The Gut is not only the Target but a Source of Inflammatory Mediators Inhibiting Gastrointestinal Motility During Sepsis

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Key Words

Septic shock • Motility • $\text{TNF}\alpha$ • Cytokines

The gastrointestinal tract is the source and the target of inflammatory mediators released during sepsis causing paralytic ileus.

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Abstract

Background: Sepsis is a common problem in intensive care patients leading to multi-organ failure and gastrointestinal paralysis. **Aim:** The aim of the study was to investigate whether the gastrointestinal tract is not only the target but also the source of inflammatory mediators inhibiting gastrointestinal motility. **Methods:** Mesenteric lymph was obtained from rats in which a sepsis was induced by lipopolysaccharide (LPS) intraperitoneally. Gastrointestinal motility was recorded following mesenteric lymph- or $\text{TNF}\alpha$ infusion into the jugular vein of separate healthy recipient rats using the strain gauge transducer technique. **Results:** Infusion of sepsis lymph significantly impairs gastric and colonic motility, decreasing the motility-index in the stomach and colon by about 57% and 21% respectively in comparison to baseline motility. Among other inflammatory mediators, $\text{TNF}\alpha$ plays an important role in mediating the inhibitory effect of mesenteric lymph on gastrointestinal motility during sepsis. **Conclusions:**

Introduction

Sepsis is the main cause of death in critically ill patients and is associated with severe problems such as multi-organ failure. One major issue during sepsis and septic shock is severely impaired gastrointestinal function, especially impaired gastrointestinal motility, clinically recognized as paralytic ileus.

Paralytic ileus may enhance the accumulation of bacteria within the gut, promoting bacterial translocation, since microcirculation of the gut wall and especially of the mucosa is significantly altered during sepsis.

A further problem of gastroparesis is the risk of acute or chronic aspiration leading often to severe pneumonia.

Impaired gastrointestinal motility therefore is one of several reasons why infections occur during sepsis. The mechanisms of this phenomenon are not clearly understood up to now. However, gut derived mediators, released during peritonitis or sepsis, play an important role in gastrointestinal motility by either local, systemic or neuronal activation [1]. Inflammatory mediators released from immune cells within the gut wall are drained predominantly from the lymphatic system. Mesenteric lymph mediators from the gastrointestinal tract enter the systemic circulation via the thoracic duct, leading to distant organ impairment, such as septic pulmonary or liver dysfunction [2]. We have recently demonstrated that mesenteric lymph obtained from animals during LPS-induced septic shock is capable of inhibiting gastric motor function [3]. Little is known, however, about the interaction of mediators, released from the gut into mesenteric lymph during sepsis, on small intestinal and colonic motility.

The aim of the study was to investigate whether the gastrointestinal tract is not only the target but also a potential source of inflammatory mediators inhibiting gastrointestinal motility. From the clinical point of view, gastroparesis and impaired colonic motility represent a major problem in critically ill patients, since enteral nutrition is often not sufficiently possible, leading to reflux, aspiration, and bacterial translocation as a consequence of impaired passage in the large bowel [4, 5].

During peritonitis or sepsis, parenteral nutrition is often necessary, as the enteral route is not possible due to gastroparesis and reduced colonic transit. It has been clearly shown, though, that enteral nutrition is beneficial in critically ill patients, leading to reduced mortality [6]. In this context, enteral feeding should be performed when feasible, even in critically ill patients. Detailed research investigating the problems of reduced motility during severe illness is therefore necessary.

Materials and Methods

Animals

Male Sprague–Dawley rats (Charles River, Kieslegg, Germany), maintained on regular laboratory chow, were housed under controlled conditions of illumination (12:12 hours light/dark cycle), humidity, and temperature (21°C). Rats were fasted overnight but allowed water ad libitum before all surgical and experimental procedures. The institutional guidelines for the care and use of laboratory animals

were followed throughout the study.

Mesenteric lymph collection

The methods involved in mesenteric lymph collection have already been published [3]. In brief, rats (260–300 g) were anesthetized with ketamin (Deltaselect, Germany, 100 mg/kg body weight) and xylazin (Bayer, Germany, 5 mg/kg body weight), and the superior mesenteric lymph duct, draining the area of the superior mesenteric artery, was cannulated with a polyvinyl tube (Medical Grade, 0.50 mm inner diameter [ID], 0.80 mm outer diameter [OD], Dural Plastics, Sydney, Australia), fixed with a drop of cyanoacrylate glue (Krazy Glue, Elmers Products Inc. Columbus, OH, USA) and externalized through a small incision in the right flank. A second cannula (silicone elastomer, 1 mm ID, 2.15 mm OD) was passed through a small incision of gastric fundus into the duodenum, and externalized through a surgical incision in the left flank. After surgery, rats were placed in Bollman cages, and a glucose-saline solution (glucose 0.2 mol/L, NaCl 145 mmol/L, and KCL 4 mmol/L) was infused continuously through the duodenal cannula at a rate of 3 ml/hour to equalize volume and energy losses via the lymph, which was draining freely. Rats were allowed to recover from surgery for 12 h. The mesenteric lymph flow depends on the amount of fluid provided for the small bowel. Therefore a steady lymph flow of 2.5 ± 0.5 ml/hour at an intestinal perfusion rate of 3 ml/h indicated a non-obstructive drainage of the mesenteric lymph with an appropriately positioned lymph cannula.

After the recovery period, abdominal lymph was collected during intestinal glucose-saline infusion 2 hours before and 6 hours after either saline (1 ml, ip, n=15, control lymph) or lipopolysaccharide (LPS; Escherichia coli, serotype 0111:B1, Sigma, Steinheim, Germany, 5 mg/kg in 1 ml, ip, n=15, sepsis lymph) was injected intraperitoneally (ip). Lymph was collected in ice-chilled tubes, centrifuged, pooled for the animals within one group, and frozen and stored at -80°C for further experimental use.

TNF α detection in mesenteric lymph and systemic blood

Rats were anesthetized and the mesenteric lymph duct was cannulated as previously described. Additionally, a polyvinyl tube was placed into the superior cava vein. Sepsis was induced using LPS in the anesthetized rats as previously described (n=6); the control group was treated with physiological saline i.p. (n=6). Lymph and blood samples were taken every 30 min up to 180 min. The blood was centrifuged (4°C at 2000 rpm) in order to obtain blood serum.

TNF α was measured in lymph and serum samples using standard rat TNF α ELISA kits (Biosource, Camarillo, CA).

Immunoprecipitation of TNF α in mesenteric lymph

The immunoprecipitation of TNF α in mesenteric lymph was performed using a selective goat anti-rat IgG-TNF α antibody (SIGMA, Saint Louis, USA) without cross reactivity to other cytokines. Mesenteric lymph was incubated with the antibody at a concentration of 1:500 overnight at 4°C . The mesenteric lymph sample was then centri-

fused with 2000 g, while the TNF α concentration in the supernatant was measured with the TNF α ELISA kit (Biosource, Camarillo, CA). A concentration of TNF α below the detection threshold confirmed the successful immunoprecipitation of TNF α in mesenteric lymph.

Recording of gastrointestinal motility

We previously described the technique of strain gauge transducers in measuring gastrointestinal motility [7, 8]. In brief, two miniature strain gauge transducers (Type EA-06-062 DN-350 E, Measurements Group, Raleigh, NC, final size 3.14 mm) were glued together with tetrahydrofuran (M-Bond 610-E, Measurements Group), while insulated copper wires (0.127 mm diameter, Measurements Group) were soldered to the transducers, and coated with epoxy resin (M-Bond 43 B-E, Measurements Group). The strain gauge transducers were immersed in silicone paste (Wacker, Munich, Germany), placed between two reinforced silicone sheets (0.178 mm, Silastic, Dow Corning), hardened for 24 h in a mold, and trimmed to their final size of 4x6 mm [7].

The strain gauge transducers were sutured in anesthetized rats (ketamine (100 mg/kg, Parke Davis, Berlin, Germany), and xylazine (15 mg/kg, Bayer, Leverkusen, Germany) on the gastric corpus, the jejunum, and colon parallel to the circular muscle layer. The copper wires of the strain gauge transducers were exteriorized through a surgical incision of the flank, and tunnelled subcutaneously to the neck. The wires were soldered to connecting plugs and wrapped in a little leather bag sutured to the dorsal skin of the rats to avoid damage by the rats. Rats were allowed to recover from strain gauge implantation at least for 3 days before motility recordings were initiated [8].

Gastric, small intestinal and colonic motility was recorded following intravenous (i.v.) infusion of physiological saline (2 ml/h, n=5), control lymph (2 ml/h, n=5) or sepsis lymph (2 ml/h, n=5) for 2 hours. Awake rats were therefore placed in light restraint cages (Bollman cages) and strain gauge transducer wires were connected to a wheatstone bridge (2100 System, Measurements Group, Raleigh, NC). The signals were simultaneously recorded by a personal computer with an A/D board (Contec Microelectronics, San Jose, CA) and a multichannel data recorder (BD 300 Kipp & Zonen, Delft, Holland). Control- or sepsis lymph was infused in randomized orders in the same animal on consecutive days allowing the rats to recover from lymph infusion.

In separate sets of experiments the effect of TNF α on gastric and colonic motility was tested. Physiological saline (2 ml/h, n=5), 0.5% bovine serum albumin (BSA) (2 ml/h, n=5) or sepsis lymph (2 ml/h, n=5) with a TNF α concentration of 1500 pg/ml was intravenously infused and gastric and colonic motility was recorded online as described above. Additionally TNF α in a concentration corresponding to sepsis lymph (Biosource international, Camarillo, CA, 1500 pmol/ml), diluted in 0.5% BSA (Serva feinbiochemica, Heidelberg, Germany, 2 ml/h, n=4), or TNF α free sepsis lymph after immunoprecipitation of TNF α was infused at 2 ml/h on consecutive days while gastric and colonic motility was recorded (each group n=5).

Motility recordings were analysed with previously published data using dedicated software (Intestinal Data Acquisition and Analysis, version 3.40.15, Standard Instruments, Karlsruhe, Germany), calculating the area under the curve (motility index, MI), the contraction frequency, and the mean contraction amplitude [8]. Baseline motility was analysed for 60 min in 5-min intervals and the mean of these 12 time segments was set at 100%. The motility after i.v. infusion of physiological saline/vehicle, control lymph, sepsis lymph, TNF α , and TNF α free sepsis lymph was analysed for 2 hours in 5-min intervals while substrates were infused. The motility index of the stomach, small bowel and colon during substrate infusion is expressed as the percentage of baseline motility.

Statistics

Data are presented as mean $\pm$ standard deviation of the mean. In all experiments the same animal served as its own control. The differences between the groups were analysed with a paired Student's t test (Prism Program (Version 3.02)).

Results

Effects of sepsis lymph on gastrointestinal motility

Infusion of sepsis lymph (SL) significantly impairs gastric motility. The motility-index decreased about 57% and 61% following infusion of SL in comparison to baseline motility as well as control lymph (CL), respectively. In the colon, the motility index increased to about 45% after the infusion of CL; however, this increase was statistically not significant. In contrast, infusion of SL decreased the motility index of the colon to about 21.2% and 45.5% in comparison to baseline motility as well as to CL, respectively. Infusion of CL or SL produced no significant effect on small bowel motility (Fig. 1a).

Infusion of sepsis lymph decreased the contraction frequency of the jejunum significantly by about 21.9% and 20.2%, as compared to the baseline motility index and the motility index after control lymph infusion, respectively. However, the contraction frequency was not altered in the stomach or in the colon after infusion of sepsis lymph (Fig. 1b).

The mean gastric contraction amplitude was significantly decreased after the infusion of sepsis lymph, as compared to both the mean contraction amplitude under control conditions and after the infusion of control lymph. In contrast, infusion of sepsis lymph did not alter the mean contraction amplitude of the jejunum or the colon (Fig. 1c).

Fig. 1. (a.) The motility of the stomach, the jejunum and the colon is presented as motility index (area under contraction treatment / area under contraction baseline $\times 100$). Baseline motility is illustrated in white bars. The motility index after infusion of control lymph and after infusion of sepsis lymph is illustrated in grey and black bars, respectively. Infusion of sepsis lymph reduced significantly the motility index of the stomach and markedly reduced the colonic motility index as compared to both the baseline motility index and the motility index obtained after control lymph infusion (* $p < 0,05$; ** $p < 0,001$). (b.) The contraction frequency (contractions/minute) of the stomach, jejunum and the colon is presented analogue to Fig. 1a. Infusion of control or sepsis lymph had no effect on the contraction frequency of the stomach or the colon, whereas infusion of sepsis lymph significantly decreased the contraction frequency of the jejunum (* $p < 0,01$). (c.) The mean contraction amplitude (mV) of the stomach, the jejunum and the colon is presented analogue to Fig. 1a. Mean contraction amplitude of the stomach was significantly decreased after infusion of sepsis lymph compared to both the mean contraction amplitude of baseline motility and the mean contraction amplitude of the control lymph group (* $p < 0,001$).

TNF α in mesenteric lymph and systemic blood circulation

TNF α is continuously released from the gut into the mesenteric lymph during sepsis for the investigated time period of 180 min (Fig 2a). In contrast, the TNF α concentration of systemic blood is bell-shaped, with a peak concentration of 2105 ± 873 pg/ml at the time point of 120 min. Thereafter the concentration of TNF α is seen to decrease continuously (Fig. 2b). In the saline treated controls the TNF α levels in mesenteric lymph were between 13.6 ± 2.6 pg/ml and 46.3 ± 29.9 pg/ml and in the superior cava vein between 11.1 ± 9.2 and 51.3 ± 29 during the observed time period.

TNF α and gastrointestinal motility

Sepsis lymph was collected for six hours from animals to detect TNF α concentration in the pooled sepsis lymph samples (mean TNF α concentration of the $n=6$ lymph samples: 1533 ± 197 pg/ml). The TNF α concentration of the pooled lymph sample was adjusted to 1500 pg/ml. This sepsis lymph produced a similar inhibition of gastric and colonic motility (Fig. 3a) as previously shown in Fig. 1 (a-c).

The gastric motility index was significantly reduced by 49.9% after infusion of TNF α (equivalent dose to sepsis lymph) as compared to vehicle infusion.

The motility index of the colon was reduced by 33.1% after infusion of TNF α ; however, this reduction was statistically not significantly different to the motility index of the vehicle group (Fig. 3b).

Immunoprecipitation of TNF α in sepsis lymph abolished the inhibitory effect of sepsis lymph on both gastric- and colonic motility (Fig. 3c).

Fig. 2. (a + b.) The TNF α concentration of mesenteric lymph (a) and systemic blood (superior cava vein, b) is demonstrated in pg/ml upon i.p. LPS injection. The TNF α concentration increased continuously in mesenteric lymph up to 180 min, while within the systemic blood circulation the TNF α concentration peaked after 120min and decreased thereafter continuously. * $p < 0.05$ vs. mesenteric lymph.

Discussion

Sepsis is one of the leading causes of death worldwide, even in the current era of broad spectrum antibiotics and highly sophisticated intensive care treatment [9].

One of the major problems during sepsis is the overwhelming response of the immune system to the triggered inflammation. The plentiful release of pro-inflammatory mediators can cause distant organ impairment such as pulmonary-, kidney-, or liver dysfunction [2]. Moreover, intestinal paralysis, which has thus far been frequently investigated, is seen as causing a variety of problems such as vomiting, aspiration pneumonia and bacterial translocation from the gut lumen to the

Fig. 3. (a.) The motility index of the stomach and colon is presented after infusion of physiological saline (baseline, white bar) and after infusion of sepsis lymph (black bar). In the stomach and colon there was a significant decrease of the motility index after infusion of sepsis lymph in comparison to the baseline motility index (* $p < 0.01$). (b.) The motility index of the stomach and colon is presented after infusion of vehicle (0.5% BSA, white bar) and after infusion of TNF α (1500 pg/ml, black bar). Baseline motility index (without treatment) is set to 100% as shown in Fig. 3a and 3c, but not demonstrated in this figure, since the comparison between vehicle treatment and TNF α bears more importance here. There was a significant decrease of the motility index in the stomach after infusion of sepsis lymph in comparison to the vehicle group (* $p < 0.05$). (c.) The motility index of the stomach and colon is presented after infusion of physiological saline (white bar) and after infusion of TNF α free sepsis lymph (black bar). No differences were found in the motility indexes after infusion of TNF α free sepsis lymph compared to baseline motility.

blood [10]. The exact mechanisms, however, behind intestinal paralysis have yet to be clearly understood.

The gastrointestinal tract hosts the majority of immune cells within the body [11]. During sepsis or septic shock, the immune cells within the wall of the gastrointestinal tract release loads of pro-inflammatory mediators. These gut-derived inflammatory mediators are predominantly released into the mesenteric lymph rather than into the portal blood, entering the systemic circulation into the jugular vein via the thoracic duct [2]. Previously, we have shown that gut-derived mediators released into mesenteric lymph during sepsis can cause distant organ impairment such as pulmonary dysfunction [12].

It is likely that inflammatory mediators released from the gastrointestinal tract during sepsis have a certain impact on gastrointestinal motility. There are three possibilities of explaining how mediators released within the gastrointestinal wall influence gastrointestinal motility: 1.) The inflammatory mediators such as $\text{TNF}\alpha$ act locally, having a direct effect on the smooth muscle cells or enteric neurons within the gut wall, or initiate an inhibitory neuronal pathway inhibiting motility. 2.) The inflammatory mediators act globally by entering the systemic circulation via the thoracic duct, influencing gastrointestinal motility after re-circulation by directly inhibiting smooth muscle cells, activating an inhibitory neuronal pathway within the gut wall or initiating an inflammatory burst within the gut wall leading to inhibition of motility. 3.) The inflammatory mediators act globally by activating higher motility centers in the brainstem and causing a central inhibition of motility e.g. via the vagus nerve.

Certain evidence has been documented in the literature indicating that inflammatory mediators released within the gut act directly on smooth muscle cells [13] or activate a neuronal inhibitory pathway [14]. In the present study, we used an established animal model to investigate the global role of gut-derived inflammatory mediators released into mesenteric lymph during sepsis. Mesenteric lymph was collected from healthy or septic rats and was re-infused into separate healthy recipient rats. Within this framework, we have been able to rule out a direct local effect of inflammatory mediators on smooth muscle cells within the gut wall. All effects of gut-derived lymphatic mediators investigated in this model must have been the result of a systemic response. However, in this animal model we were not able to distinguish between a response within the gastrointestinal tract and a response of inhibitory regulatory neuronal

pathways.

Infusion of sepsis lymph into healthy rat recipients significantly inhibited gastric motility and markedly reduced colonic motility, whereas jejunal motility was marginally influenced. Interestingly, these findings match clinical observations. During paralytic ileus due to sepsis, gastric paresis and reduced colonic motility lead to many difficulties such as gastric reflux, the risk of aspiration pneumonia, and the possibility of bacterial translocation from the colon [10]. In intensive care patients, an application of a nasogastral tube is therefore often necessary, and intravenous stimulation of the bowel is often performed. However, colonic motility, in contrast to small intestinal motility, is often less influenced by pro-kinetics. In this context it is worth commenting on the fact that the mesenteric sepsis lymph was collected from the superior mesenteric lymph duct, draining the jejunum and proximal ileum. These mesenteric lymph mediators released from the small bowel during sepsis inhibited, interestingly enough, mainly the motility of the stomach and the colon after reinfusion, while motility of the small bowel was hardly influenced. A similar “pan-enteric field effect” was investigated in an ileus model of selective small bowel manipulation, which leads to a cellular invasion of immune cells and inhibition of motility in the unmanipulated stomach or colon [15].

Previously, we analyzed the pro-inflammatory mediators in mesenteric lymph released from the gastrointestinal tract during LPS-induced sepsis. Among others, the classic pro-inflammatory cytokines, such as $\text{TNF}\alpha$, Interleukin 1β (IL 1β), and Interleukin 6 (IL6), were seen to increase. $\text{TNF}\alpha$ increased up to 200-fold in mesenteric lymph during sepsis [12]. $\text{TNF}\alpha$ is a primary and potent mediator of inflammation, capable of inducing other pro-inflammatory cytokines, such as IL6, elicits metabolic and haemodynamic changes, as well as causing end-organ dysfunction [16]. $\text{TNF}\alpha$ is synthesized mainly by macrophages and dendritic cells upon stimulation with LPS, which is recognized by the Toll like receptor 4 (TLR4) located on the surface of the immune cells [17]. The activation of TLR4 leads to an activation of the nuclear factor- κB (NF- κB), stimulating the synthesis of pro-inflammatory cytokines [18]. $\text{TNF}\alpha$ is known to inhibit smooth muscle contractions [19] and is capable of inhibiting gastrointestinal motility when injected centrally [20]. It was shown that $\text{TNF}\alpha$ binding protein protects animals from LPS induced intestinal dysmotility [21]. Moreover there is evidence that $\text{TNF}\alpha$ or cytokine reducing drugs such as semapimod are able to prevent inflammation related cytokine

release and postoperative ileus [22, 23]. This underlines the importance of TNF α in its influence on gastrointestinal motility.

In the present study, we were able to detect TNF α in both the mesenteric lymph and the systemic blood taken from the superior cava vein. In both the blood and the mesenteric lymph, TNF α increased significantly after sepsis was induced, although the TNF α concentration remained elevated in the mesenteric lymph and decreased continuously in the systemic blood after 120 min. The interpretation of this data remains speculative. It is possible that the main source of TNF α during this septic shock model is the gut rather than the liver. Macrophages and dendritic cells within the gut wall release TNF α into the interstitium during sepsis which is predominantly drained by the mesenteric lymph. Since the lymph flow is very low compared to the blood flow the concentration of mediators might be higher in the lymph compared to the blood and the release is more consistent.

In this context, we further investigated the effect of TNF α in mesenteric lymph on gastrointestinal motility. We were able to show that (1) sepsis lymph inhibited both gastric and colonic motility, (2) TNF α in a concentration corresponding to sepsis lymph significantly inhibited gastric motility while reducing colonic motility, and (3) TNF α free sepsis lymph had no influence on gastric or colonic motility. The mechanism of TNF α on gastrointestinal motility is not completely understood. As mentioned above, circulating TNF α is capable of inhibiting both smooth muscle activity in the gut and central neural pathways regulating gastrointestinal motility by passing the blood brain barrier [20, 24, 25]. However, it is also possible that TNF α itself plays a minor role in inhibiting gastrointestinal motility, as it instead stimulates other cells releasing mediators which inhibit gastrointestinal motility.

In conclusion, the gastrointestinal tract releases inflammatory mediators during sepsis that are capable of causing distant organ failure, including paralytic ileus.

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