Nitric oxide-mediated injury of interstitial cells of Cajal and intestinal dysmotility under endotoxemia of mice

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ABSTRACT
Gastrointestinal dysmotility is frequently observed under septic conditions, yet its precise mechanisms remain to be elucidated. In this study, we have investigated the mechanisms of intestinal dysmotility by lipopolysaccharides (LPS) and the role of the interstitial cells of Cajal (ICCs) in motility disorders using a mouse endotoxin model. The injection of LPS caused time- and dose-dependent decreases in the intestinal contractility, which was associated with similar time- and dose-dependent decreases in the number of KIT-positive fibroblast-like cells located in the intermuscular layer. iNOS inhibitors, l-NAME and aminoguanidine (AG), but not 7-nitroindazole (7NI), a specific nNOS inhibitor, inhibited the LPS-induced decreases in both the contractility and the number of KIT-positive cells. A spontaneous NO releaser, FK409, not only diminished spontaneous electrical potential and phasic contractions, but also decreased the number of KIT-positive cells. Pretreatment with gadolinium inhibited the activation of macrophages and the induction of iNOS in intestinal resident macrophages, and restored the number of KIT-positive cells and intestinal contractions. These results suggested that NO produced from intestinal macrophages via iNOS induced by LPS, may be involved in the ICCs injury and intestinal dysmotility under septic conditions.

Sepsis is still the most common cause of death in intensive care units (ICU) (1). Paralytic ileus or gastrointestinal stasis is frequently observed under septic conditions. The gastrointestinal abnormalities under these conditions are characterized by a decrease in gastric emptying and delayed intestinal transit (26). Most phenomena under septic conditions can be reproduced by the administration of bacterial products including lipopolysaccharides (LPS), the signals of which are transduced via specific receptors on the plasma membrane of leukocytes, such as Toll-like receptor-4 (TLR4); thus, LPS is considered to be a major player in sepsis and gastrointestinal stasis (4, 12, 18, 21). Gastrointestinal dysmotility under septic conditions accompanies other forms of morbidity, and results in prolonged hospitalization, increased medical costs, and an increase in mortality rate. Although several pathogenic mechanisms including local inflammation, cytokines, such as tumor necrosis factor (TNF) or interleukin-1 (IL-1), and an increase in nitric oxide (NO), have been proposed for motility disorders under sepsis (4, 5, 18, 30), the central mechanisms of gastrointestinal dysmotility under these conditions have not been elucidated.

Interstitial cells of Cajal (ICCs), which mediate input from the enteric motor nervous system to smooth muscle, are the pacemaker cells in the gastrointestinal tract (9). ICCs generate spontaneous electrical slow waves and regulate rhythmic peristalsis. Loss of ICCs has been reported in animal mod-
nels and several human diseases including diabetes and chronic intestinal pseudo-obstruction, which typically develop gastroparesis and intestinal dysmotility (10, 23, 25). In animal experiments, blocking KIT signaling of fetal animals by a variety of techniques, causes a loss of ICCs, resulting in dysmotility of the stomach and intestine. The major mediators that control gastrointestinal motility are acetylcholine and substance P for excitation and NO and vasoactive intestinal peptide (VIP) for inhibition (2). Neurons expressing neuronal NOS or producing NO are suggested to be involved in the relaxation of gastrointestinal smooth muscles. Although ICCs maintain normal gastrointestinal motility and LPS, as well as sepsis, causes gastrointestinal dysmotility, neither the role nor the mechanisms of ICCs in gastrointestinal dysmotility under septic conditions have been elucidated.

In this study, we evaluated ICC injuries via LPS injection and investigated the mechanisms of ICC impairment using a mouse endotoxin model. Our results suggest that LPS induces the expression of inducible NOS (iNOS) in intestinal resident macrophages, from which increased NO production may cause ICC impairment, resulting in gastrointestinal dysmotility.

MATERIALS AND METHODS

The procedures involving animal care and sample preparation were approved by the Animal Experimental Committee of Osaka University Graduate School of Medicine and were performed in accordance with the NIH guidelines, and the regulations and guidelines for the care and use of laboratory animals of the Osaka University Graduate School of Medicine.

Chemicals. The following chemicals were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA.): Lipopolysaccharide (LPS: Escherichia coli; L3880), Nω-nitro-L-arginine methyl ester (L-NAME), amino-guanidine (AG), 7-nitroindazole (7NI), inhibitors of nitric oxide synthesis. L-arginine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All chemicals used were of analytical grade. The FK409 was kindly provided from Shionogi Co. Ltd. (Osaka, Japan).

Animals. BALB/cA mice aged 7–8 weeks were purchased from Clea Japan, Inc. (Tokyo, Japan) and were provided with water and standard laboratory chow ad libitum. After overnight fasting, the mice were used for the following experiments.

Experimental protocol. LPS dissolved in 0.1 mol/L phosphate-buffered saline (PBS) or PBS as a control was injected into the abdominal cavities of BALB/cA mice at indicated doses. The mice were euthanized by cervical dislocation after 6, 12, 18, 24, and 48 h by either LPS or PBS injection. The intestines were immediately prepared for tension recording and histologic examinations. L-NAME (1 mg/mL), AG (5 mg/mL), or 7NI (1 mg/mL) dissolved into PBS was injected into the abdominal cavity at indicated doses.

To inactivate resident macrophages, we used gadolinium (Gd). Gd (7 mg/kg) was injected twice into the abdominal cavity, at 48 and 24 h, prior to the LPS injection. LPS was then injected at 10 mg/kg. At indicated time points, the intestine was dissected.

Tension recording. The intestine was cut into segments 10 mm in length, which were then immersed in a bath filled with 37°C Takemasa Tyrode buffer (28). The Takemasa Tyrode consisted of NaCl 145 mM, glucose 11.1 mM, NaHCO 3 4.8 mM, CaCl 2 • 2H 2 O 1.5 mM, KCl 2.6 mM, MgCl 2 • 6H 2 O 0.73 mM, and NaH 2 PO 4 • 2H 2 O 0.33 mM. Using the apparatus shown in Fig. 1 (19, 27), the intestinal segments were placed in a bath containing Takemasa Tyrode solution, which was kept in 37°C and oxygenated. One end of the segment was fixed to an isometric force transducer and the other end was connected to a pressure transducer. The longitudinal contractions were recorded in the isometric force transducer, and the circular contractions were recorded in a pressure transducer under a hydrostatic pressure of 1.0 cm H 2 O. The serosal electromyography was recorded via a platinum electrode in a glass tube filled with...
Tyrode solution. The intestines were allowed to equilibrate in the chamber for 20 min with a 200 mg preload. Simultaneous recordings were then performed.

Sample preparation for histochemical and morphological analyses. After the mice were anesthetized intraperitoneally with pentobarbital (25 mg/kg), the ileum and jejunum were rapidly dissected and fixed overnight with 4% paraformaldehyde buffered with 0.1 M phosphate buffer (pH 7.2) (PB) containing 4% sucrose for light microscopy and with 2% paraformaldehyde-2% glutaraldehyde buffered with 0.1 M PB for conventional transmission electron microscopy, as previously described (13–15). For light microscopy, the samples of cryosections were embedded in an OCT compound after cryoprotection with 15 and 30% sucrose solutions and cut into 10 μm sections with a cryostat (CM3050; Leica Microsystems, Nussloch, Germany). Sections were placed on silan-coated glass slides and stored at −80°C until used. Samples for electron microscopy were postfixed with 2% OsO4 with 0.1 M PB, block-stained in 1% uranyl acetate, dehydrated with a graded series of alcohol, and embedded in Epon 812. Silver sections were cut with an ultramicrotome (Ultracut N; Reichert-Nissei, Tokyo, Japan), stained with uranyl acetate and lead citrate, and observed with an electron microscope (H-7100; Hitachi, Tokyo, Japan).

Immunohistochemistry. For detection of the KIT-positive cells using a c-kit antibody, frozen sections were immunostained as previously described (15, 16). Briefly, the samples were treated with 0.3% H2O2 in methanol for 30 min and incubated with 2% normal goat serum for 20 min at room temperature (RT). They were then incubated at 4°C with the first monoclonal antibody of the c-kit (1 : 100; eBioscience, Inc., San Diego, CA, USA) overnight. Further incubations were performed with biotinylated goat anti-mouse IgG for 1 h, and finally with peroxidase-conjugated streptavidin (VECTASTAIN ABC KIT; Vector Laboratories, Burlingame, CA) for 1 h at RT. After each step, the sections were rinsed thoroughly in 0.1 M phosphate-buffered 0.5 M saline (pH 7.2) that contained 0.1% Tween 20 (TPBS) (Sigma). Staining for peroxidase was performed using 0.0125% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Dojindo Laboratories, Kumamoto, Japan) and 0.002% H2O2 in 0.05 M Tris-HCl buffer (pH 7.6) for 10 min. The KIT-positive cells in the intermuscular layer were counted throughout specimens and were expressed as cells/cm in the longitudinal axis of the intestine.

Whole-mount immunohistochemistry. A segment of the ileum was dissected from each of the BALB/cA mice and placed in dishes filled with 0.1 mol/L PBS at 37°C. The segments were washed of their content and opened up along the mesenteric border, then pinned flat, mucosal side up. The segments were fixed in 4% paraformaldehyde overnight and incubated in 2% bovine serum albumin (BSA) (Sigma) for 20 min at 20°C; then they were immunostained with the primary antibodies (rat anti-mouse F4/80 antigen (1 : 50) and rabbit anti-nitric oxide synthase II (1 : 200)) for 48 h at 4°C followed by incubation with the second antibodies (goat anti-rabbit and rat IgG coupled with FITC and Alexa Fluor 594, respectively). After each process, the tissues were washed with 0.1 mol/L PBS (pH 7.2). Immunostained tissues were mounted serosal side up and examined with a laser scanning confocal microscope (OLYMPUS LSM-GB200; Olympus Co. Tokyo, Japan). Whole-mount preparations were used to count the KIT-positive cells. KIT-positive cells in the intermuscular layer were counted throughout the slides using three different sections from each mouse, which were then averaged for each.

Western blotting. Intestinal resections from each mouse were homogenized in 2 mL of 0.05 M Tris-buffered 0.15 M saline containing 1% Triton X-100 and a protease inhibitor mixture (Boehringer Mannheim, Indianapolis, IN) using a Politron homogenizer at 80% of the maximal speed. After being centrifuged twice at 10,500 × g for 10 min at 4°C, the supernatant was measured for protein concentration using the BCA protein assay system (Pierce, Rockford, IL), and immunoblotting was performed. Each sample was separated by 10% SDS-PAGE. Electrophoretic transfer of proteins from polyacrylamide gels to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Tokyo, Japan) was performed according to a previously established method (29). The sheets were soaked in PBS containing 5% BSA (Sigma) to block nonspecific binding and then incubated overnight with anti-nitric oxide synthase II (1 : 1,000) or subunit β of mitochondrial F1F0ATPase (1 : 1,000) (17). The membranes were washed 3 times for 10 min in PBS containing 0.1% Tween 20 and then further incubated for 1 h at RT with horse-radish peroxidase-labeled secondary antibody (pig anti-rabbit IgG; DAKO, Glostrup, Denmark) diluted 1 : 1,000. After three washes in PBS with 0.1% Tween 20, the membranes were treated with a chemi-
luminescent ECL kit (Amersham, Arlington Heights, IL) according to the manufacturer’s recommended protocol.

Statistics. The results are expressed as the mean ± standard deviation (SD) unless otherwise noted. The experiments were performed at least 3 times unless mentioned otherwise. Statistical analysis was performed using the Kruskal-Wallis test and one-way, as well as repeated-measure analysis of variance (ANOVA), with Scheffe’s or Newman-Keuls’ post-hoc test. \( P \) values less than 0.05 were considered significant.

RESULTS

LPS-induced decrease in spontaneous intestinal movement

We prepared intestinal segments of the jejunum and ileum from saline- or LPS-injected mice to measure the autonomous contractions of the small intestine using a Tension recording system and serosal myoelectrography (Fig. 1). The intestinal segments from both saline-injected mice and control mice (no preparation) showed spontaneous phasic contractions with regular cycles of 25–30 contractions/min. Intraperitoneal bolus injection of LPS (10 mg/kg), however, caused irregular and attenuated contractile activities (Fig. 2A). This reduction in contractility appeared time-dependent, and the lowest degree of contractility was observed 12–18 h after LPS injection. Thereafter, contractility was restored. Intestinal preparations from control and saline-injected mice showed normal slow waves corresponding to phasic contractions of the longitudinal muscles and clusters of spike potentials corresponding to the circular muscle contractions (Fig. 2B-a). In contrast, both slow waves and spike potentials were reduced by LPS injection (Fig. 2B-b). In accordance with the diminished and irregular spike potentials, intraluminal pressure was reduced by LPS injection.

Next, we examined time- and dose-dependent changes in spontaneous phasic contractions by quantitative measurements. As shown in Fig. 3A, the phasic contractions were decreased time-dependently by LPS injection, although there was no change induced by saline injection. The nadir of the phasic contractions was observed at 12 h after the LPS injection. Contractility was restored within 48 h. The decrease in contractility attenuated by LPS was dose-dependent (Fig. 3B). Doses of more than 10 mg/kg of LPS could not be obtained due to lethality in mice within 24 h.

![Figure 2](image1)

**Fig. 2** Intestine contractions after LPS injection. LPS (10 mg/kg) dissolved in saline or an equivalent amount of only saline (sham) was administered intraperitoneally (i.p.) to BALB/cA mice and then intestinal segments were isolated to measure spontaneous contractions. (A) Intestine contractions measured by an isometric force transducer before LPS injection (control) (a), after 6 (b), 12 (c), 18 (d), and 24 (e) h from LPS i.p. (B) Intestinal contractions were simultaneously measured before (a) and 18 h after LPS i.p. (b). The upper, middle, and lower lines are the serosal myoelectrography, the intestinal contractions measured by an isometric force transducer, and the intraluminal pressure, respectively.
Morphological changes after LPS injection
Morphological changes by LPS (10 mg/kg) were examined, and at each time point, no significant morphological change was observed by hematoxylin-eosin staining among the control, the saline- and LPS-injected mice. Next, we examined the KIT-positive cells in the intermuscular layer by KIT-immunostaining. KIT-positive fibroblast-like cells were mainly observed in the intermuscular layer and less frequently in the submucosal layer of the control mice, and the former appeared to correspond to the interstitial cells of Cajal. With LPS injection, KIT staining was diminished even in KIT-positive cells, and the number of KIT-positive cells was apparently

![Figure 3](image1.png)

**Fig. 3** Time- and dose-dependent decrease in intestinal contractions after LPS injection. (A) Time-dependent changes in intestinal contractile forces measured by an isometric force transducer. Vertical bars represent the means ± SD (n = 3). (B) LPS dose-dependent changes in intestinal contractile forces measured by an isometric force transducer. Each bar indicates a contractile tension measured at 18 h after saline or LPS injection. Vertical bars represent the means ± SD (n = 3).

![Figure 4](image2.png)

**Fig. 4** Immunohistochemical and electron microscopic changes in ICCs after LPS injection. Intestinal tissues after intraperitoneal administration of LPS (5 mg/kg) dissolved in saline (B, D) or an equivalent amount of saline (sham) (A, C). (A, B) Immunohistochemistry for KIT. By LPS treatment, the number of KIT-immunoreactive cells in the intermuscular layer was apparently decreased. Inset in A indicates a typical KIT-positive ICC. (C, D) Electron microscopy revealed that LPS induced caveole formation, lipid droplets (white arrowheads) and plasma membrane ruffling in the ICCs and smooth muscle cells around myenteric plexus. Scale bars: 10 μm (A, B) and 2 μm (C, D). The arrows indicate the ICCs.
disorders was examined using several NO inhibitors. LPS injection induced iNOS expression (green color in Fig. 6B) in cells scattered in subserosal, submucosal and muscle layers (Figs. 6A and 6B). The cells expressing iNOS were also positive for the macrophage marker (red color in Fig. 6B), suggesting that LPS injection induced iNOS in intestinal macrophages. The addition of L-NAME, a non-selective iNOS inhibitor, did not alter the iNOS induction in macrophages of control and LPS-injected mice (Figs. 6C and 6D, respectively and Fig. 7B) but suppressed an LPS-induced decrease in phasic contractions of the intestines, both manometrically and electrically (Fig. 7C). L-NAME also inhibited a decrease in the number of KIT-positive cells in the intermuscular layer (Fig. 7A). L-NAME itself did not influence KIT staining (Fig. 7A), intestinal contractions (Fig. 7C) and iNOS induction (Fig. 7B). Similar results were obtained for a more specific iNOS inhibitor, AG, but not for 7NI, a specific nNOS inhibitor (Fig. 7C). Thus, iNOS induction in intestinal macrophages appeared to be involved in LPS-induced dysmotility.

We examined the effects of NO on intestinal contractions and the number of KIT-positive fibroblast-like cells using spontaneous NO releaser, FK409 (6). In in vitro experiments, the addition of FK409 to prepared jejunal segments decreased the spontaneous electrical potential in the myoelectrography, di-

![Fig. 5](image-url)  
**Fig. 5** Time- and dose-dependent changes in the number of KIT-positive fibroblast-like cells in the intermuscular layer. (A) 5 mg/kg of LPS dissolved in saline (black) or an equivalent amount of saline (white) was administered intraperitoneally to BALB/cA mice. At indicated times, KIT-positive fibroblast-like cells in the intermuscular layer were counted. Vertical bars represent the means ± SD (n = 3). (B) At 12 h after intraperitoneal administration of indicated doses of LPS dissolved in saline or an equivalent amount of saline, KIT-positive cells in the intermuscular layer were counted. Vertical bars represent the means ± SD (n = 3).
minimized phasic contractions, and the intestine was then transiently relaxed (Fig. 8A). These phenomena were also observed in the intestinal preparations that were pre-treated by tetrodotoxin to block neural transmission, suggesting that the effects of FK409 are not considered to be caused by NO as a neurotransmitter. Next, we injected FK409 into mice. There were no changes in the respiration and circulation including arterial blood pressure. In the intestine, however, similar to LPS injection, FK409 significantly decreased the number of KIT-positive cells as well as contractile activities (Figs. 8B and 8C).

Because intestinal macrophages and iNOS induced by LPS appeared to be involved in LPS-induced intestinal dysmotility, we next examined the roles of intestinal resident macrophages using a macrophage/Kupffer cell inhibitor, gadolinium (Gd). Gd pretreatment also restored the number of KIT-positive fibroblast-like cells as well as intestinal contractions even in the presence of LPS (Figs. 9A and 9B). Pretreatment with Gd inhibited the LPS-induced iNOS induction in resident macrophages, although the number of intestinal macrophages was increased (Fig. 9C). These results suggested that intestinal resident macrophages activated by LPS were involved in LPS-induced intestinal dysmotility.

Together, our results indicated that LPS induced iNOS induction and subsequent NO production in the intestinal macrophage and that increased NO played a key role in the motility disorder after LPS injection.

DISCUSSION

Gastrointestinal dysmotility is frequently observed under septic conditions and postoperative states (21, 26, 32), yet its precise mechanisms remain to be elucidated. Local inflammation with leukocyte infiltration in the gastrointestinal tract and systemic inflammation appeared to be involved in prolonged motor disturbance during sepsis. The mechanism of intestinal dysmotility under septic conditions has been investigated using animal models of LPS administration or cecal ligation and puncture (CLP). The latter is more clinically relevant but appears to

![Fig. 6](image_url) iNOS induction in intestinal macrophages after LPS administration. A segment of the ileum was dissected from each mice, and was opened along the mesenteric border, then, was mounted serosal side up. After fixation and immunostaining, the tissues were examined with a laser scanning confocal microscope focusing on the intermuscular layer. The right side of each photograph indicates the proximal end of the intestinal segment, and the left side the distal end. Before (A) and 8 h after intraperitoneal administration of LPS (B), L-NAME (C) or LPS and L-NAME (D), intestinal segments were immunostained for F4/80 (red) and iNOS (green). iNOS was induced in F4/80-positive macrophages after LPS administration (B) and iNOS induction by LPS was still observed by L-NAME co-administration (D). Bars indicate 20 μm.
the small intestine in a time- and dose-dependent manner, and these phenomena were coincident with the attenuated staining of KIT in KIT-expressing fibroblast-like cells in the intermuscular layer (ICC-like cells). Under these conditions, however, minimal morphological changes appeared in either smooth muscle cells or KIT-positive fibroblast-like cells, as shown by HE staining and electron microscope.

ICCs in the small intestine are considered to perform a pacemaker function for intestinal motor activity by generating an electrical slow wave and by mediating cholinergic and nitrergic neurotransmissions (2, 9). ICCs in particular express KIT protein and are usually identified by microscope via KIT-immunohistochemistry. We have pursued ICCs as KIT-positive fibroblast-like cells in the muscular layer to eliminate mast cells. Losses of KIT expression and decreases in KIT-positive ICCs are associated with motility disorders and are coincidentally found in human gastrointestinal diseases including diabetic...
ICC injury by NO

In surgery, a loss of intestinal motility has been associated with acute disruption of ICC networks and a decrease in slow waves and phasic contractions (32). This postoperative gastrointestinal dysmotility has been suppressed by inhibiting iNOS or COX-2 activities (11, 31). These data suggest a role of iNOS induced by LPS or by surgical insult. In this study, we have shown that LPS injection not only increased a number of infiltrating leukocytes but also induced iNOS expression in macrophages in the smooth muscle layers. iNOS inhibitors, such as L-NAME and AG, restored intestinal motility and the number of KIT-positive fibroblast-like cells, but a specific nNOS inhibitor, 7NI, did not. Inactivation of resident macrophages by Gd pre-treatment significantly prevented the activation of iNOS in macrophages, restored KIT-positive cells, and sustained spontaneous intestinal contractility. Furthermore, a NO donor, FK409, inhibited spontaneous contractions of prepared intestinal segments. Importantly, in vivo administration of the drug not only decreased the number of KIT-immunostaining cells but also reduced intestinal contractility as observed by LPS administration. When NO concentrations rise, the gas may affect cell compo-

gastroparesis, slow transit constipation, chronic intestinal pseudo-obstruction, and others (7, 20, 25). In these experimental conditions, we have found that contractile changes are always accompanied by comparable changes in the KIT-positive cells in the intermuscular layer, and that the fibroblast-like cells were still found in the intermuscular layer even after LPS treatment and recovered KIT-positivity within 48 h. These data indicate that KIT-positive fibroblast-like cells may be transiently injured by LPS, as represented by transient KIT disappearance, which may result in a decrease in spontaneous intestinal movement.

Although several candidates, such as cannabinoid, 5HT4 receptor, TNF-alpha, IL-1, prostaglandins, iNOS and NO, have been reported, neither consensus nor conclusion has been obtained for the major causative factors of sepsis-induced ileus (3, 4, 18, 30). Gastrointestinal dysmotility under sepsis is accompanied by leukocyte infiltration in the gastrointestinal wall, which consists mainly of activated macrophages and neutrophils in the tunica muscularis and around the myenteric plexus, as observed in this study. Elevated levels of iNOS expression accompany the activation of macrophages. Intestinal dysmotility due to LPS has been restored by the addition of iNOS inhibitors or by the silencing of iNOS activity (4). In surgery, a loss of intestinal motility has been associated with acute disruption of ICC networks and a decrease in slow waves and phasic contractions (32). This postoperative gastrointestinal dysmotility has been suppressed by inhibiting iNOS or COX-2 activities (11, 31). These data suggest a role of iNOS induced by LPS or by surgical insult. In this study, we have shown that LPS injection not only increased a number of infiltrating leukocytes but also induced iNOS expression in macrophages in the smooth muscle layers. iNOS inhibitors, such as l-NAME and AG, restored intestinal motility and the number of KIT-positive fibroblast-like cells, but a specific nNOS inhibitor, 7NI, did not. Inactivation of resident macrophages by Gd pre-treatment significantly prevented the activation of iNOS in macrophages, restored KIT-positive cells, and sustained spontaneous intestinal contractility. Furthermore, a NO donor, FK409, inhibited spontaneous contractions of prepared intestinal segments. Importantly, in vivo administration of the drug not only decreased the number of KIT-immunostaining cells but also reduced intestinal contractility as observed by LPS administration. When NO concentrations rise, the gas may affect cell compo-

**Fig. 8** Effects of FK409 on intestinal contractility and KIT-positive fibroblast-like cells. (A) Serosal electromyography using a suction electrode (upper lane) and spontaneous contractions of longitudinal muscles (lower lane) are shown. At the indicated time (arrow), FK409 (50 mg/L) was added to the Tylox buffer. (B) KIT-positive cells in the intermuscular layer were counted 12 h after the intraperitoneal injection of saline (sham), LPS (10 mg/kg), or FK409 (50 mg/kg). Vertical bars represent the means ± SD (n = 3). (C) Spontaneous contractile force of longitudinal muscles at 12 h after saline (sham), LPS (10 mg/kg), or FK409 (50 mg/kg) injection. Vertical bars represent the means ± SD (n = 3).
ments by modifying ion channels, transporters, and other enzymes (22). These results may indicate that NO produced by iNOS in activated intestinal macrophages may be a major player in the intestinal contractile dysfunction that is caused by LPS.

The limitations of this paper may include an experimental model that used a one-shot injection of LPS, which may not reflect human septic conditions in clinical practice. LPS administration is most commonly used to elucidate the mechanisms of septic conditions in animal experiments. A one-shot injection is a simple model to investigate the effects of LPS in vivo, although such conditions may not be present in clinical medicine. Another limitation may be the use of an intestinal preparation to measure contractility. This may not reflect in vivo changes in gastrointestinal motor activities. Significant correlation has been reported between motility changes in intestinal preparations and gastrointestinal transit as well as pathophysiological changes (8, 24). Our results show no direct evidence for the causal association of either a decrease in KIT-immunoreactivity or a decrease in intestinal contractility. Previous investigations have shown that a decrease in KIT-immunoreactivity is involved in gastrointestinal motility disorders in humans (7, 20, 25), suggesting that a reduction in KIT-immunoreactivity may be one of the causative biomarkers for intestinal dysmotility.

In summary, using intestinal preparations isolated from LPS-treated mice, we investigated the mechanism of intestinal dysmotility by LPS. We have found that the administration of a one-shot injection of LPS caused a dose- and time-dependent decrease in the number of KIT-immunoreactive cells and a concomitant suppression of spontaneous intestinal contractions, which were restored either by iNOS inhibitors or by the inactivation of resident macrophages by Gd. FK409, a NO donor, which caused similar changes in intestinal motility and morphology. These results suggested that NO produced from activated intestinal macrophages may play a major role in LPS-induced pacemaker dysfunction and

Fig. 9 Effects of gadolinium pre-treatment on KIT-positive fibroblast-like cells and spontaneous contractions. Gadolinium (Gd: 7 mg/kg) was intraperitoneally injected at 48 and 24 h before LPS administration. At 12 h following LPS (10 mg/kg) or saline injection, the intestine was prepared. (A) The number of KIT-positive fibroblast-like cells. Gadolinium partly restored the number of KIT-positive cells under LPS treatment. Vertical bars represent the means ± SD (n=3). (B) Spontaneous contractile force. Gadolinium, which had no effect on spontaneous contractions, restored contractile force under LPS treatment. Vertical bars represent the means ± SD (n=3). (C) A whole segment was mounted serosal side up and was examined using a laser scanning confocal microscope focusing on the intermuscular layer. The right side of each photograph indicates the proximal end of the intestinal segments, and the left side the distal end. Immunohistochemistry for F4/80 (red) and iNOS (green). The intestine pretreated by gadolinium showed an increased number of enlarged macrophages following LPS administration (b) compared with sham administration (a), whereas those macrophages had little immunoreactivity for iNOS. Bars indicate 20 μm.
subsequent intestinal dysmotility.

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