Mechanisms of polymicrobial sepsis-induced ileus

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Submitted 20 August 2003; accepted in final form 8 April 2004

Overhaus, Marcus, Sandra Tögel, Michael A. Pezzone, and Anthony J. Bauer. Mechanisms of polymicrobial sepsis-induced ileus. Am J Physiol Gastrointest Liver Physiol 287: G685–G694, 2004; 10.1152/ajpgi.00359.2003.—Sepsis frequently occurs after hemorrhage, trauma, burn, or abdominal surgery and is a leading cause of morbidity and mortality in severely ill patients. We performed experiments to delineate intestinal molecular and functional motility consequences of polymicrobial sepsis in the clinically relevant cecal ligation and puncture (CLP) sepsis model. CLP was performed on male Sprague-Dawley rats. Gastrointestinal transit, colonic in vivo pressure recordings, and in vitro muscle contractions were recorded. Histochemistry was performed for macrophages, monocytes, and neutrophils. Inflammatory gene expressions were quantified by real-time RT-PCR. CLP delayed gastrointestinal transit, decreased colonic pressures, and suppressed in vivo circular muscle contractility of the jejunum and colon over a 4-day period. A leukocytic infiltrate of monocytes and neutrophils developed over 24 h. Real-time RT-PCR demonstrated a significant temporal elevation in IL-6, IL-1β, monocyte chemoattractant protein-1, and inducible nitric oxide synthase, with higher expression levels of IL-6 and inducible nitric oxide synthase in colonic extracts compared with small intestine. Polymicrobial CLP sepsis induces a complex inflammatory response within the intestinal muscularis with the recruitment of leukocytes and elaboration of mediators that inhibit intestinal muscle function. Differences were elucidated between endotoxin and CLP models of sepsis, as well as a heterogeneous regional response of the gastrointestinal tract to CLP. Thus the intestine is not only a source of bacteremia but also an important target of bacterial products with major functional consequences to intestinal motility and the generation of cytokines, which participate in the development of multiple organ failure.

inducible nitric oxide synthase; cecal ligation and puncture; lipopolysaccharide; proinflammatory cytokines

SEPSIS FREQUENTLY OCCURS AFTER hemorrhage, trauma, burn, or abdominal surgery. It is a leading cause of morbidity and mortality in severely ill patients (1). Intra-abdominal sepsis also develops secondary to pancreatitis, appendicitis, diverticulitis, perforated ulcers, or an infarcted bowel (23). Not only does the intestine harbor the systemically invading organisms (5), but it also is a major target of bacterial-induced organ dysfunction. It has been hypothesized that one of the sequelae of the gut’s response to bacteremia is its participation in generating copious amounts of proinflammatory mediators, which participate in causing systemic inflammatory response syndrome and multiple organ failure (20, 24).

Exogenous lipopolysaccharide (LPS) from gram-negative bacteria is known to be a causative factor of sepsis-induced ileus, and the use of LPS as a selective stimulus for ileus is mechanistically valuable (3, 6, 7, 12, 19). It has previously been shown that LPS initiates an inflammatory cascade of events that consists of the activation of the normally quiescent network of resident intestinal muscle macrophages (6, 28) and the elaboration of a plethora of inflammatory cytokines, chemokines, and substances (nitric oxide and prostaglandins) from the muscularis externa. This inflammatory milieu subsequently results in the recruitment of circulating leukocytes (33) and thus the further release of leukocyte-derived substances. Many of these factors, such as nitric oxide and prostaglandins, are known to mechanistically be involved in causing ileus through their direct alteration of the kinetic properties of intestinal smooth muscle cells (22, 30). Additionally, a strong case can be made for the influence of LPS-induced cytokines (IL-6, IL-1β, and tumor necrosis factor-α) in altering enteric neuromuscular transmission as key factors during sepsis (2, 10, 19).

Although much has been learned from the LPS injection models, they have been criticized for various reasons. Typically, exogenous LPS produces serum cytokine responses that are transient and are of a much greater magnitude than those observed in septic patients. LPS also causes a severe hypodynamic circulatory response without the initial hyperdynamic circulatory response as is seen clinically. Additionally, pharmacological agents and manipulations that have been shown to be effective in exogenous LPS animal models have failed in clinical trials (4, 9, 26). For instance, calcium antagonists improve endotoxin survival, but they increase mortality in the cecal ligation and puncture (CLP) sepsis model (13). Thus it has recently been concluded by general agreement that LPS injection may be an adequate model of endotoxic shock but not of sepsis (26). Because of these limitations, experimental models other than LPS injection have been developed to more precisely mimic the human disease process. One such model is the CLP model, which appears to closely mimic both qualitatively and quantitatively the clinical observations of vascular reactivity and inflammation induced during polymicrobial peritonitis, bacteremia, and systemic sepsis (9, 35).

Although there have been a number of studies that have focused on characterizing the hepatic, pulmonary, and systemic inflammatory responses that contribute to the pathogenesis of sepsis in the CLP model (26), unfortunately, only one study could be identified that investigated the effects of CLP on intestinal motility (18), and no molecular studies have been performed on the intestinal muscularis to our knowledge. The one motility study used rat ileal longitudinal muscle, which is...
now known to be relatively unsensitive to LPS products (6, 7, 34), and a nonselective nitric oxide inhibitor that also blocks constitutive enteric neural nitric oxide as well as all other nitric oxide synthases (18). Thus little functional and molecular data concerning polymicrobial sepsis leading to bowel dysmotility and gut-derived cytokine production exist. The experiments on the small and large intestine in the present study were designed to gain mechanistic insight into the molecular and functional motility consequences of polymicrobial sepsis in the clinically relevant polymicrobial cecal ligation and puncture sepsis model.

MATERIALS AND METHODS

Experimental groups and operative procedure. Sprague-Dawley male rats were obtained from Harlan (Indianapolis, IN). The University of Pittsburgh Institutional Animal Care and Use Committee approved the protocol. Rats were housed in a pathogen-free facility that is accredited by the American Association for Accreditation of Laboratory Animal Care and complies with the requirements of humane animal care as stipulated by the US Department of Agriculture and the Department of Health and Human Services. The rats were maintained on a 12:12-h light-dark cycle and provided with commercially available rat chow and tap water ad libitum.

Rats were anesthetized with continuous isoflurane inhalation. The operative procedures were performed under sterile conditions. Four groups of animals were studied (control, laparotomy, sham CLP, and CLP). CLP animals underwent a small midline laparotomy; the cecum was everted and partially ligated with a 4-0 silk tie and punctured once with a 21-gauge needle. CLP was performed to induce peritonitis in Sprague-Dawley rats weighing ~230 g each. After induction of peritonitis, the rats were killed and studied at different time points (postoperative day 1, 2, 3, 4; n = 6 for each group) for functional studies and at different times (1, 3, 6, 24, 48 h; n = 6 per group) postoperatively for molecular studies. Age-matched rats not receiving an operation served as controls. Sham groups underwent laparotomy and cecal ligation without puncture or laparotomy with only minor cecal handling without either ligation or puncture.

Functional studies. Operated animals were anesthetized with isoflurane a second time at the time of death, and the abdominal wound was reopened. The entire bowel was then removed and placed in cold preoxygenated Krebs-Ringer buffer (KRB). Mechanical activity was measured as previously described (14). A segment of midjejunal or colon was pinned in a dissecting dish containing ice-cold, preoxygenated KRB; the segment was opened along the mesentery. The mucosa was removed, and the remaining muscularis was cut into strips (1 × 10 mm) parallel to the circular muscle layer and suspended in standard horizontal mechanical organ chambers that were continuously perfused with preoxygenated KRB solution maintained at 37°C. One end of each strip was tied to a fixed post, and the other was attached to an isometric force transducer (WPI, Sarasota, FL). In the organ chamber, each strip was allowed to equilibrate for 1 h. Strips were then incrementally stretched to the length at which maximal contraction occurs. Dose-response curves were generated by exposing the muscles to increasing concentrations of the muscarinic agonist bethanechol (0.3–300 μM) for 10 min with intervening 10-min wash periods. The functional significance of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) inhibition was determined by measuring changes of in vitro circular muscle spontaneous contractility in response to selective iNOS inhibition using L-N(ω-arginine) (L-N(ω-Aarginine)) (L-NIL; 30 μM) and the COX-2 inhibitor 5,5-dimethyl-3-(3-fluorophenyl)-4-(methylsulfonyl)-phényl2(5H)-furanone (DFU; 5 mg/l). Contractile activity was calculated as grams per square millimeters per second by converting weight and length of the strip to square millimeters of tissue.

Intestinal transit was measured in control and operated animals at different times postoperatively by evaluating the intestinal location of nonabsorbable fluorescein isothiocyanate (FITC)-labeled dextran (molecular weight = 70,000) as previously described (14). Animals were anesthetized and given FITC-dextran (200 μl of 5 mg/ml stock solution). Two hours after administration, the contents of the entire gastrointestinal tract were divided into the stomach, 10 equal small intestinal segments, cecum, proximal colon, middle colon, and distal colon. Each segment was opened and mixed vigorously with 2 ml of KRB solution to obtain a supernatant containing the FITC-dextran. The supernatant was centrifuged at 10,000 rpm to force the intestinal chyme to a pellet. The fluorescence in duplicate aliquots of the cleared supernatant was read in a multwell fluorescence plate reader (Gemi-niXS, Molecular Devices, Sunnyvale CA; excitation 488 nm and emission 525 nm) for quantification of the fluorescent signal in each bowel segment. A median distribution histogram of the fluorescence contained in each bowel segment was then plotted for analysis of this transit. This provided an accurate, nonradioactive measurement of intestinal transit without further surgical intervention.

To demonstrate the in vivo role of proinflammatory mediators, we performed in vivo gastrointestinal transit experiments with the specific iNOS inhibitor L-NIL and the COX-2 inhibitor DFU. We performed the CLP operation as described above. After 21 h, we injected L-NIL (50 μg/kg) and DFU (5 mg/kg) intraperitoneally and performed the transit procedure at 24 h as described above (n = 5).

Distal colonic in vivo motility was recorded in awake, restrained rats 24 h after colonic manipulation, sham operations, and controls (n = 5 each) with an intracolonic balloon-tipped catheter as previously described (25). Pressures were recorded and analyzed using the MacLab data acquisition package (ADInstruments, Castle Hill, Australia) and a Macintosh G3 computer. After a 20- to 30-min period of acclimatization, animals underwent recording for periods of 2–3 h.

Histochemistry and immunohistochemistry. Muscularis whole mounts were prepared as described earlier (16). Midjejunal and midcolonic segments were cut from the bowel and immersed in KRB in a Sylgard-filled glass dish at 4°C. The length and width of each jejunal segment were measured with a caliper, and the segment was gently pinned along the mesenteric border. The bowel was opened along the mesentery and stretched to 150% of the length and 250% of the width. Mucosa and submucosa were scraped off under microscopic observation (Wild, Heerbrugg, Switzerland), and the normal, 5-cm opened segments of jejunum were fixed in 100% ethanol for 10 min. Each segment was washed twice in KRB, and the mucosa-free muscularis whole mounts were finally cut into 1 × 1 cm pieces and used for staining procedures.

Histochemical staining for myeloperoxidase (MPO) was used to detect polymorphonuclear neutrophils, which had infiltrated into the freshly prepared muscularis whole mounts using a mixture of 10 μg of Hanks-Yates reagent (Polysciences, Warrington, PA), 10 ml of KRB, and 100 μl of 3% hydrogen peroxide (Sigma-Aldrich, St. Louis, MO) for 10 min. The reaction was stopped with cold KRB. MPO-positive cells were counted at a magnification of ×200.

Muscularis whole mounts were also used for immunohistochemical analysis of the jejunal and colon muscularis. Each whole mount was incubated for 24 h at 4°C in the primary antibody (ED1 and ED2) followed by three 10-min washes in buffer. The specimens were then incubated in the appropriate secondary antibody at 4°C for 4 h and washed three times for 10 min in buffer. Whole mounts were placed on a coverslip and inspected by fluorescent microscopy (Nikon FXA, Fryer, Huntley, IL).

Quantification of gene expression. The time course of postoperative mediator mRNA expression was analyzed using SYBRgreen two-step real-time RT-PCR (PE Applied Biosystems, Foster City, CA). Total mRNA extraction was performed as previously described using the guanidinium-thiocyanate phenol-chloroform extraction method.
The RNA pellets were resuspended in RNAsecure resuspension solution (Ambion, Austin, TX). After the resuspension, a DNase treatment was carried out (DNA-free reagent, Ambion). Then, equal aliquots (20 H9262 g) of total RNA from each sample, quantified by spectrophotometry, were processed for complementary DNA (cDNA) synthesis.

Primers were designed according to published sequences (17, 31, 36, 37) or using Primer Express software (PE Applied Biosystems) and purchased from Life Technologies (Rockville, MD). GAPDH was used as an endogenous control. The sequences of the real-time PCR primers are listed in Table 1. The efficiency and equality of the real-time PCR primer pairs were determined by amplifying serial dilutions of colonic muscularis cDNA. For each target gene, different MgCl2 (2–5 mM) concentrations were tested to optimize the PCR amplification. Agarose gel electrophoretic analysis was used to verify the presence of a single product and to ensure that the amplified product corresponded to the size predicted for the amplicon. Each sample was estimated in triplicate. The PCR reaction mixture was prepared using the SYBRgreen PCR Core Reagents (Applied Biosystems). PCR conditions on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems) were as recommended by the manufacturer. Relative quantification was performed using the comparative cycle threshold (CT) method as described previously by Schmittgen et al. (27) (see also User Bulletin no. 2, PE Applied Biosystems).

Small intestinal extracts from the laparotomy only group did not display altered in vivo or in vitro gastrointestinal motility or display an increased MPO-positive leukocyte recruitment into the intestinal muscularis. Molecular changes as evaluated by the comparative CT values of this group were not significantly different for IL-1 H9252, tumor necrosis factor- H9251, monocyte chemoattractant protein (MCP)-1, COX-2, or iNOS compared with unoperated controls. IL-6 mRNA levels were increased from 1.15 H11006 0.40 to 208.8 H11006 44.27-fold. However, this value was 1% of the upregulation caused by CLP. Therefore, this additional group was not studied further, and the sham CLP groups, along with unoperated animals, were used for statistical comparison to the CLP-induced polymicrobial septic group of animals.

Table 1. Nucleotide sequences of oligonucleotide primers with their accession number

<table>
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<tr>
<th>Target Gene</th>
<th>Primer Sequences (5’ to 3’)</th>
<th>Accession No.</th>
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<tbody>
<tr>
<td>GAPDH</td>
<td>Sense: ATGGCACAGGCTCAAGGCTGAG</td>
<td>NM_017008</td>
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<tr>
<td></td>
<td>Antisense: CGTCCTGGGAAGATGCTGAT</td>
<td></td>
</tr>
<tr>
<td>COX-2</td>
<td>Sense: GCTCGGATGTCCTTCGAG</td>
<td>AF233596</td>
</tr>
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<td></td>
<td>Antisense: AAGGATTTGCTGATGCTG</td>
<td></td>
</tr>
<tr>
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<td>NM_012611</td>
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<tr>
<td></td>
<td>Antisense: CCAAGCATATTTGACTTCC</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Sense: AAGGATTTTCACGACACCC</td>
<td>X66539</td>
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<td></td>
<td>Antisense: CACCTGGTCAGCCACTC</td>
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<tr>
<td>IL-1β</td>
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<td>M26744</td>
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<td>MCP-1</td>
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COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; MCP, musculocutaneous perforator.

Fig. 1. Transit histogram for the distribution of nonabsorbable FITC-labeled dextran along the gastrointestinal tract 2 h after oral ingestion in control, sham, and cecal ligation and puncture (CLP)-treated animals. A: in control animals, the nonabsorbable marker accumulated in the last 3 segments of the intestine [geometric center (GC) = 9.3]. Sham animals had a similar distribution pattern (GC = 8.9; P = 0.365). CLP caused a significant delay in intestinal transit (GC = 4.9; P = 0.0032). B: plot of the calculated geometric center value for control, sham 24 h, and CLP at postoperative days 1–4 (CLP-1 to -4, respectively). The plot demonstrates the in vivo functional temporal recovery of gastrointestinal transit over a period of 4 days after the initial CLP-induced transit delay.
RESULTS

Gastrointestinal motility. We first sought to characterize functional alterations in gastrointestinal motility in response to nonlethal CLP-induced enteric polymicrobial bacteremia because this has not been previously accomplished (4, 35). Gastrointestinal transit was measured using orally administered FITC-labeled dextran after a period of 2 h in control animals, sham-operated animals, and CLP animals on PODs 1–4 (Fig. 1).

In control animals, the fluorescence-labeled dextran was transported down the intestine to its distal end with the peak detected in the distal ileum. The average calculated geometric center for controls was 9.3 ± 0.55 for 15 segments of the gastrointestinal tract. The transit distribution histograms and calculated geometric centers for sham-operated animals with cecal eversion (8.9 ± 0.55, P > 0.05) were not significantly different from controls on postoperative day 1. In contrast, CLP-induced polymicrobial sepsis caused a significant delay in gastrointestinal transit. After a 2-h transit period, the peak fluorescence in the CLP animals only reached the midjejunum 24 h after operation (Fig. 1A). The CLP animals had a significantly slower average calculated geometric center of 4.9 ± 0.95 (P = 0.0032). This initial period of ileus, however, resolved itself over the following 4 days, as gastrointestinal transit gradually normalize with the peak fluorescence again distributed mostly to the distal ileum on postoperative day 4 (Fig. 1B). Interestingly, the simultaneous inhibition of iNOS and COX-2 in vivo restored transit almost completely to

Fig. 2. Representative distal colonic pressure recordings obtained from a control (A), sham-operated (B), and CLP-manipulated (C) animal 24 h after surgery. CLP-induced sepsis caused a significant reduction in colonic contraction amplitude and frequency compared with control and sham-operated animals (P < 0.05).
control values. The fluorescein-labeled dextran reached the distal ileum expressed by a geometric center of 8.16 ± 1.08, which shows no significant differences to control values.

The above transit distribution histograms after 2 h primarily measured the functional movement of FITC-dextran through the stomach and small intestine. Therefore, to investigate in vivo colonic function after CLP, we performed intracolonic balloon pressure recordings in control, sham, and CLP animals after 24 h. Representative distal colonic pressure recordings from each group of animals are illustrated in Fig. 2.

The colonic musculature of control and sham animals generated relatively regular large prolonged phasic contractions with superimposed spike contractions. The amplitude of the distal colonic pressure waves averaged 67.4 ± 13.2 and 65.3 ± 11.6 cmH2O for control and sham animals. The prolonged large phasic contractile frequency averaged 0.72 ± 0.14 (controls) and 0.6 ± 0.09 (sham) per minute. Twenty-four hours after CLP, colonic contractile amplitudes were significantly reduced by 56.8% (29.8 ± 11.6 cmH2O, \(P < 0.01\)) and were more variable in amplitude and frequency compared with the control and sham groups. Colonic contractile frequencies of the prolonged large phasic contractions were also significantly less frequent (0.43 ± 0.15 per minute, \(P = 0.0133\)) in CLP animals.

In the next series of experiments, muscularis function was measured directly by determining the in vitro capacity of jejunal and colonic circular smooth muscle strips to respond to the cholinergic agonist bethanechol using standard organ bath techniques. As illustrated in Fig. 3, bethanechol exposure generated a dose-dependent increase in circular muscle contractile activity of the small bowel and colon.

In accordance with the above in vivo experiments, jejunal and colonic muscle strips prepared 24 h after CLP exhibited a significant reduction in muscle contractility. Circular muscles from the mid-small bowel of control animals generated contractions with a mean contractile force area of 2.15 ± 0.15 g·mm−2·s−1 at a concentration of 100 µM bethanechol, whereas jejunal circular muscle strips from CLP animals showed a significant impairment in the bethanechol-stimulated dose-response curve (contractile force of 1.08 ± 0.28 g·mm−2·s−1 at 100 µM) (Fig. 3A). A similar inhibitory effect of CLP on the bethanechol dose-response curve was obtained using circular muscle strips cut from the midregion of the colon (Fig. 3B). Colonic circular muscle strips from CLP animals generated contractions with a mean contractile force area of 2.36 ± 0.43 g·mm−2·s−1 at 100 µM bethanechol, which represented a 50% decrease in muscularis function compared with control animals (4.76 ± 0.56 g·mm−2·s−1 at a concentration of 100 µM bethanechol).

**Leukocytic infiltration.** Our previous studies have demonstrated that deterioration in muscle function after insult is frequently caused by the presence of leukocytes within the gastrointestinal muscularis. Therefore, leukocytic infiltrates within the small and large intestinal muscularis were investigated in whole-mount preparations 24 h after the induction of CLP-induced sepsis. We focused on macrophages, monocytes, and polymorphonuclear neutrophils because these leukocytes possess the ability to release smooth muscle kinetically active substances that directly or indirectly modulate the gut muscularis (7, 11, 15, 19, 29). As previously reported, ED2+ leukocytes (macrophages) form a dense network of resident phagocytes within the normal muscularis of the small and large intestines Fig. 4, A and B.

Morphologically, macrophages from control animals were stellate in appearance, exhibiting well-spread pseudopodia extending from a nuclear cytoplasmic enlargement. Visually, the number of ED2+ cells within the intestinal muscularis of these organs did not change 24 h after CLP; however, the macrophages did appear to alter their morphology. In an irregular clustering pattern, macrophages from CLP animals appeared to retract their pseudopodia, taking on a less sprawling morphology. A typical example of this change in muscularis macrophage morphology can be seen in Fig. 4C.

As shown in Fig. 4D, jejunal whole mounts stained for the ED1 antigen revealed the presence of only a few round-shaped monocytes in control and sham animals. Additionally, as previously observed, this antibody also lightly stained the stellate-shaped resident muscularis macrophages. In the animals subjected to CLP, however, numerous ED1+ monocytes could be microscopically detected as newly extravasated leukocytes into the jejunal muscularis syncytium (Fig. 4E).

In contrast to the control jejunum, colonic muscularis whole mounts displayed a large number of constitutively present...
ED1\(^+\) cells, as well as the lightly stained ED1\(^+\) muscularis macrophages (Fig. 4F). These cells clustered heavily around tightly adherent small vascular structures that coursed along the submucosal edge of the muscularis externa. In fact, the irregular distribution and large basal presence of the rounded ED1\(^+\) cells made it difficult to quantitatively count an increase in ED1\(^+\) cells in colonic whole mounts prepared from CLP animals.

As shown in Fig. 5 and quantified in the histogram of Fig. 6, MPO-positive neutrophils were present only occasionally in control whole mounts of the jejunum and colon (16). Although the sham procedure of cecal eventration increased the number of extravasated PMNs in the muscularis of both organs, this polymorphonuclear neutrophil infiltrate was even more significantly enhanced in CLP animals.

Inflammatory molecular events. To determine the possible upregulation of inflammatory mediators, which could participate in causing leukocyte recruitment and functional muscle impairment (15, 29, 33), we performed quantitative real-time RT-PCR on isolated muscularis extracts of the small and large intestine for IL-6, IL-1\(\beta\), MCP-1, iNOS, and COX-2. Inflammatory gene mRNAs were measured over a time course of 0–48 h comparing control, sham operated, and CLP muscle extracts. For all mediators, except COX-2, small intestine and colonic extracts from CLP animals entered the exponential amplification phase earlier than those of the control and sham-operated animals, demonstrating a higher starting template concentration (Figs. 7 and 8).

In general, each mediator followed a similar temporal pattern of upregulation in both organs with a rapid induction within 1 h after CLP that remained significantly elevated through 48 h after the CLP procedure. Of note, when the small intestine was compared with the colon, however, a >100-fold induction of iNOS was measured from colonic extracts compared with a fourfold induction measured from small intestinal extracts (Fig. 8).
The functional contractile significance of the induction of iNOS and COX-2 mRNAs within the jejunal and colonic intestinal muscularis by CLP was investigated using the relatively selective iNOS inhibitor L-Nil (30 μM) and the COX-2 inhibitor DFU (5 mg/l). Circular jejunal and midcolonic muscle strips were harvested and prepared for organ bath recordings. After the equilibrated spontaneous contractions were recorded for a period of 30 min, muscles were acutely superfused with both L-Nil and DFU for a further 30-min period. Application of the iNOS and COX-2 blockers caused a 62% increase over jejunal circular muscle control activity and an 181% increase in colonic circular muscle spontaneous contractions over control colonic muscles exposed to the blockers.

**DISCUSSION**

Unfortunately, although many agents have shown promise in septic animal models, clinical trials of these treatments failed, and patient mortality rate with severe sepsis and subsequent multiorgan failure remains >20%. One critical reason for this discrepancy could be the use of clinically inappropriate animal models that do not closely mimic the septic human patient (4). Comparatively, CLP appears to be a reliable and clinically relevant animal model of the human septic condition. Indications for the relevance of the CLP model are that the endoge-
nous polymicrobial nature of the induced infection is similar to clinical bacteremia and symptomatically after CLP animals develop an initial hyperdynamic cardiovascular response similar to what is observed in human sepsis (9, 26, 35). In this nonlethal CLP study, we observed the development of an intra-abdominal abscess surrounding the ligated and perforated cecum. The morphological appearance of perforation coverage and abscess formation mimics the clinical situation of a secondary intra-abdominal sepsis, such as after diverticulitis or gastric ulcer perforation with omental coverage (8). Like in our laboratory’s previous studies using LPS-induced ileus (6, 7, 28, 33), we investigated the hypothesis that local inflammatory events within the gut muscularis externa and a subsequent inhibition of intestinal smooth muscle contractility mechanistically participate in the systemic inflammatory response syndrome and gastrointestinal ileus. Our aim was to investigate motility and cellular and molecular events within not only the small intestine, but also the colon, which has previously not been studied. Additionally, recovery from CLP-induced gastrointestinal ileus was also examined.

Functionally, we observed a substantial decrease in upper gastrointestinal transit after CLP, as indicated by the transit distribution histograms. Similarly, in vivo colonic pressure activity was suppressed, as well as in vitro jejunal and colonic muscle strip mechanical responses to bethanechol. The 50% degree in muscle inhibition was relatively similar between the jejunal and colonic regions of the gastrointestinal tract, as caused by our nonlethal CLP model. Interestingly, gastrointestinal transit started to recover after 24 h and reached a normal transit pattern again 4 days after the induction of sepsis. Thus, from a functional standpoint, nonlethal CLP suppressed circular muscle contractility to a similar extent and duration as did a single nonlethal injection of endotoxin (6, 7, 28). As reported

Fig. 7. Real-time RT-PCR analysis of proinflammatory mediator mRNAs. Cycle threshold (CT) values were calculated in control, sham, and 1, 3, 6, 24, and 48 h after CLP-induced sepsis in the small bowel and colon. A: in the jejunum, IL-6 peaked after 3 h, whereas in the colon the peak was at 6 h. The fold increase showed regional differences, and was 3 times higher in the small bowel. B: IL-1β expressions in the small bowel and colon showed a similar temporal upregulation after CLP and a similar fold increase in the small bowel and the colon with a first peak at 3 h and a possible second peak at 48 h. C: monocyte chemoattractant protein (MCP)-1 peaked at 6 h in the small bowel and at 3 h in the colon after CLP. The small bowel had a second peak at 48 h. Fold increases were similar in both intestinal regions.

Fig. 8. Real-time RT-PCR analysis of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 mRNAs. CT values were calculated in control, sham, and 1, 3, 6, 24, and 48 h after CLP-induced sepsis in the small bowel and colon. A: in the jejunum, iNOS demonstrated a significant but relatively small increase, peaking at 6 h and declining back toward normal levels at 48 h. Colonic muscularis iNOS mRNA expression pattern was different in the colon with its maximum expression at 48 h, which exceeded the relative maximum increase in the small bowel by 25-fold. B: COX-2 levels demonstrated only minor induction after CLP-induced sepsis in the small bowel or the colon. In the colon, COX-2 mRNA expression had a tendency to decline during the first 6 h but rebounded to control levels again at 24 h, whereas in the small bowel, COX-2 had 2 moderate peaks at 1 and 48 h with a 3-fold increase compared with controls.
in the one previous study using a lethal model of CLP (18), ileal longitudinal muscle decreased its function by 33%. The decreased sensitivity of longitudinal compared with circular muscle to CLP has also been observed with endotoxin (6, 34).

Muscle dysfunction in both the polymicrobial CLP and endotoxin models of sepsis is associated with a significant recruitment of neutrophils and monocytes into the jejunal muscularis 24 h after the initiation of sepsis (6, 7, 28). Quantitatively, however, neutrophil recruitment caused by CLP represented a greater percentage of the jejunal muscularis leukocytic infiltrate compared with the endotoxin model, which initiated a nearly pure monocytic recruitment into the jejunal muscularis (7). This more complex leukocytic infiltrate might be expected due to the intraperitoneal release of numerous bacterial products after polymicrobial CLP sepsis. The colonic muscularis was also significantly infiltrated with neutrophils during CLP. But unlike the small intestine, the control colonic muscularis contained a large population of resident ED1+ round-shaped monocytes, which clustered in a distinct perivascular pattern. A monocytic increase after CLP was difficult to ascertain in the colon, because of their large basal presence and irregular perivascular distribution.

We have previously demonstrated that MCP-1 is a critical chemokine involved in the recruitment of monocytes into the jejunal muscularis (33). Acute LPS injection causes a strong early 3-h 280-fold induction in jejunal MCP-1 as measured by real-time RT-PCR over control levels. Even a lower 30-fold induction appeared quite organ specific. Small intestinal extracts demonstrated peak mRNA induction at 6 h, whereas the colonic signal peaked much later at 48 h. It is difficult to explain this temporal difference in iNOS induction, but it could be because the colon is more anatomically insulated from the small intestine and, therefore, less rapidly exposed to the bacterial leakage from the punctured cecum. However, a region-specific heterogeneous response of the constituents of the different muscularis extracts cannot be ruled out. Here we demonstrated for the first time that a large population of ED1+ monocytes with a perivascular distribution is constitutively present in the colon. The presence of these phagocytes, in addition to the ED2+ resident muscularis macrophages could explain the higher degree of iNOS induction within colonic extracts compared with the small intestine, which does not display this pattern of resident monocytes. We have also observed this regional difference in iNOS expression after surgical manipulation of the intestine, which our laboratory used to investigate the mechanisms of postoperative ileus (21, 32). However, in contrast to iNOS, COX-2 was more highly induced in the small intestine compared with the colon, although the regional difference was not as striking. The molecular induction of iNOS and COX-2 was functionally correlated with jejunal and colonic contractile responses after synaphe blockade with L-NIL and DFU. Spontaneous circular muscle contractile activity recorded from both the jejunum and colon harvested from CLP animals increased significantly over control muscle responses to synaphe blockade. Additionally, simultaneous in vivo administration of iNOS and COX-2 inhibitors almost completely restored the transit after CLP, supporting the in vitro data of L-NIL and DFU usage followed by an improved muscle activity outcome. Also reflected in contractile activity was the observation that colonic activity increased to a greater percentage of control activity compared with the jejunum, thus demonstrating that both regions of the gastrointestinal tract were tonically inhibited by the upregulation of iNOS and COX-2 synthases and also that functional activity correlated with a greater iNOS induction within the colonic muscularis.

In summary, the above data demonstrates that polymicrobial CLP sepsis induces a complex inflammatory response within the intestinal muscularis with the recruitment of leukocytes and elaboration of mediators that inhibit intestinal muscle function. Interestingly, differences were elucidated between LPS and CLP models of sepsis as well as a heterogeneous regional response of the gastrointestinal tract to CLP. Thus the intestine is not only a source of bacteremia but also an important target of the bacterial products with major functional consequences to intestinal motility and the generation of cytokines, which could participate in the development of multiple organ failure.

ACKNOWLEDGMENTS

The authors thank Jessica Whitcomb for excellent technical support regarding the in vivo transit and immunohistochemistry experiments.

GRANTS

This work was supported by National Institute of of General Medical Sciences Grants R01-GM-58241 and P01-GM-53789.

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