflex pathways (black). Mediators involved in the different cell populations are marked in the same color as the cell type involved. NO: Nitric oxide; PG: Prostaglandins; SP: Substance P; CGRP: Calcitonin gene-related peptide; GABA: γ-aminobutyric acid; NGF: Neuropeptides, growth factors; PAF: Platelet-activating factor; TRPV: Transient receptor potential channel of the vanilloid subtype; nAChR: Nicotinic acetylcholine receptor.

of postoperative ileus, resulting in secretion of molecules such as lymphocyte function-associated antigen-1 and intercellular adhesion molecule-1 (ICAM-1), again attracting more leucocytes within the intestinal muscularis and therefore maintaining the inflammatory cascade[13,21,57]. In the postoperative model, the important role of intestinal residential macrophages was definitely proven by the work of Wehner *et al*[61], showing that depletion and inactivation of the macrophages in rats and mice prevented intestinal inflammation and postoperative ileus.

In addition to residential macrophages and inflammatory leucocytes, mast cells are also put forward as important cells in the induction and maintenance of the inflammatory cascade and its effects on motility. There is evidence for bidirectional communication between mast cells and neurons in the gastrointestinal tract[62-64]. de Jonge *et al*[65] proved elegantly that the degranulation of connective tissue mast cells is a key event in the establishment of the intestinal infiltrate in the abdominal wall in a murine model of postoperative ileus. The importance of mast cells in the pathogenesis of postoperative ileus could be translated to the human situation; The *et al*[66] showed that intestinal handling triggered mast cell activation as well as leucocyte infiltration in patients undergoing an abdominal hysterectomy. In a pilot trial with the mast cell stabiliser ketotifen, the same authors demonstrated that ketotifen improved the surgery-induced delay in gastric emptying of liquids in humans[67]. The number and activity of both residential macrophages and mast cells in LPS-treated mice is upregulated (personal communication)[68]. More studies on the role of mast cells and their mediators need to be performed in models of sepsis-induced ileus.

The question as to whether the main initiator of the inflammatory reaction is the residential macrophage or the mast cell remains unresolved. In a recent paper, Boeckxstaens *et al*[40] suggest a role for peritoneal mast cells adjacent to mesenteric blood vessels. Activated by neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) released from the adjacent afferent neurons, mast cells are able to release proinflammatory mediators into the peritoneal cavity diffusing into the blood vessels and increasing mucosal permeability. In turn, luminal bacteria and/or bacterial products enter the gastrointestinal wall and activate the resident macrophages triggering intracellular signaling pathways, leading to transcription of inflammatory molecules, cytokines, chemokines and adhesion molecules[40]. Others support a role for the residential macrophages as the first responders and conductors which would orchestrate the inflammatory events after surgical manipulation or endotoxin exposure[21]. More importantly, the interplay between these initiating cells and the nervous system should be further investigated as both the mediators released from mast cells and from residential macrophages are able to affect neuronal signaling within and from the gastrointestinal wall (Figure 1)[21,62].

Neuro-immunomodulatory pathways involved in the pathogenesis of inflammatory-mediated ileus

The gastrointestinal tract is richly innervated by an intrinsic enteric nervous system and by an extrinsic autonomic nervous system consisting of parasympathetic vagal and pelvic neurons and sympathetic splanchnic neurons. During inflammation of the gut, there is a complex multidirectional interaction between immune and inflammatory cells, neurons and smooth muscle cells (Figure 1)[21,69]. Wang *et al*[53] proved that inflammatory mediators released from the gut during endotoxemia were able to affect jejunal afferent discharge in the rat. Both LPS itself and mesenteric lymph fluid collected after injection of LPS were able to increase the afferent discharge. Liu *et al*[70] demonstrated an increased discharge in capsaicin-sensitive

mesenteric vagal afferents following systemic LPS. It was also shown that the endotoxin-induced delay in gastric emptying in rats could be suppressed by systemic capsaicin, by local application of capsaicin to the vagal nerve (but not to the celiac ganglion), and by a CGRP receptor antagonist[31]. Our own group also provided evidence for a role of afferent neurons in the motility disturbances induced by endotoxin in mice[25]. Neuronal afferent involvement was demonstrated by the beneficial effect of hexamethonium and capsaicin, and the effect of the afferent neurons was mediated by CGRP and the TRPV1 receptor. In a postoperative murine model, the involvement of both vagal and spinal afferent neurons in the inhibition of gastrointestinal motility was shown, with a differential effect of COX2 inhibition on the two types of afferent neurons[71]. All these results underline the importance of an initial activation of afferent neurons leading to the activation of inhibitory neuronal reflex pathways in gastrointestinal motility disturbances induced by sepsis or surgical manipulation of the intestine.

Activation of the vagovagal pathway is able to modulate inflammation in the gastrointestinal tract on the one hand and motility on the other hand. The cholinergic nervous system is able to attenuate the production of proinflammatory mediators and to inhibit inflammation; this mechanism is known as the cholinergic anti-inflammatory pathway[69,72,73]. The cholinergic anti-inflammatory surveillance system starts with the activation of vagal sensory afferent fibers by proinflammatory cytokines, secreted by innate immune responses stimulated *via* exogenous and endogenous molecular products of infection and injury such as LPS. Information is transmitted to higher brain centres. In the brain, vagal efferent fibers are activated; signaling back to the gastrointestinal tract. Acetylcholine inhibits the cytokine release directly *via* the α7 nicotinic acetylcholine receptor (nAChR) expressed on macrophages. Animal models showed that the anti-inflammatory effect is not exclusively mediated *via* macrophages but that other immune cells such as dendritic cells and mast cells may also be involved[73]. However, anti-inflammatory properties of vagal activation were also shown in murine isolated intestinal and peritoneal macrophages in the light of inflammatory surveillance: whereas acetylcholine stimulated the phagocytic potential of the macrophages, it inhibited the immune reactivity, as evidenced by reduction of NF-κB and proinflammatory cytokines and stimulation of IL10 production *via* nAChR α4/β2[74]. On the other hand, there is also evidence for indirect modulation of inflammatory processes *via* postganglionic neuromodulation of immune cells in primary immune organs such as the spleen[69,72,75]. In a lethal rat endotoxemia model, direct electrical stimulation of the peripheral vagus nerve was shown to inhibit tumor necrosis factor (TNF) synthesis in the liver, attenuate peak serum TNF levels and prevent the development of shock[76]. In a rat cecal ligation and puncture model, stimulation of the caudal vagal trunk prevented the induced hypotension, alleviated the hepatic damage and plasma TNFα production but had no effect on liver NF-κB activation[77]. In a murine sepsis model, transcutaneous vagal nerve stimulation reduced TNFα levels and improved survival[78]. In a murine postoperative ileus, stimulation of the vagal nerve ameliorated surgery-induced inflammation and ileus, whereas AR-R17779, an α7AChR agonist, prevented postoperative ileus and reduced the inflammatory cell recruitment in a similar mouse model[79,80].

Furthermore, sympathetic nerves might be involved in the neuroimmunomodulation of the different functions of the gastrointestinal tract. Hamano *et al*[81] showed that yohimbine, an α2-adrenergic receptor antagonist, improved endotoxin-induced inhibition of gastrointestinal motility in mice. They suggest the mechanism of action is related to the activation of α2-adrenergic receptors on macrophages downregulating the expression of iNOS. However, it could not be excluded that gastric emptying was improved *via* inhibition of the presynaptic α2-adrenergic receptors on cholinergic vagal nerves. Under normal conditions, these receptors decrease the release of acetylcholine and thereby reduce gastrointestinal motility[81]. Nevertheless, Vanneste *et al*[82] could demonstrate that presynaptic α2-adrenergic receptor control of cholinergic nerve activity was unchanged in a rat model of postoperative ileus. Also in the postoperative ileus model, a beneficial effect of spinal cord stimulation at the level of T5-T8 segments was recently shown on gastric emptying, although the mechanism of action remains to be unravelled[83]. An interaction of sympathetic neurotransmitters with the gut immune system, glial cells and gut flora was recently suggested, in correlation with the vagal immunomodulatory mechanisms in conditions of inflammation or ileus[84].

A cell population that might be relevant to consider in this neuroimmunomodulatory framework is the enteric glial cell population. Enteric glial cells are part of the enteric nervous system, along with neurons and interstitial cells of Cajal (ICC); originating from the neuroectoderm. They form a widespread network in the gastrointestinal wall where they outnumber the neuronal cell population at the level of the myenteric plexus, the submucosal plexus and the interconnecting nerve strands. Glial cells are small, star-shaped cells with numerous processes extruding from the epithelium and can be identified by the presence of specific proteins such as glial fibrillary acidic protein (GFAP), vimentin, S100B and glutamine synthetase. Glia contain precursors for neurotransmitters such as GABA and NO, express receptors for certain purinoceptors, express cytokines - interleukin (IL)-1β, IL-6, TNFα - and neuropeptides such as neurokinin A and substance P after activation[85]. Enteric glial cells can directly or indirectly modulate neuromuscular transmission, gastrointestinal motility and secretion. They also control - together with enteric neurons - intestinal barrier functions and gut immune homeostasis. Glial cells should therefore be recognised as important players in the multidirectional interactions between neurons, immune cells and intestinal epithelium (Figure 1)[85,86]. Ablation of glial cells in adult transgenic mice results in a fulminant and lethal jejunoileitis characterized by an increased myeloperoxidase activ-

ity, degeneration of myenteric neurons and intraluminal hemorrhage, pointing towards an important role of enteric glia in the maintenance of bowel integrity[87].

LPS administration in mice increases the expression of S100B in the intestine, which is indicative of an upregulation of glial cells. This effect of LPS is reversed by the cannabinoid cannabidiol, paralleled by a decrease in glial cell hyperactivation and a decrease in mast cells and macrophages (personal communication)[68]. The role of enteric glia and S100B in human gastrointestinal inflammation has been recently investigated in human biopsies, showing increased S100B in the duodenum of patients with celiac disease and in rectal biopsies of patients with ulcerative colitis; both associated with an increase in iNOS protein expression and nitrite production[88,89]. Changes in enteric glial cells and their markers (GFAP and S100β) have also been described in inflammatory bowel disease (IBD)[86].

Intracellular signaling pathways involved in the pathogenesis of inflammatory-mediated ileus

Intracellular signaling pathways play an important role in the initiation of the inflammatory immune response. Luminal bacteria and/or bacterial products enter the gastrointestinal wall and activate the resident macrophages, inducing phosphorylation of MAP kinase (ERK1/2, JNK and p38), thereby activating intracellular transcription factors such as NF-κB, STAT3, Egr-1 and NF-IL6 in both macrophages and leucocytes[21,40,79,90,91]. This leads to the induction of numerous inflammatory molecules (iNOS and COX2), cytokines (TNFα, IL1β, IL6), chemokines (MCP-1, GM-CSF, MIP1α, VEGF) and adhesion molecules (ICAM-1).

Inhibition of protein tyrosine kinases (PTK), resulting in the phosphorylation of tyrosine residues on proteins, occurs at multiple steps in the signaling cascade. Tyrphostin AG 126, a PTK inhibitor, reduces the inflammatory mediator expression induced by surgical manipulation probably through inhibition of the transcription factor NF-κB[92]. Wehner *et al*[93] showed the contribution of early p38-MAPK activation in murine postoperative ileus by the use of the macrophage specific inhibitor, semapimod. A role for Egr-1 was demonstrated in murine postoperative ileus in Egr1 knockout mice and in mice treated with the PPARγ agonist, rosiglitazone[90,91]. In addition, the beneficial effects of CO-releasing molecules in the development of postoperative ileus seem to be mediated by interference with p38 and ERK1/2 activation[94]. These data provide evidence for a key role of activation of transcription factors in postoperative ileus.

These intracellular signaling pathways also play a crucial role in endotoxic ileus. However, during sepsis and endotoxemia, TLRs come into sight and might represent an important pathogenic tool. Cells of the innate immune system recognize microorganisms and/or parts of microorganisms and initiate responses through PAMP binding to PRRs. TLRs are a family of PRRs (similar to, for instance, NOD), while PAMPs are often cell-wall molecules. LPS, a specific PAMP from Gram-negative bacteria, is known as a potent TLR4 ligand[9,95]. Specific TLR pathways (TLR2, TLR4, TLR5) are under investigation in the pathogenesis of gastrointestinal ileus in sepsis models[96-98].

Downstream intracellular signaling pathways after TLR4 activation involve different adaptor molecules (for a schematic overview see[97,99]). The bacterial molecules are presented more efficiently to the innate immune system by the complex of LPS-binding protein, CD14 and MD2, forming an essential part of the LPS-receptor next to TLR4[95,100]. Further on, stimulation of TLR recruits the adaptor molecule, myeloid differentiation primary response gene 88 (MyD88), to the receptor complex, leading to the activation of IL1R-associated protein kinases and TNF-receptor-associated factor 6 to finally activate NF-κB and MAP kinases, resulting in the production of proinflammatory cytokines and chemokines[97,99,101,102]. MyD88 plays a key role in the cytokine production in response to TLR ligands. Nevertheless, several other adaptor proteins are involved, such as TIR-domain-containing adaptor protein/MyD88 adaptor-like (TIRAP/Mal), TIR-domain-containing adaptor inducing IFNs (TRIF), TRIF-related adaptor molecule and sterile α- and armadillo-motif-containing protein[99,101,103].

Buchholz *et al*[96] recently showed the involvement of TLR4 pathways in endotoxin-induced ileus. They hypothesise that endotoxin-induced ileus is induced by TLR4 signaling in nonhematopoietic cells in the early phase (6 h after injection of LPS), whereas at high doses of LPS and at later time points both hematopoietic and nonhematopoietic TLR4 signaling contributes. The molecular response attributed to the hematopoietic cells points towards a role for residential macrophages and potentially also leucocytes in the late phase. The role of mast cells has not been investigated so far, to our knowledge. But which nonhematopoietic non-bone marrow-derived cells could then be involved in TLR4 signaling? Related to gastrointestinal disturbances, possible candidate cells are smooth muscle cells, intrinsic neurons and ICC[96]. No data are yet available, to our knowledge, regarding the expression of TLR4 on ICC. However, functional TLR4 expression is described on smooth muscle cells and myenteric plexus cells in the murine and human intestine, with expression of TLR4 on both neurons and glial cells in mice. TLR4 expression seems absent in enterocytes[104,105]. Outside the gastrointestinal wall, TLR4 receptors are also expressed in dorsal root ganglia primary sensory neurons[105] and in the rat nodose ganglion[106]. The enteric nervous system, therefore, can be directly implicated in intestinal immune defence towards intestinal microbiota.

Very recently, a dominant role for the MyD88-dependent signaling pathway in early endotoxin-induced murine ileus was shown, as MyD88 deficient mice were completely protected from endotoxin-induced ileus and the induction of the inflammatory cascade, whereas TRIF deficiency only partially protected the mice from ileus[98].

A study by Kuno *et al*[107] reported a beneficial effect on LPS-induced changes in colonic motility in the guinea pig after administration of TAK-242, a selective TLR4 signal transduction inhibitor, illustrating again the potential therapeutic options of interference with the TLR4 pathway.

POSSIBLE NOVEL THERAPEUTIC STRATEGIES

The current treatment strategies for sepsis as described in the Sepsis Guidelines 2008 were described in the first part of this paper. They largely rely on general supportive measures such as fluid resuscitation, cardiovascular support and antimicrobial treatment. The use of corticosteroids is controversial and the use of recombinant activated protein C is reserved for patients with severe sepsis and a high risk of death[2,3,10,108,109]. More specific anti-inflammatory therapy such as antilipopolysaccharide treatment and blocking of proinflammatory cytokines such as TNFα and IL1β were ineffective or have failed to improve mortality so far[108]. Selective digestive tract decontamination is not recommended in the sepsis guidelines[3]. Selective digestive tract decontamination reduces infections (mainly pneumonia) and mortality in a general population of critically ill and trauma patients, however no studies are available in patients with severe sepsis or septic shock. The juries were split on the issue of selective gut decontamination with equal numbers in favor and against the recommendation of the use of selective gut decontamination. They agreed that further research was needed in patients with severe sepsis or septic shock and they voted against inclusion in the current guidelines[3].

Therapeutic interventions related to gastrointestinal motility, secretion and epithelial barrier function might be effective as well. As ileus plays a pathogenic role in the maintenance of sepsis and multiple organ failure and in the occurrence of secondary infections, prokinetic therapy might be of value. Theoretically, ileus could be overcome, increasing gastrointestinal motility thereby reducing bacterial stasis, bacterial overgrowth and bacterial translocation and so interrupting the activation of the inflammatory cascade. Motility can, for instance, be enhanced by stimulation of excitatory neuronal pathways or by direct smooth muscle effects. For the treatment of postoperative ileus *per se*, comprehensive reviews are available in the literature[13,40,110,111]. So far, treatment of postoperative ileus is also largely supportive and based on a multimodal approach including fluid restriction, optimal (epidural) analgesia, minimally invasive surgical procedures, early mobilization and early oral feeding.

Before going into more detail regarding potential therapeutic targets, two general considerations about drug development need to be taken into account. First of all, it has been clearly shown over the last few years that the transition of promising drug targets in experimental animal models towards beneficial clinical trials is hard, difficult to predict and few products have been commercialized[112,113]. For instance, with regard to the therapeutic pipeline for irritable bowel syndrome (IBS), another disorder associated with motility and sensitivity disturbances and a possible pathogenic role for inflammation, several drugs could not finalize the research developmental trajectory towards clinical use: the neurokinin receptor antagonists and fedotozine, a peripheral κ-opioid receptor agonist, could not prove clinical efficacy in phase ⅡB and phase Ⅲ trials, although promising results were shown in experimental studies. Other drugs have been withdrawn despite clinical benefit due to safety reasons, impacting the risk-benefit ratio, such as tegaserod and alosetron. The definition of clear end points and biomarkers with clinical relevance emphasizing symptoms and quality of life need to be considered[112,113]. Secondly, interference with the patients' immune system could affect their first line defence against other infections and could affect patient wound healing[114]. In the 1990s, several controlled clinical trials of immunomodulators in severe sepsis were undertaken but failed to show benefit or even increased mortality[11,108].

As evidence for a bidirectional communication between the neuroendocrine and immune systems accumulates in the pathogenesis of inflammatory ileus (for hypothetical scheme, see Figure 1), and also in IBD and IBS, interference with the immune system seems a promising therapeutic strategy[40,64,114-117]. The interplay between the epithelial barrier function, intestinal motility and secretion, and the cellular function of immunocytes can be influenced by neuronal and immune mediators with therapeutic potential. Several potential target cells, mediators or intracellular pathways can be proposed such as mast cells, residential macrophages, glial cells, the cholinergic anti-inflammatory pathway, afferent neurons, intracellular signaling pathways such as egr-1, p38 and TLR4 transduction inhibitors, together with a plethora of neuroactive substances released by damaged or inflamed tissue such as cytokines (IL-1β, IL-6), chemokines, prostaglandins and leukotrienes, neuropeptides, growth factors (NGF), hormones, histamine, tryptase, *etc*[40,114-116]. In the field of inflammation-associated ileus, therapeutic strategies combining anti-inflammatory with prokinetic properties might have more potential for future drug development.

Ghrelin is one of these compounds with therapeutic potential, as it has been shown to possess anti-inflammatory properties together with prokinetic activity[114,118-120]. Ghrelin and the ghrelin receptor are expressed by lymphocytes, monocytes and dendritic cells. Activation of the ghrelin receptor results in an inhibition of proinflammatory cytokine expression and an increase in survival in various inflammatory disease models[114,118]. Furthermore, ghrelin and ghrelin receptor agonists are proven to be prokinetic in animal models of delayed gastric emptying and in patients with gastroparesis of different origins (for review see[119]). Experimental studies with ghrelin and clinical trials with synthetic ghrelin agonists (TZP-101, TZP-102) and a selective growth hormone secretagogue (ipamorelin) are currently ongoing. Ipamorelin, a ghrelin mimetic, proved beneficial in a postoperative ileus model in the rat[121]. In a phase ⅡB trial, the ghrelin agonist TZP-101 accelerated recovery of the gastrointestinal tract after (partial) colectomy compared to placebo[122]. Specifically related to sepsis, it was shown that ghrelin ameliorates the gut barrier dysfunction by reducing serum HMGB1 and by activation of the vagus nerve *via* central ghrelin receptors[123]. We have shown the prokinetic potential of ghrelin and the ghrelin receptor agonist, growth hormone releasing peptide 6, in a septic mouse model[26]. In a simi-

lar model, Dixit *et al*[124] showed potent anti-inflammatory effects of ghrelin on the mRNA expression of IL-1β, IL-6 and TNFα in the liver, spleen, lungs and mesenteric lymph nodes of LPS-treated mice, associated with an attenuation of the LPS-induced anorexia. The combination of prokinetic and anti-inflammatory properties enhances the potential of ghrelin-related drugs in inflammation-induced ileus. These effects are hypothesized to be mediated by the anti-inflammatory cholinergic pathway and by interactions with immune cells[118,119,120,124].

Another intestinal hormone with potential in the treatment of postoperative ileus is glucagon-like peptide 2 (GLP-2). Activation of GLP-2 was recently reported to ameliorate inflammation and intestinal dysmotility associated with surgical manipulation of the bowel in a murine model[125]. The beneficial effects on the proinflammatory milieu were more pronounced in the mucosa compared to the intestinal muscularis and the authors speculate that the protective effect of GLP-2 is associated with mucosal inflammation and barrier dysfunction, not excluding interference with the anti-inflammatory vagal pathway[125].

The endocannabinoid system is involved in the regulation of physiological and pathophysiological responses in the gastrointestinal tract such as food intake, emesis, gastric protection, gastric secretion, visceral sensation, gastrointestinal motility, intestinal inflammation and cell proliferation[126]. CB1 and CB2 receptors are the classical receptors for all kinds of cannabinoid agonists, whereas non-CB receptor-mediated effects of cannabinoids are also described[126]. Generally it is accepted that CB1 activation inhibits gastrointestinal motility in different regions of the gastrointestinal tract, whereas the role of CB2 receptors in the control of physiological motility is less clear. Both CB1 and CB2 receptors have been shown to play a role in motility in pathophysiological inflammatory conditions[126]. In septic ileus in mice and rats, CB1 and CB2 receptor antagonists protected against LPS-induced changes in motility (*in vitro* and *in vivo*), without affecting the increase in TNFα, but reduced the increase in IL6 in the group treated with a low dose of LPS[127]. We demonstrated that septic ileus in mice was associated with an upregulation of intestinal CB1 but not CB2 receptors and an increase in fatty acid amide hydrolase (FAAH), which is the principal catabolic enzyme for fatty acid amides. Cannabidiol, a non-psychotropic cannabinoid without significant binding activity to CB1 or CB2 receptors, however, further decreased the LPS-induced motility disturbances *in vivo*[128]. Very recently, we showed that LPS-induced sepsis in mice resulted in a hyperactivation of glial cells, an increase in intestinal mast cells, macrophages and TNFα. These effects were abrogated by cannabidiol treatment and associated with a decrease in S100B expression, suggesting a crucial role of glial cells (personal communication)[68].

As mast cells and residential muscular macrophages are proposed as key players in the pathogenesis of ileus, targeting these cells might also show therapeutic potential. Depletion and inactivation of macrophages in rodents prevented intestinal inflammation and postoperative ileus[61]. As interfering with immune responses could also affect wound healing as stated above, it is important to investigate the effect of macrophage depletion on the healing process. Very recently, it was shown that pharmacological and genetic inhibition of muscularis macrophages in mice did not affect intestinal anastomotic healing[129]. Whether this approach is translational to the human situation remains questionable. However, interference with the macrophages could occur at several levels. Additionally, interference with the TLR4 receptor, as described above, offers a therapeutic potential.

An interesting way to downregulate macrophages is to interfere with the cholinergic anti-inflammatory pathway; for instance, by the use of α7 nicotinic acetylcholine receptors agonists, direct vagal stimulation or the use of acetylcholine esterase inhibitors[40,69]. Electrical stimulation of the vagal nerve attenuates systemic inflammation in rodent models of endotoxemia, cecal ligation and puncture, and intestinal manipulation[76-79]. Pretreatment with the α7 nAChR, AR-R17779, prevented postoperative ileus and the inflammatory reaction in mice[80]. Nicotine itself has also been tested in clinical trials for IBD, however its use is jeopardized by its toxicity[117,130]. Electrical stimulation of the vagus nerve remains an invasive procedure and pharmacological interference with acetylcholine receptors might have side-effects; both treatment strategies need further optimization. Recently, stimulation of the vagal nerve in a rodent postoperative model by enteral administration of lipid-rich nutrition was shown to be beneficial and to be mediated by a CCK-dependent vagal mechanism[131].

With regard to mast cells, a first randomized and placebo-controlled pilot study in humans undergoing major abdominal surgery for gynecological malignancy showed that ketotifen, a mast cell stabilizer with histamine 1 receptor blocking activity, restored gastric emptying, ameliorated abdominal cramping and tended to improve colonic transit[67]. Mast cells can also be modulated at several levels including development, homing, secretory phenotype, stabilization, interference with membrane receptors or downstream pathways, or blocking the effect of the mediators released[132]. Potential drugs or drug targets are cromolyn, ketotifen, Syk kinase inhibitors; even TLR antagonists and blockers of the mediators released by mast cells such as tryptase, proteases, chymase, prostaglandins, leukotrienes, cytokines and growth factors, chemokines and neuropeptides (CRF and substance P)[63,132].

CONCLUSION

The impact of the gastrointestinal tract on the initiation and maintenance of inflammation and secondary infections involved in the pathogenesis of multiple organ failure is generally accepted. Therapeutic interventions related to gastrointestinal motility can therefore be effective in sepsis treatment, as bacterial translocation and activation of the inflammatory cascade can be put on hold. This hypothesis stresses the important link between gastrointestinal inflammation and motility.

Both endotoxic and postoperative animal models offer the opportunity to study the role of, and interference with, inflammation related to gastrointestinal motility and sensitivity. The complex interplay within the gastrointestinal wall between mast cells, residential macrophages and glial cells on the one hand, and neurons and smooth muscle cells on the other hand, forms the basis for further research towards novel therapeutic strategies. Many molecules have potential to intervene with this complex cellular interplay at the level of intracellular signaling pathways, chemokines, cytokines, neuroactive substances and mediators involved in afferent neuronal signaling and the anti-inflammatory vagal pathway. The combination of anti-inflammatory properties and prokinetic properties within one drug seems the most promising route for translational research.

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