Intestinal permeability is reduced and IL-10 levels are increased in septic IL-6 knockout mice

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Wang, Quan, Cheng Hui Fang, and Per-Olof Hasselgren. Intestinal permeability is reduced and IL-10 levels are increased in septic IL-6 knockout mice. Am J Physiol Regul Integrative Comp Physiol 281: R1013–R1023, 2001.—Sepsis is associated with increased intestinal permeability, but mediators and mechanisms are not fully understood. We examined the role of interleukin (IL)-6 and IL-10 in sepsis-induced increase in intestinal permeability. Intestinal permeability was measured in IL-6 knockout (IL-6−/−) and wild-type (IL-6+/+) mice 16 h after induction of sepsis by cecal ligation and puncture or sham operation. In other experiments, mice or intestinal segments incubated in Ussing chambers were treated with IL-6 or IL-10. Intestinal permeability was assessed by determining the transmucosal transport of the 4.4-kDa marker fluorescein isothiocyanate conjugated dextran and the 40-kDa horseradish peroxidase. Intestinal permeability for both markers was increased in septic IL-6+/+ mice but not in septic IL-6−/− mice. Treatment of nonseptic mice or of intestinal segments in Ussing chambers with IL-6 did not influence intestinal permeability. Plasma IL-10 levels were increased in septic IL-6−/− mice, and treatment of septic mice with IL-10 resulted in reduced intestinal permeability. Increased intestinal permeability during sepsis may be regulated by an interaction between IL-6 and IL-10. Treatment with IL-10 may prevent the increase in mucosal permeability during sepsis.

potential mediators setting these mechanisms in motion include nitric oxide (42), platelet-activating factor (40), and proinflammatory cytokines, such as interferon-γ and tumor necrosis factor (TNF; 1, 40, 42). An additional cytokine that has been proposed to cause an increase in intestinal permeability is interleukin (IL)-6 (18, 49). The role of IL-6 in the development of mucosal injury, however, is less clear than that for other cytokines. In a recent study, increased intestinal permeability in a model of hemorrhagic shock was associated with upregulated local and systemic IL-6, but no direct evidence was provided that IL-6 caused mucosal dysfunction (49). In other studies as well, an association between IL-6 levels and intestinal permeability was found without establishment of a cause-effect relationship (18). Results from some experiments even suggest that IL-6 may protect the intestinal mucosa from the consequences of systemic inflammation (32). Thus additional studies are needed to test the role of IL-6 in the regulation of intestinal permeability. This is particularly important because recent studies suggest that IL-6 is produced locally in the gut mucosa during sepsis and endotoxemia (26) and that the enterocyte is a significant source of IL-6 in these conditions (25, 29, 48).

In the present study, we tested the role of IL-6 in sepsis-induced mucosal dysfunction by determining intestinal permeability in IL-6 knockout and corresponding wild-type mice. Because IL-6 deficiency may result in altered levels of other cytokines, in particular IL-10, which has been proposed to exert protective effects during sepsis and endotoxemia (21, 36, 38, 52), the role of IL-10 in mediating changes in intestinal permeability was examined as well.

MATERIALS AND METHODS

Experimental animals. Male IL-6 gene knockout mice (IL-6−/−; B6/129S-IL6, stock #002254) and their wild-type counterparts (IL-6+/+; B6/129S F2/J, stock #101045) were purchased from Jackson Laboratories (Bar Harbor, ME). Male IL-10 gene knockout mice (IL-10−/−; B6/129 P2-IL10, stock #002251) and their wild-type counterparts (IL-10+/+; C57BL/6J, stock #000664) were purchased from the same

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IN ADDITION TO ITS PRIMARY functions of digestion and absorption of nutrients, the intestinal mucosa serves as an important barrier to prevent the absorption of toxins, antigens, and microorganisms across the intestinal wall. There is evidence that this barrier function of the intestinal mucosa is impaired in patients and experimental animals with multiple trauma (33), burn injury (7, 8), hemorrhagic shock (10, 49), and sepsis (47), and it has been proposed that the increased mucosal permeability may contribute to the development of multiple organ failure and mortality in these conditions (9).

Multiple factors may contribute to loss of mucosal integrity in shock, trauma, and sepsis, including oxygen radicals, intracellular hypoxia and acidosis, nitric oxide, and malnutrition (reviewed in Ref. 14). The
source. The mice (body wt 20–30 g) were housed in a room with an ambient temperature of 22°C and a 12:12-h light-dark cycle. The mice were allowed to acclimatize in the laboratory for 1 wk before experiments. The experiments were conducted and the animals were cared for in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals. The experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

Induction of sepsis. Sepsis was induced in IL-6 knockout and wild-type mice by cecal ligation and puncture (CLP) as described previously (26). In short, mice were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg) administered intramuscularly. A midline abdominal incision was performed, and the cecum was ligated with a 3–0 silk just below the ileocecal valve and was punctured twice with an 18-gauge needle. Other mice were sham operated, i.e., they underwent laparotomy and manipulation but no ligation or puncture of the cecum. All mice were resuscitated with sterile saline (40 ml/kg) administered subcutaneously on the back at the time of surgery. Mice had free access to water, but food was withheld after the surgical procedures to avoid any influence of differences in food intake between the groups on intestinal permeability. Mucosal permeability was measured 4 and 16 h after CLP or sham operation. These time points were based on previous studies in which circulating levels of IL-6 were increased at 4 h and remained elevated up to 16 h after CLP in mice (26).

Bacterial cultures. Culture of peritoneal fluid was performed in IL-6+/+ and IL-6−/− mice 16 h after sham operation or CLP. The abdominal area was cleaned with betadine, after which 1 ml of sterile saline was injected intraperitoneally. The abdomen was massaged for 20 s, after which the abdomen was opened and peritoneal fluid was aspirated in a sterile syringe. The fluid was serially diluted and aliquots were cultured on trypticase soy agar (TSA) plates under aerobic conditions for 24 h or on TSA agar plates with 5% blood under anaerobic conditions for 48 h. After culture, the number of colonies was counted and results were expressed as colony forming units (CFU) per milliliter of peritoneal fluid. Bacterial species were identified by cross morphological appearance and standard microbiological techniques.

Treatment of mice with IL-6 or IL-10. To further test the role of IL-6, mice were treated with three doses of 100 μg/kg of human recombinant IL-6 (Endogen, Woburn, MA) or corresponding volumes (0.5 ml/mouse) of sterile saline administered intraperitoneally over 16 h with 8-h intervals. Measurements of intestinal permeability were started 1 h after the last IL-6 injection. The mice had free access to water, but food was withheld after the first injection to avoid any influence of reduced food intake on intestinal permeability in IL-6-injected mice. The protocol for IL-6 treatment used here was based on a previous report in which treatment of mice with the same amounts of IL-6 resulted in circulating IL-6 levels similar to those seen in septic mice (46).

The effect of IL-10 on sepsis-induced intestinal permeability was examined by injecting 400 μg/kg of mouse recombinant IL-10 (R&D Systems, Minneapolis, MN) or corresponding volumes of sterile phosphate-buffered saline (pH 7.4) intraperitoneally 30 min before CLP. The dose of IL-10 used here was based on a previous report in which treatment of mice with 400 μg/kg of recombinant IL-10 protected the animals from lethal endotoxemia (17).

Intestinal permeability in vivo. Intestinal permeability was determined by measuring the appearance in blood of two markers administered by gavage as described previously (47). Mice were anesthetized by inhalation of Metophen (Schering-Plough Animal Health, Union, NJ), after which 20 ml/kg body wt of phosphate-buffered saline (pH 7.4) containing 22 mg/ml fluorescein isothiocyanate conjugated dextran (FITC-dextran, molecular mass 4.4 kDa; Sigma Chemical, St. Louis, MO) and 1 mg/ml horseradish peroxidase (HRP; molecular mass 40 kDa; Sigma Chemical) were administered by gavage. A blood sample (∼150 μl) was obtained in a capillary tube 2 h after administration of the markers by orbital retrobulbar puncture. A second blood sample was obtained by cardiac puncture 5 h after administration of the markers and just before death. The amounts of markers administered and the time points for measurement of their concentrations in blood were based on studies reported previously (5, 7, 41, 47, 50).

The blood samples were centrifuged (3,000 rpm at 4°C) for 20 min. Plasma (50 μl) was mixed with an equal volume of phosphate-buffered saline (pH 7.4) and added to a 96-well microplate (Maxisorp, Nalge Nucleon, Denmark) that had been precoated with goat anti-HRP antibody (1:5,000 dilution; Sigma Chemical). The concentration of fluorescein was determined by spectrophotofluorometry (CytoFlu 2300, Millipore, Bedford, MA) with an excitation wavelength of 485 nm (20 nm band width) and an emission wavelength of 530 nm (25 nm band width) using serially diluted samples of the marker as standard. The plate was then incubated for 2 h, after which the wells were washed three times with Tris-buffered saline (pH 7.6) and 0.2% Tween 20. HRP activity was determined by adding 100 μl of reagent (TMB One-Step Substrate System, S1600, Dako, Carpenteria, CA) for 15 min and stopping the reaction with 100 μl 2N H2SO4. The concentration of HRP was determined by spectrophotometry at 550–450 nm.

Intestinal permeability in vitro. In vitro permeability was measured in segments from the distal ileum mounted in Ussing chambers. Segments of intestine (2–3 cm) were opened along the mesenteric border, rinsed with Krebs-Ringer bicarbonate buffer (pH 7.4), and pinned as intact sheets between siliconized Ussing half-chambers (0.5 cm2 exposed surface area). The Ussing chambers were equipped with two calomel voltage-sensitive electrodes and two Ag-AgCl current passing electrodes (Easy Mount Diffusion Chamber System, Physiologic Instruments, San Diego, CA). Both the mucosal and serosal reservoirs of the chamber contained 5 ml oxygenated Krebs-Ringer bicarbonate buffer (pH 7.4). The buffer was oxygenated (95% O2–5% CO2) continuously during the experiment and kept at 37°C by a water-jacketed system.

The electrical potential difference between the mucosal and serosal surface was determined by using the calomel electrodes, and electrical resistance was determined by changing voltage across the mounted intestinal wall and measuring the change in short-circuit current. The electrical resistance was calculated by Ohm’s law. The potential difference is an indicator of tissue viability, and the resistance is considered to reflect tissue integrity (16).

After the preparations had stabilized for 20 min and baseline potential difference and resistance had been established, FITC-dextran (2.2 mg/ml) and HRP (0.1 mg/ml) were added to the mucosal reservoir. Samples (0.22 ml) were taken (and replaced with identical volumes of fresh medium) at 20-min intervals during 180 min from the serosal reservoir for measurement of FITC-dextran and HRP as described above. In some experiments, human recombinant IL-6 (100 ng/ml) was added to the serosal reservoir at the start of the 180-min experimental period, and in other experiments, mouse recombinant IL-10 (25 ng/ml) was added to the serosal reser-
voir. The potential difference and electrical resistance were stable through the 180-min incubation when tissue from both septic and nonseptic IL-6+/+ and IL-6−/− mice was incubated (data not shown).

Intestinal transit. Because intestinal permeability in vivo can be influenced by the length of time during which the mucosa is exposed to the markers, it was important to determine intestinal transit under the different experimental conditions. This was done by measuring the appearance of a 70-kDa FITC-dextran marker in consecutive segments of the small intestine 30 min after gavage with 0.1 ml 5 mM FITC-dextran (34). Mice were anesthetized as described above, the abdomen was opened, and the small intestine was excised from the pylorus to the cecum. The intestine was divided into eight segments of equal length, and each segment was flushed with 3 ml 0.05 M Tris-buffered saline solution (pH 10.3). The samples were centrifuged at 1,200 rpm for 5 min, and the amount of FITC-dextran in each segment was measured as described above and expressed as percentage of total amount of FITC-dextran administered.

Plasma and tissue cytokine levels. Plasma TNF-α, IL-1β, IL-6, and IL-10 levels were determined with commercially available ELISA kits for mouse TNF-α, IL-1β, IL-6 (MiniKit, Endogen, Woburn, MA), and IL-10 (Mouse IL-10 DuoSet, Genzyme Diagnostics, Cambridge, MA). Cytokine levels in mucosa from ileum were measured after preparation of mucosal samples as described in detail previously (26).

Statistics. Results are presented as means ± SE. Analysis of variance followed by Student-Newman-Keuls test or Student’s t-test was used for statistical analysis. Survival data were analyzed by χ² test.

RESULTS

In initial experiments, plasma IL-6 was measured 16 h after sham operation or CLP in IL-6 knockout (IL-6−/−) and wild-type (IL-6+/+) mice. Plasma IL-6 levels were below the level of detection in all groups of mice, except the septic wild-type mice in which plasma IL-6 concentration was 8.8 ± 0.7 ng/ml. IL-6 concentrations in ileal mucosa of IL-6+/+ mice were increased from 3.2 ± 0.5 to 7.8 ± 0.7 ng/g 16 h after CLP, in line with previous results from this laboratory (26). There was no increase in mucosal IL-6 levels detectable in septic IL-6−/− mice. The nondetectable IL-6 levels noted here in plasma from IL-6+/+ mice 16 h after sham operation are in line with a previous study from our laboratory (26). In that study, plasma levels of IL-6 were undetectable 8 and 16 h after sham operation and were present at very low concentrations 4 h after sham operation. In other studies, plasma IL-6 levels were detectable after sham operation in mice at different time points (3, 12, 44).

Septic mice showed signs of illness in the form of piloerection, exudate around the eyes and nostrils, and moderate diarrhea. There was no significant difference in mortality rate between IL-6−/− and IL-6+/+ mice in early sepsis (up to 24 h after CLP). At later time points (up to 7 days), however, mortality rate was higher among septic IL-6−/− than IL-6+/+ mice (Fig. 1). No sham-operated mice died.

The number of bacteria in peritoneal fluid 16 h after CLP was 10⁶–10⁷ CFU/ml with no difference between IL-6+/+ and IL-6−/− mice. In peritoneal fluid from sham-operated mice, 10–10² CFU/ml were found, consistent with contamination. The most predominant bacteria in peritoneal fluid of septic mice were Escherichia coli, streptococcus, staphylococcus, enterococcus, and lactobacillus with no apparent difference between IL-6+/+ and IL-6−/− mice. Intestinal permeability for both FITC-dextran and HRP was increased 16 h after CLP in IL-6+/+ mice as indicated by higher plasma concentrations 2 and 5 h after administration of the markers (Fig. 2). In sharp contrast, sepsis did not result in increased intestinal permeability for any of the markers in the IL-6−/− mice, suggesting that the effect of sepsis on mucosal integrity may, at least in part, be mediated by IL-6.

Because the transmucosal uptake of the markers used here could be influenced by the time during which the mucosa is exposed to the markers, it was important to determine intestinal transit time under the different experimental conditions. On the basis of the appearance of the 70-kDa FITC-dextran marker in consecutive segments of the small intestine after administration of the marker by gavage, intestinal transit was slower in septic than in sham-operated mice (Fig. 3). Because the same difference in transit time between sham-operated and septic mice was seen in both IL-6+/+ and IL-6−/− mice, it is not likely that the delayed intestinal transit in septic mice was the cause of increased permeability in septic wild-type mice. This interpretation was further supported in in vitro experiments using Ussing chambers and in which permeability was higher in segments of distal ileum from septic than from sham-operated IL-6+/+ mice (Fig. 4). Thus the differences in intestinal permeability seen between the four groups of mice in the initial experiments (see Fig. 2) probably did not reflect differences in intestinal transit time.
Although the results described above suggest that increased intestinal permeability during sepsis may be mediated by IL-6 and that this effect of IL-6 does not primarily reflect changes in intestinal transit time, it is not known if changes in permeability reflect a direct effect of IL-6. To further test the potential role of IL-6 in the regulation of mucosal permeability, we next treated nonseptic, unoperated IL-6+/+ and IL-6−/− mice with recombinant IL-6. Administration of three doses of 100 μg/kg intraperitoneally of the cytokine did not result in significant increase in intestinal permeability for any of the markers (Fig. 5). (Although the ~50% increase in permeability for FITC-dextran in IL-6-treated IL-6−/− mice was not statistically significant, the data suggest that IL-6 may have certain effects on gut permeability in this group of mice.) The protocol for IL-6 treatment used here was identical to that used in a recent study in which we found that plasma levels of IL-6 after this treatment were similar to those seen in septic mice (46).

In additional experiments, segments of ileum were incubated in Ussing chambers in the absence or presence of 100 ng/ml of IL-6. Also in these experiments, IL-6 did not influence intestinal permeability (Fig. 6).

Fig. 2. Plasma levels of 4.4-kDa FITC-dextran (A) and 40-kDa horseradish peroxidase (HRP; B) 2 and 5 h after administration of the markers by gavage in sham-operated and septic IL-6+/+ and IL-6−/− mice; n = 6 or 7 for each data point. *P < 0.05 vs. sham and vs. corresponding CLP group in IL-6−/− mice.

Fig. 3. Intestinal transit of a 70-kDa FITC-dextran marker administered by gavage in sham-operated and septic IL-6+/+(A) and IL-6−/− mice (B). The amount of FITC-dextran was measured in the stomach (G) and in 8 consecutive equal-length segments (S1–S8) of the small bowel from the pylorus to the ileocecal junction 30 min after administration of the marker. Results are means ± SE from 5 or 6 mice in each group.
in both IL-1β and TNF-α was substantially augmented in IL-6 \(-/-\) mice (Fig. 8). Because proinflammatory cytokines have been implicated in mucosal injury during sepsis (14), it is not likely that the reduced intestinal permeability noticed in IL-6 \(-/-\) mice was related to the high levels of IL-1β and TNF-α. Increased IL-1β and TNF-α levels, however, may in part explain the increased mortality in septic IL-6 \(-/-\) mice.

In contrast, IL-10 is an important anti-inflammatory cytokine that has been shown to be protective in various organs and tissues during inflammation (21, 36, 52) and that can improve animal survival during sepsis and endotoxemia (38). Changes in IL-10 levels in IL-6 \(-/-\) mice and the effect of IL-10 on intestinal permeability during sepsis are not known. We therefore next measured plasma IL-10 levels in sham-operated and septic mice. As can be seen in Fig. 9A, IL-10 levels were increased in septic mice and this increase was ~20 times greater in septic IL-6 \(-/-\) than in IL-6 +/- mice. Mucosal IL-10 levels were approximately eight times higher in IL-6 \(-/-\) than in IL-6 +/- mice but were not further increased by sepsis (Fig. 9B).

Because these results suggest that the improved intestinal permeability noticed in septic IL-6 \(-/-\) may be related to high IL-10 levels (rather than or in addition to reduced IL-6 levels), we next treated septic IL-6 +/- mice with IL-10. Administration of 400 μg/kg of IL-10 significantly reduced intestinal permeability in vivo for both FITC-dextran and HRP (Fig. 10). When segments of ileum from septic IL-6 +/- mice were treated in vitro in Ussing chambers with 25 ng/ml of IL-10, permeability for both markers was substantially reduced (Fig. 11).

To further elucidate the potential roles of IL-6 and IL-10 in sepsis-induced increase in intestinal permeability, we next measured plasma IL-10 levels in sham-operated and septic mice.
ability, additional experiments were performed in which intestinal permeability was measured at an earlier time point (4 h) after CLP or sham operation. The rationale for this experiment was results in previous studies indicating that IL-6 levels are increased earlier than IL-10 levels in sepsis (43, 44). Therefore, if increased intestinal permeability in sepsis is related to an interaction between IL-6 and IL-10 levels, it would be expected that an increase in intestinal permeability occurs early in sepsis. Indeed, results in our experiments showed that intestinal permeability was increased as early as 4 h after CLP (Fig. 12).

The potential role of IL-10 in the regulation of intestinal permeability during sepsis was further examined by comparing the effect of sepsis on intestinal permeability in IL-10 wild-type and knockout mice. The increase in FITC-dextran permeability was significantly greater in IL-10 $^-/-$ than in IL-10 +/- mice, supporting (but not proving) a role of IL-10 in protection of gut mucosa during sepsis (Fig. 13A). In contrast, the sepsis-induced increase in HRP permeability was not different in IL-10 $^-/-$ and IL-10 +/- mice (Fig. 13B). Although this result may seem contradictory to the results in which administration of IL-10 in vivo or in vitro reduced permeability for both markers (see Figs. 10 and 11), it should be pointed out that the situation in IL-10 $^-/-$ mice may be complicated by changes in other cytokines as well (as described above for IL-6 $^-/-$ mice).

**DISCUSSION**

Increased intestinal permeability during sepsis, as noted here, was reported in previous studies as well, both in experimental animals (27, 35, 47, 51) and
humans (28). Loss of mucosal integrity was observed in additional conditions characterized by systemic inflammation, including hemorrhagic shock (10, 49), trauma (33), burn injury (7, 8), and intestinal ischemia (16). These observations support the important role of the intestine in the overall response to critical illness and systemic inflammation and are consistent with the concept that impaired mucosal integrity may be a mechanism of morbidity and mortality in these conditions (9, 14). The present finding of increased mortality in IL-6 $^{-/-}$ septic mice, despite reduced intestinal permeability, may therefore seem surprising but may reflect the complex situation in septic IL-6 $^{-/-}$ mice, characterized not only by reduced gut permeability but by increased levels of the proinflammatory cytokines IL-1$\beta$ and TNF-$\alpha$ as well. The results from the bacterial cultures performed here suggest that the difference in mortality rates between septic IL-6 $^{+/+}$ and IL-6 $^{-/-}$ mice were not related to differences in severity of the bacterial infection. Higher mortality rate in septic IL-6 $^{-/-}$ than in septic IL-6 $^{+/+}$ has been reported by others as well (20).

The two markers employed in the present study to examine intestinal permeability have been used in several previous studies, both in vivo and in vitro (5, 7, 41, 50). Although the mechanisms of their transmucosal...
Sal transport are not completely understood, FITC-dextran is frequently used as a marker of paracellular transport, whereas HRP mainly crosses the intestinal epithelium transcellularly. The results in the present study suggest that sepsis is associated with an increase in both para- and transcellular permeability. The exact cellular mechanisms of increased mucosal permeability during sepsis remain to be determined. In particular, the role of the tight junctions will be important to examine as well as the relationship between increased permeability of markers and bacterial translocation.

The main purpose of the present study was to test the role of IL-6 in sepsis-induced increase in intestinal permeability. To achieve that goal, two experimental approaches were used: 1) the effect of sepsis on intestinal permeability was determined in IL-6−/− mice and 2) the effect of IL-6, administered in vivo to mice or added in vitro to intestine mounted in Ussing chambers, on mucosal permeability was examined.

The finding that intestinal permeability was increased in septic IL-6+/+ mice but not in septic IL-6−/− mice suggests that IL-6 is involved in the loss of mucosal integrity during sepsis and supports previous reports in which an association was found between high IL-6 levels and increased mucosal permeability. Results from the experiments in which mice or intestinal segments were treated with IL-6 suggest that IL-6 alone does not give rise to increased mucosal permeability and that IL-6 does not have a direct effect on intestinal mucosa. Thus the role of IL-6 in sepsis-induced intestinal permeability probably reflects an indirect effect of the cytokine. It is possible, for example, that IL-6 interacts with other cytokines or other...
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substances released during sepsis. The result of a further increase in intestinal permeability after administration of IL-6 to septic mice noted here would support that possibility.

An alternative mechanism by which high levels of IL-6 may play a role in sepsis-induced intestinal permeability could be that IL-6 suppresses the levels of cytokine(s) or substance(s) that normally exert a protective effect on the mucosa. The present results of high levels of IL-10 in septic IL-6 /– mice and improved mucosal permeability after treatment with IL-10 of mice in vivo or intestinal segments in vitro suggest that the relationship between IL-6 and IL-10 levels may be important for the development of mucosal dysfunction during sepsis. An important role for the balance between inflammatory mediators in the development of severity of and mortality in sepsis was pointed out by others as well (45).

An apparently conflicting result was the finding that IL-10 levels were increased in septic IL-6 /+ mice (albeit to a much smaller extent than in IL-6 /– mice) concomitantly with increased intestinal permeability. This observation suggests that increased intestinal permeability during sepsis does not simply reflect suppressed IL-10 levels, but it is possible that the ratio between IL-6 and IL-10 is an important factor for the maintenance of mucosal integrity. Thus during sepsis in IL-6 /+ mice, the relative increase in IL-6 levels was greater than the increase in IL-10 levels, increasing the ratio between IL-6 and IL-10. Further studies are needed to better define the mechanism(s) by which IL-6 and IL-10 influence intestinal integrity during sepsis.

Results from experiments in which IL-6 /– mice are used need to be interpreted with caution for several reasons. Concentrations of other cytokines, both pro- and anti-inflammatory, may be altered in IL-6 /– mice as exemplified by the high IL-1β, TNF-α, and IL-10 levels in the present report. Altered IL-1 and TNF levels in IL-6 /– mice have been observed in other studies as well (13, 19). In addition, results noted in IL-6 /– mice may be model dependent. For example, previous reports provided evidence that the inflammatory response to tissue injury caused by turpentine injection was severely compromised in IL-6-deficient mice, whereas the systemic inflammation caused by endotoxin was not influenced by the absence of IL-6 (13, 19).

The high levels of IL-10 in IL-6 /– mice noted here in both plasma and ileal mucosa as well as the protective effect of IL-10 in vivo and in vitro on sepsis-induced mucosal dysfunction are novel observations. The results support recent studies in which IL-10 improved barrier function in cultured intestinal epithelial cells (22, 23). The mechanism of the protective effect of IL-10 is not known from the present study. Other reports suggest that inhibited release of interferon-γ from immune cells in the mucosa (22, 23) and reduced matrix metalloproteinase activity (30) may be mechanisms by which IL-10 preserves mucosal integrity. Additional potential mechanisms include downregulated activity of “inflammatory” transcription factors, including nuclear factor (NF)-κB, activator protein-1, and NF-IL6 (11, 37).

Although several reports support the concept that IL-10 exerts a protective effect in sepsis and endotoxemia, contradictory results have been reported as well. For example, in a study by Remick et al. (31), exogenous IL-10 failed to decrease the mortality or morbidity in septic mice. In a recent study by Song et al. (39), treatment of mice with anti-IL-10 antibody improved survival in mice rendered septic by CLP. It is not known from that study if treatment with anti-IL-10 antibody influenced intestinal permeability. It is interesting to note, however, that mortality was increased in a group of mice with high IL-10 levels in the present study (although these mice had high IL-1β and TNF-α levels as well) and was reduced in septic mice treated with anti-IL-10 antibody in the study by Song et al.

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(39). It is obvious that more studies are needed to better define the role of IL-10 in the response to sepsis. It should be noted that although the present study suggests that high IL-6 levels are associated with mucosal injury and dysfunction during sepsis, results in other reports are consistent with a protective effect of IL-6 in gut mucosa (32) and other tissues as well, including liver and lung (4, 6, 24). The reason for these apparently contradictory results is not known at present, but it may be due to differences in experimental models and animal species between the present and other studies. For example, Rollwagen et al. (32) reported that IL-6 exerted a protective effect on intestinal mucosa in a hemorrhagic shock model in mice that may differ substantially from a model of septic peritonitis such as used in the present study.

In summary, the present results suggest that IL-6 plays a role in sepsis-induced increase in mucosal permeability, at least under the present experimental conditions, and that this effect of IL-6 may reflect an interaction with other cytokines released during sepsis rather than a direct effect of IL-6 on the mucosa. Results from experiments in IL-6 −/− mice suggest that an interaction between IL-6 and IL-10 may prevent the increase in mucosal permeability during sepsis. It should be emphasized that the present results do not rule out that other factors, e.g., gut hypoperfusion, may play a role in the increased gut permeability during sepsis.

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