Enteral feeding decreases gut apoptosis, permeability, and lung inflammation during murine endotoxia

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Alschcer, Kurt T., P. Terry Phang, Treena E. McDonald, and Keith R. Walley. Enteral feeding decreases gut apoptosis, permeability, and lung inflammation during murine endotoxia. Am J Physiol Gastrointest Liver Physiol 281: G569–G576, 2001.—We tested the hypothesis that endotoxia and fasting are associated with increased gut apoptotic activity, gut permeability, and inflammation in a distant organ. Fed or fasted CD-1 mice were studied 6 h after intraperitoneal injection of either saline (sham) or endotoxin (4 mg/kg of 0111:B4 Escherichia coli lipopolysaccharide). We found that endotoxin increased gut caspase-3 and -6 activity by 4.9 ± 0.6- and 4.5 ± 0.5-fold, respectively (P < 0.001), and increased terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling (TUNEL) staining of mucosal cells (P < 0.05). Feeding decreased caspase-3 activity by 40% (P < 0.05) and decreased endotoxin-induced TUNEL staining (P < 0.05). Endotoxin increased gut poly(ADP-ribose) polymerase activity by 15% (P < 0.05). Endotoxin increased gut permeability by 44% (P < 0.05), an effect reduced 36% by feeding (P < 0.05). Similarly, endotoxin increased pulmonary neutrophil infiltration (6.0 ± 1.0-fold, P < 0.001) and increased lung interleukin (IL)-6 (5.9 ± 0.1-fold, P < 0.001) and macrophage inflammatory protein (MIP)-2 expression (290 ± 40-fold, P < 0.001), whereas feeding decreased this effect by 43% for neutrophils, 40% for IL-6 (P < 0.05), and 35% for MIP-2 (P < 0.05). Thus endotoxin increased gut apoptotic activity, gut permeability, and pulmonary inflammation. Enteral feeding may decrease the distant organ inflammation by reducing gut apoptosis, thereby maintaining gut mucosal function during endotoxia.

Sepsis; endotoxin; caspase; poly(ADP-ribose) polymerase

Sepsis and the systemic inflammatory response in both animals and humans are associated with gut mucosal damage and dysfunction (8, 31). Gut dysfunction during sepsis is a common problem, resulting in loss of gut mucosal barrier selectivity, increased permeability to various hydrophilic solutes (4), and translocation of bacterial products into the circulation (7, 8), which may then further increase the inflammatory response in distant organs, resulting in multiple organ dysfunction and death (30). As a result, the gut is viewed by many as an “engine” that drives sepsis. One possible contributory mechanism to endotoxin-induced gut mucosal damage is increased apoptosis (33). Septic inflammatory mediators enhance apoptosis in a large number of cell lines (17, 22, 26). In intact animals, increased cardiac and hepatic apoptosis during sepsis may contribute to sepsis-related dysfunction of those organs (5, 19, 22). Thus it is reasonable to postulate that increased gut apoptosis may also occur during sepsis and that excessive mucosal cell death may contribute to the observed gut mucosal atrophy, damage, and impaired gut barrier function.

In experimental models of sepsis, enteral feeding has been shown to reduce mucosal atrophy and improve gut immunity (24), and clinically, enteral feeding reduces septic morbidity in postoperative and post-trauma patients (23, 28, 36). If, as we postulated above, sepsis induces gut mucosal apoptosis leading to increased gut permeability and translocation with increased inflammation in distant organs, then feeding could conceivably have beneficial effects by affecting the underlying mechanism: feeding may reduce gut apoptosis during sepsis. Indeed, if these steps, 1) increased apoptosis, 2) increased gut permeability, and 3) increased inflammation in distant organs, are causally related, then feeding, if it affects one step, must affect all steps.

Accordingly, we tested the effect of endotoxin in fed versus fasted mice using a two by two experimental design. We did not wish to study established starvation (>1–3 days in mice (12, 46)]. Instead, we chose to study a moderate degree of food restriction comparable to the moderate limitation of food intake that may frequently be found in critically ill patients. On the basis of preliminary autopsy studies that showed the fasted mouse gut lumen to be empty, we chose a 16-h fast. We first measured caspase-3 activity as an important final common mediator of the intracellular apoptotic cascade and caspase-6 activity because it is activated by caspase-3 and hence provides additional evidence of caspase-3 activity (15, 37, 41). As a further measure of the induction of apoptotic pathways, we also measured poly(ADP-ribose) polymerase (PARP) expression and activity because recent investigations have shown that PARP, an enzyme involved in DNA repair and a final...
effect or in the apoptotic pathway, may play a role in gut dysfunction during sepsis (40). We used terminal deoxynucleotidyltransferase nick-end labeling (TUNEL) staining of gut sections to determine which cells were involved. To assess gut dysfunction, we measured gut permeability to dextran molecules. Whether increased gut permeability in fasted mice can result in an increase in the inflammatory response in a distant organ is unknown. Therefore, we measured the number of neutrophils in lung sections and measured the concentration of both interleukin (IL)-6 and macrophage inflammatory protein (MIP)-2 in samples of lung using ELISA. We chose IL-6 because it is a proinflammatory cytokine directly related to mortality in human sepsis (32, 34) and chose MIP-2 because it is the murine functional analog to IL-8, a key neutrophil chemotactic cytokine in the lungs in human sepsis and acute respiratory distress syndrome (18, 27). We chose endotoxin injection, not as an accurate model of clinical sepsis, but rather to investigate the mechanism in this well-studied, reproducible model that results in increased gut permeability (3, 9, 14, 35, 42).

METHODS

Animals. The protocol was approved by the University of British Columbia Committee on Animal Care. Five-week-old male CD-1 mice obtained locally were housed in groups of five in standard cages and supplied ad libitum with laboratory chow and water until they entered the study. The environment had controlled temperature and humidity with a 12:12-h light-dark cycle. During the feeding protocol, a wire grid floor was added to the cages to eliminate coprophagia.

Experimental protocol. Four groups of 10 mice each were studied: Fed/Sham, Fasted/Sham, Fed/Endotoxin, and Fasted/Endotoxin. Animals in the fed groups received rodent chow ad libitum (Lab Diet Rodent Diet 5001, PMI International, Brentwood, MO) throughout the experiment; the fasted mice were denied food for 16 h before either saline (sham) or endotoxin injection. We chose a 16-h fast because preliminary time-course experiments showed that it took 16 h to clear the mouse gut of solid food after eating, and we wanted to study the gut in the presence and absence of food. All groups had free access to water at all times. The animals were then briefly anesthetized with 3% halothane and given a 1-ml subcutaneous injection of sterile normal saline and a 0.25-ml intraperitoneal injection of either sterile normal saline (sham) or endotoxin (4 mg/kg of 0111:B4 Escherichia coli lipopolysaccharide (LPS) dissolved in normal saline). Six hours later, the animals were again anesthetized, and the entire small bowel was rapidly harvested and irrigated with 3 ml of ice-cold normal saline to clear the intestinal contents. The gut and lungs were snap-frozen in liquid nitrogen and stored at −80°C. The animals were euthanized by exsanguination while anesthetized.

Caspase activity. Frozen whole gut tissue was homogenized for 30 s in 2 ml of ice-cold buffer containing 50 mM Tris·HCl, pH 8.0, 25 mM MgCl₂, and 0.1 mM phenylmethylsulfonyl fluoride. The iced homogenate was then sonicated, and centrifuged at 5,000 rpm for 15 min at 4°C, and the supernatant was assayed for protein concentration (BCA assay, Pierce, Rockford, IL). This supernatant was diluted to 2 mg protein/ml and assayed for caspase-3 and -6 activity with a colorimetric assay (R&D Systems, Minneapolis, MN). Gut protein samples were incubated with either the p-nitroanilide (p-NA)-conjugated caspase-3-specific peptide Asp-Glu-Val-Asp-p-NA (DEVD-p-NA) or the caspase-6-specific equivalent Val-Glu-Ile-Asp-p-NA (VEID-p-NA) as substrate. The cleavage of the peptide by caspase released the chromophore p-NA, which was quantitated spectrophotometrically at 504 nm in a microplate reader (Rainbow Reader, SLT Lab Instruments). The results were expressed as fold increase in caspase activity over the Fed/Sham control group.

PARP activity. To determine PARP activity, we used a radiolabeled NAD-based enzymatic assay (R&D Systems). Samples of gut protein (10 μl each), prepared as described in Caspase activity (2 mg/ml total protein), were incubated with 2 μl of 1 μCi/μl [32P]-labeled NAD (New England Nuclear, Boston, MA), 10 μl of 10× PARP buffer, 10 μl of 1 mM NAD, and 68 μl of distilled water. After exactly 1 min, the reaction was stopped with 900 μl of ice-cold 20% TCA, passed through a GF/C glass fiber filter prewetted with 10% TCA, and washed four times with 3 ml of cold 10% TCA and twice with cold 85% ethanol, leaving the precipitated chains of radiolabeled PARP on the filter. A scintillation counter (Beckman LS 8200, Beckman Coulter) was then used to quantify the radioactivity on the filter to determine PARP activity, expressed in nanomoles per minute per microliter. A positive control used damaged DNA and stock PARP enzyme.

PARP Western blot analysis. We performed Western blot analysis using the gut protein samples described in PARP activity. Two hundred micrograms of each gut protein sample, 5 μl of molecular weight (MW) marker, and 7.5 μl of HeLa cell extract were loaded on a 7.5% SDS-PAGE gel. After separation by gel electrophoresis at 200 V for 55 min, the protein was then transferred to a nitrocellulose membrane, washed, and blocked. The primary PARP antibody consisted of 2 μg/ml of rabbit anti-mouse polyclonal IgG (Upstate Biotechnology, Lake Placid, NY). This antibody was specific for both the intact (116 kDa) and cleaved (85 kDa) forms of PARP. After a horseradish peroxidase-conjugated secondary goat anti-rabbit antibody (Upstate Biotechnology) was used, the blots were imaged (ECL, Hyperfilm, Amersham) and densitometry was performed (Eagle Eye II, Stratagene, La Jolla, CA).

TUNEL staining. TUNEL staining was carried out with the TdT-FragEL DNA fragmentation detection kit (Onco-gene, Cambridge, MA). Positive and negative controls consisted of gut samples either treated with 0.1 mg/ml of DNase or incubated without TdT, respectively. TUNEL staining was performed according to the manufacturer’s instructions, with the following exception; the permeabilization step was carried out with 40 μg/ml of proteinase K for 30 min at 37°C. A single blinded observer determined the number of apoptotic cells per high-power field and whether the cells were mucosal epithelial or other cells.

Measurement of intestinal mucosal macromolecular permeability. In separate but identical experiments, we measured the passage of macromolecules from the lumen of the small intestine to the systemic circulation as previously described (13). Four groups of mice were studied: Fed/Sham (n = 10), Fasted/Sham (n = 10), Fed/Endotoxin (n = 10), and Fasted/Endotoxin (n = 9). Following the feeding and injection protocol described in Experimental protocol, the mice were anesthetized with inhaled halothane 5 h after sham or endotoxin injection. Via a midline laparotomy, a 21-gauge plastic catheter was secured in place with a 5-0 silk opening of the catheter. Intestinal contents were gently expressed manually from this gut segment to clear the gut lumen. Absence of lumen contents in this gut segment was
confirmed at autopsy. To create a closed segment of small bowel, a 5-0 silk suture was used to tie off the terminal ileum. Both renal pedicles were ligated with 5-0 silk to prevent urinary excretion of the fluorescent probe. Once the surgical procedure was completed, 0.6 ml of freshly mixed 25 mg/ml fluorescein-labeled dextran MW 4,000 (FD-4; Sigma, Oakville, ON, Canada) in normal saline was slowly injected into the gut lumen, avoiding any distension of the gut wall. The entire procedure took 15 min to complete, during which the animal remained anesthetized. To keep the animal warm and protect the dye from light, the intestine was covered with a blanket of aluminum foil. After 45 min (6 h after sham or endotoxin injection), 0.5 ml of blood was collected by cardiac stab, anticoagulated with citrate dextrose, and centrifuged at 5,000 rpm for 10 min, and the supernatant was diluted 1:2 in PBS (pH 7.3). The concentration of FD-4 was determined with a fluorescence spectrophotometer (LS-50; PerkinElmer, Palo Alto, CA) with the following parameters set: excitation wavelength, 492 nm; excitation slit width, 2.5 nm; emission wavelength, 515 nm; emission slit width, 10 nm; and integration time, 10 s. A standard curve relating FD-4 concentration to fluorescence intensity was generated for each day's experiment by adding known amounts of FD-4 to PBS.

**Tissue neutrophils.** Four-micrometer sections of paraffin-embedded lungs or gut were deparaffinized and rehydrated. Sections were autoclaved in citrate buffer for 20 min to allow for antigen retrieval. Cooled sections were stained for neutrophils as previously described (44) with rat anti-mouse neutrophil antibody (Serotec) diluted 1:500 in Tris-buffered saline plus 1% BSA (fraction V; Sigma). Biotinylated rabbit anti-rat IgG (DAKO) diluted 1:300 was selected as the secondary antibody. The number of neutrophils per ×40 field was determined in five random fields/mouse.

**Measurement of lung cytokines.** We used ELISA to measure the concentration of both IL-6 and MIP-2 in lung tissue. Lung tissue stored at −80°C was homogenized for 30 s (Tissue-Tearor, BioSpec, Bartlesville, OK) in 1 ml of ice-cold 1X PBS, pH 7.4. The samples were then centrifuged at 1,500 rpm for 10 min at 4°C, and the supernatant was assayed for protein concentration (BCA assay, Pierce). All samples were then made up to a uniform protein concentration in PBS. The ELISA antibodies for IL-6 were chosen on the basis of their ability to be paired (IL-6: MP5-20F3 and MP5-32C11, PharMingen, San Diego, CA). MIP-2 was measured with a mouse MIP-2 Quantikine M immunoassay kit (R&D Systems).

**Statistics.** All results are expressed as means ± SE. We used a two-factor (±feeding and ±endotoxin) analysis of variance to determine whether these factors had a statistically significant effect, choosing $P < 0.05$ as significant. When a significant difference was found, we used an unpaired Student's $t$-test that was corrected for multiple comparisons using a sequentially rejective Bonferroni procedure to identify specific differences.

**RESULTS**

**Gut apoptosis.** Six hours after endotoxin injection, caspase-3 activity increased $4.9 \pm 0.6$-fold ($P < 0.001$, endotoxin groups compared with sham groups; Fig. 1) and caspase-6 activity increased $4.5 \pm 0.5$-fold ($P < 0.001$; Fig. 1) relative to sham groups, indicating that endotoxin activates apoptotic pathways in the gut. Enteral feeding decreased caspase-3 activity by $41\%$ in the endotoxin groups ($P < 0.05$; Fig. 1). In sham groups not treated with endotoxin, feeding did not alter caspase-3 activity. Similarly, feeding decreased gut caspase-6 activity in both sham and endotoxin groups, although these differences were not statistically significant. TUNEL staining also demonstrated an endotoxin-induced increase in total apoptotic cells in the fasted group that was inhibited by feeding ($P < 0.05$; Fig. 1).
In particular, apoptosis of mucosal epithelial cells was markedly inhibited by feeding (\( P < 0.05 \); Figs. 1 and 2). However, cells in all layers of the gut wall contributed to TUNEL staining, including infiltrating leukocytes, although there was no difference in the number of neutrophils infiltrating the gut wall among the four groups (1.0 ± 0.2 neutrophils/×40 field). Thus enteral feeding ameliorates endotoxin-induced apoptosis.

### Gut PARP expression and activity

PARP is cleaved and inactivated by CPP32 caspases, which include caspase-3 and -6. However, PARP activity increases in response to DNA strand breaks. DNA strand breaks increase with caspase-3 and subsequent endonuclease activity (45) and are believed to increase in enterocytes during inflammatory injury (21). Therefore, we measured both PARP protein expression and PARP activity in gut tissue to resolve the net effect of increased caspase activity on the competing potential outcomes of decreased PARP expression and increased PARP activity. Gut PARP protein expression at 6 h mirrored the activity of caspase-3 and -6, the proteins that cleave PARP; that is, endotoxin decreased PARP expression by 60% (Fig. 3). Feeding resulted in greater PARP expression in both sham and endotoxin groups, but this difference was not statistically significant. We did not observe the 85-kDa bands characteristic of cleaved PARP at 6 h, but 85-kDa bands were detectable at 12 h (Fig. 3). In parallel with the increase in PARP expression, feeding also significantly increased PARP activity at 6 h (\( P < 0.05 \); Fig. 4). In contrast, endotoxin, which decreased PARP expression, resulted in an 18% increase in gut PARP activity (\( P < 0.05 \); Fig. 4). Thus although PARP expression is reduced by endotoxin administration, possibly due to the observed increased CPP32 caspase activity that cleaves PARP, endotoxin administration increases PARP activity similar to other models of sepsis (40).

### Gut mucosal macromolecular permeability

Whether the above evidence of increased gut apoptosis is related to impaired gut barrier function is unknown. Whether reduction in endotoxin-induced apoptosis by feeding then reduces gut permeability during endotoxemia is also unknown. Therefore, we measured gut macromolecular permeability by quantifying the transfer of fluorescein-labeled dextran MW 4,000 (FD-4) from the gut lumen into the systemic circulation. Gut permeability increased by 44% in the endotoxin groups (\( P < 0.01 \); Fig. 5). Furthermore, fasted mice had 38% higher gut permeability than fed mice (\( P < 0.01 \)) in both sham and endotoxin groups (Fig. 5). These results indicate
that even an overnight fast may be enough intervention to significantly affect gut permeability and, furthermore, that fasting adds to endotoxin-induced increases in gut macromolecular permeability.

**Lung inflammatory response to fasting and endotoxemia.** Whether increased gut permeability in fasted mice can result in an increase in the inflammatory response in a distant organ is unknown. Therefore, we measured the concentration of both IL-6 and MIP-2 in samples of lung protein extract from each group using ELISA and measured neutrophils in lung sections. Endotoxin-treated mice had a 5.9 \pm 0.1-fold increase in lung IL-6 concentration (\( P < 0.001 \); Fig. 6), consistent with the expected systemic inflammatory response (43). Enteral feeding decreased the lung IL-6 concentration by 40% in the endotoxemia groups (\( P < 0.05 \); Fig. 6). There was no difference in lung IL-6 between the fed and fasted mice that did not receive endotoxin. A similar pattern was seen with lung MIP-2 concentrations (Fig. 6). Endotoxin-treated mice had a 290 \pm 40-fold increase in lung MIP-2 concentration (\( P < 0.001 \)). As with lung IL-6, we found that lung MIP-2 concentration within the endotoxemic group was 35% less in fed mice than in fasted mice (\( P < 0.05 \)). There was no difference in lung MIP-2 between fed and fasted mice within the sham groups. Similar results were found when these whole lung ELISAs were not corrected for total sample protein. Endotoxin and feeding altered pulmonary neutrophil infiltration (\( P < 0.05 \)) in parallel with the changes observed in proinflammatory cytokine expression (Fig. 6). These results indicate that enteral feeding reduces pulmonary inflammation during endotoxemia but has no effect on pulmonary cytokine expression or neutrophils under normal circumstances.

**DISCUSSION**

The novel findings in this study are that an increase in gut apoptotic activity occurs after endotoxin infusion and is associated with increased gut macromolecular permeability and increased pulmonary cytokine expression. In this setting, the intervention of feeding decreases the extent of apoptotic activity, gut permeability, and pulmonary cytokine expression.

In other experimental settings, increased gut apoptosis results in increased gut permeability (38, 39). Further investigations suggest that increased gut permeability increases the systemic inflammatory response and involvement of distant organs (30). Together, our new results and previous observations suggest an important association between gut apoptosis, gut barrier function, and pulmonary inflammation during sepsis. Enteral feeding ameliorates increased gut apoptotic activity, which, we postulate, may be a key step in improving gut barrier function and decreasing inflammation in distant organs during sepsis.

Gut injury in sepsis and in other models of gut injury results in loss of gut barrier function to bacteria and bacterial products such as endotoxin (7, 8, 14, 20, 31). The cause and mechanism of gut dysfunction in sepsis are unclear. Gut mucosal injury seen in septic rodents is not associated with concurrent mucosal perfusion.
deficits, suggesting that ischemia may not play a prominent role in normotensive septic gut dysfunction (29). However, gut mucosal atrophy occurs during sepsis (33). We suggest that one possible mechanism for sepsis-related gut dysfunction is increased apoptosis.

Recent studies have shown that increased cardiac and hepatic apoptosis during sepsis may contribute to the sepsis-related dysfunction of those organs (19, 22). Apoptosis plays an important role in gut mucosal homeostasis under normal physiological conditions. After differentiating from stem cells located at the base of intestinal crypts, enterocytes migrate toward the villus tip, where they undergo apoptosis, detach, and are shed to complete their 3- to 5-day life cycle (16). In inflammatory states, tumor necrosis factor induces caspase activation within enterocytes, causing enterocyte detachment in the lumen and resulting in villus atrophy (33). In another model, apoptosis induced by doxorubicin results in increased gut permeability that can be reduced by inhibitors of apoptosis (39). Here we extend these results to show that the endotoxin-induced inflammatory response in mice also induces increased apoptosis in the gut and, similar to previous observations (33, 39), this is associated with gut barrier dysfunction.

The DNA repair enzyme PARP is strongly activated in enterocytes by endotoxin, both in vitro and in vivo, because of DNA breaks caused by peroxynitrite and other reactive oxygen intermediates produced as part of an inflammatory response (21) and by caspase activity (1). Our results are consistent with these findings and show that gut PARP activity is increased by endotoxin administration. Activation of PARP depletes cellular energy stores and may contribute to cell and organ dysfunction, such as the increase in gut macromolecular permeability we observed (21, 40). However, caspase-3 and -6 then result in cleavage and inactivation of PARP (2). Therefore, we measured PARP expression as evidence of caspase-3 and -6 activity. The decrease in PARP expression measured by Western blot analysis that we observed during endotoxemia is consistent with the increase in gut caspase-3 and -6 activity that we observed during endotoxemia.

Our results are consistent with the hypothesis that failure of gut barrier function may play a role in the etiology of the systemic inflammatory response and the acute respiratory distress syndrome (6, 11, 25). As anticipated, endotoxin increased pulmonary IL-6, MIP-2, and neutrophil infiltration. We note that the observed increases in pulmonary proinflammatory cytokine expression may represent increased local cytokine production, increased leakage of circulating cytokines into the lung, increased activation of alveolar macrophages, or increased trafficking of inflammatory cells to the lung. Indeed, we observed an increase in pulmonary neutrophil content, so part of the increase in pulmonary proinflammatory cytokines may be related to the increased inflammatory cell infiltrate.

Feeding had a number of important effects. We found that enteral feeding partially reversed endotoxin-induced increases in CPP32 caspase activation

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**Fig. 6.** Average lung interleukin (IL)-6 (A) and macrophage inflammatory protein (MIP)-2 (B) concentrations and number of neutrophils per ×40 field (C) are shown for each of 4 experimental groups. A: we measured IL-6 because it is a proinflammatory cytokine closely related to mortality in human sepsis. Endotoxin (filled bars) greatly increased IL-6 expression (P < 0.001) as expected. Within the endotoxin-treated groups, feeding reduced lung IL-6 expression; *P < 0.05. B: we measured MIP-2 because it is the murine functional analog to IL-8, a key chemotactic cytokine in the lungs in human sepsis and acute respiratory distress syndrome. Endotoxin greatly increased MIP-2 expression (P < 0.001) as expected. Within the endotoxin-treated groups, feeding reduced lung MIP-2 expression; *P < 0.05. C: similarly, endotoxin increased the number of infiltrating neutrophils (PMNs) (P < 0.001), an effect partially reversed by feeding (*P < 0.05). Values are means ± SE.
and reduced the number of TUNEL-stained cells in gut sections. This effect of enteral feeding on gut apoptosis has not previously been elucidated. Our subsequent finding that endotoxia increases gut macromolecular permeability supports previous observations in animals as well as humans (14, 31). Our results extend these observations by demonstrating that enteral feeding significantly decreases gut macromolecular permeability during endotoxia. A further novel finding is that feeding reduced the inflammatory response in a distant organ. These results do not prove a causal link between gut apoptosis, gut permeability, and inflammation in a distant organ; that is, the effects of feeding on gut permeability and pulmonary inflammation may be due to effects unrelated to gut apoptosis. However, the concordant effect of endotoxin on apoptosis, gut permeability, and lung inflammation, and the concordant reverse effect of feeding, are consistent with an important mechanistic connection. It is interesting to note that even this mild to moderate degree of fasting has significant physiological effects.

A number of limitations with these results should be considered. First, although endotoxin infusion is a well-established tool for investigating gut barrier dysfunction, it is not a realistic model of human sepsis. CD-1 mice are relatively sensitive to endotoxin. Different effects are possible in other strains of mice or in other species. Therefore, extrapolation of these findings to human sepsis should be limited. Second, although feeding had a beneficial effect in this model, it is important to recognize that this was not a study to assess the clinical efficacy of enteral feeding. Third, the 16-h fast used here represents a mild to moderate fasting, and analogous food deprivation is commonplace in both animals and humans when surgery or other invasive procedures are planned. This degree of fasting is not as severe as the 1- to 3-day fasts used in previous experiments (10, 12, 46). Furthermore, we did not find any differences in apoptosis, gut permeability, or pulmonary inflammation between the Fed/Sham and Fasted/Sham groups, indicating that this moderate fast by itself was insufficient to cause a significant physiological effect by these measures. Finally, although we have shown that the pattern of gut permeability in each of the four groups is paralleled by a corresponding change in pulmonary IL-6, MIP-2, and neutrophils, the precise mechanism of how increased gut macromolecular permeability leads to increased pulmonary inflammation has yet to be determined.

We conclude that gut apoptotic activity, gut permeability, and pulmonary inflammation are increased during endotoxia. Enteral feeding decreases gut apoptotic activity and is associated with improved gut barrier function and decreased inflammation in a distant organ, the lungs. These data support the hypothesis that enteral feeding may decrease distant organ inflammation by reducing gut apoptosis, thereby maintaining gut mucosal function during endotoxia.

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