Differential effect of imipenem treatment on wild-type and NK cell-deficient CD8 knockout mice during acute intra-abdominal injury

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Enoh, Victor T., Chad D. Fairchild, Cheng Y. Lin, Tushar K. Varma, and Edward R. Sherwood. Differential effect of imipenem treatment on wild-type and NK cell-deficient CD8 knockout mice during acute intra-abdominal injury. Am J Physiol Regul Integr Comp Physiol 290: R685–R693, 2006. First published November 3, 2005; doi:10.1152/ajpregu.00678.2005.—CD8 knockout mice depleted of CD8-positive T and natural killer (NK) cell-deficient mice restored CLP-induced injury and mortality (16). These findings indicate that CD8-positive T and NK cells directly mediate or facilitate the lethal inflammatory response caused by CLP. Furthermore, it appears that these cell types act additively or synergistically, because depletion of either cell type alone provides only marginal resistance to CLP-induced injury (15, 16).

A complete understanding of the mechanisms by which CD8-positive T and NK cells mediate CLP-induced injury is complicated by the complexity of the CLP model. CLP, as performed in our laboratory, entails ligation of the cecum at its base (to obstruct blood flow distal to the ligature) followed by perforation of the cecal wall by puncture with a 20-gauge needle (to release cecal contents into the peritoneal cavity), which results in cecal ischemia and soiling of the peritoneal cavity. Potential mechanisms of CLP-associated injury include bacterial peritonitis and systemic dissemination of gut-derived bacteria, as well as cecal ischemia or translocation of bacterial toxins such as endotoxin and superantigens. It is unclear which of these mechanisms of injury predominates in wild-type mice after CLP and whether CD8-positive T and NK cell-deficient mice are resistant to any particular mechanism of injury. In the present study, we hypothesized that bacterial dissemination is a major cause of injury and mortality in wild-type mice after CLP and that CD8KO/αAsGM1 mice are resistant to this mechanism of injury. To test these hypotheses, wild-type and CD8KO/αAsGM1 mice were subjected to CLP and treated with the broad-spectrum antibiotic imipenem to minimize systemic dissemination of gut-derived microorganisms. In further studies, mice were subjected to cecal ligation without puncture of the cecal wall and then treated with imipenem. The cecal ligation model was designed to cause cecal ischemia but minimize or eliminate systemic dissemination of cecal flora. Finally, in another group of mice, cecal ligation was followed by removal of cecal contents by irrigation of the cecal lumen with antibiotic-containing saline to cause cecal ischemia but remove the contribution of cecal flora to local and systemic inflammation. Survival and bacterial counts were measured in all groups to assess infection-associated mortality and bacterial burden. Temperature, acid-base balance, and cytokine production were also evaluated as indexes of physiological dysfunction and inflammation.

MATERIALS AND METHODS

Mice. Female 6- to 8-wk-old C57BL/6J wild-type and CD8 knockout (CD8KO, strain B6.129S2-Cd8a<sup>−/−</sup>) mice were purchased from Jackson Laboratory (Bar Harbor, ME). NK cells were depleted of natural killer (NK) cells by treatment with anti-asialoGM1 (CD8KO/αAsGM1 mice) are resistant to injury caused by cecal ligation and puncture (CLP). However, CLP-induced injury is complex. Potential sources of injury include bacterial dissemination, cecal ischemia, and translocation of bacterial toxins. We treated wild-type and CD8KO/αAsGM1 mice with imipenem after CLP to decrease bacterial dissemination. Additional mice were subjected to cecal ligation without puncture of the cecal wall or cecal ligation and removal of cecal contents. Imipenem treatment decreased bacterial counts by at least two orders of magnitude. However, all wild-type mice, whether treated with saline or imipenem, died by 42 h after CLP and exhibited significant hypothermia, metabolic acidosis, and high plasma cytokine concentrations. Wild-type mice subjected to cecal ligation without puncture also died, despite very low bacterial counts in blood, but wild-type mice subjected to cecal ligation and washout of cecal contents survived. In CD8KO/αAsGM1 mice subjected to CLP, imipenem treatment increased survival from 50% to 100%. After cecal ligation without puncture, long-term survival was 80–90% in CD8KO/αAsGM1 mice. Hypothermia, metabolic acidosis, and cytokine production were attenuated in CD8KO/αAsGM1 mice compared with wild-type controls. These results indicate that bacterial dissemination is not a major source of injury in wild-type mice after CLP, but the presence of gut flora in the cecal lumen is required for induction of systemic inflammation after cecal injury. CD8KO/αAsGM1 mice are resistant to any particular mechanism of injury. In the present study, we hypothesized that bacterial dissemination is a major cause of injury and mortality in wild-type mice after CLP and that CD8KO/αAsGM1 mice are resistant to this mechanism of injury. To test these hypotheses, wild-type and CD8KO/αAsGM1 mice were subjected to CLP and treated with the broad-spectrum antibiotic imipenem to minimize systemic dissemination of gut-derived microorganisms. In further studies, mice were subjected to cecal ligation without puncture of the cecal wall and then treated with imipenem. The cecal ligation model was designed to cause cecal ischemia but minimize or eliminate systemic dissemination of cecal flora. Finally, in another group of mice, cecal ligation was followed by removal of cecal contents by irrigation of the cecal lumen with antibiotic-containing saline to cause cecal ischemia but remove the contribution of cecal flora to local and systemic inflammation. Survival and bacterial counts were measured in all groups to assess infection-associated mortality and bacterial burden. Temperature, acid-base balance, and cytokine production were also evaluated as indexes of physiological dysfunction and inflammation.

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selectively depleted by intraperitoneal injection of 50 μg of anti-asialoGM1 (Cedarlane Laboratories, Hornby, ON, Canada) 24 h before CLP. Control mice were injected intraperitoneally with 50 μg of nonspecific IgG (Sigma Chemical, St. Louis, MO). All studies were approved by the Institutional Animal Care and Use Committee at The University of Texas Medical Branch and met National Institutes of Health guidelines for the care and use of experimental animals.

Cecal injury models. For CLP, mice were anesthetized with 2% isoflurane in oxygen via facemask. A 1- to 2-cm midline incision was made through the abdominal wall, and the cecum was identified and ligated with a 3-0 silk tie at its base (i.e., ~2 cm from the tip). The cecal wall was punctured once with a 20-gauge needle. The cecum was lightly squeezed to express a small amount of stool from the puncture site to ensure a full-thickness perforation. Great care was taken to preserve continuity of flow between the small and large intestines. Inspection of mice at various intervals after CLP revealed no evidence of bowel obstruction. The cecum was returned to the abdominal cavity, and the incision was closed with surgicliips. Mice were presented to the surgeon in a blinded fashion to minimize experimental bias. Sham-operated mice were anesthetized and subjected to midline laparotomy; the cecum was exteriorized and returned to the abdomen, and the wound was closed with surgicliips.

Additional mice underwent cecal ligation without puncture of the cecal wall. The procedure was performed as described above for CLP, except the cecum was not punctured with a needle. These mice were also treated with saline or imipenem as described above.

In a third group of mice, the cecum was isolated and ligated as described above, a small portion of the distal tip of the cecum was excised, and the cecal contents were removed by irrigation of the cecal lumen with 2 ml of imipenem/cilistatin. The cecum was externalized and subjected to midline laparotomy; the cecum was exteriorized and returned to the abdomen, and the wound was closed with surgicliips. The mice were injected with imipenem/cilistatin (25 mg/kg) in 1 ml of normal saline beginning immediately after CLP and every 8 h thereafter. The 18-h time point was chosen because wild-type mice exhibit significant morbidity at that time, and mortality begins 18–24 h after CLP.

Additional mice underwent cecal ligation without puncture of the cecal wall. The procedure was performed as described above for CLP, except the cecum was not punctured with a needle. These mice were also treated with saline or imipenem as described above.

In a third group of mice, the cecum was isolated and ligated as described above, a small portion of the distal tip of the cecum was excised, and the cecal contents were removed by irrigation of the cecal lumen with 2 ml of imipenem/cilistatin. The cecum was externalized for the irrigation procedure to minimize peritoneal soiling. The washed cecum was returned to the abdomen, and the wound was closed with surgicliips. The mice were injected with imipenem/cilistatin (25 mg/kg) in 1 ml of normal saline beginning immediately after CLP and every 8 h thereafter.

RNase protection assay. Heart and lung tissue was harvested and flash frozen in liquid nitrogen. Samples were stored at -80°C until used. Total RNA was isolated using TriReagent (Molecular Research Center, Cincinnati, OH). The RNase protection assay was performed using the Riboquant system (B-D Pharmingen), according to the manufacturer’s instructions. Briefly, radiolabeled RNA probes were synthesized from DNA template sets using T7 RNA polymerase, [32P]UTP, and pooled nonradiolabeled nucleotides. Isolated total RNAs (20 μg/sample) were hybridized with the purified riboprobes and subjected to RNase digestion. Protected RNA species were separated on 5% polyacrylamide sequencing gels using 0.5 × Trisborate-EDTA running buffer. Gels were run at 50-W constant power for 70 min and dried under a vacuum, and the protected fragments were visualized using autoradiography.

ELISA. Peritoneal fluid was harvested from mice by lavage of the peritoneal cavity with 5 ml of sterile saline, and heparinized blood was obtained by carotid laceration in mice anesthetized with 2% isoflurane. Plasma was harvested by centrifugation of blood (1,200 g for 10 min). IL-6 and macrophage inflammatory protein-2 (MIP-2) concentrations in peritoneal fluid and plasma were determined using an ELISA, according to the manufacturer’s protocol (eBioscience, San Diego, CA). Briefly, standards or experimental samples were added to microtiter plates that were coated with capture antibody for the cytokine of interest and incubated for 2 h. After the plates were washed, horseradish peroxidase-conjugated cytokine-specific antibody was added to each well, the reaction was incubated for 2 h, and the plates were washed again. Substrate solution was added, the plates were incubated for 30 min, and the reaction was terminated by the addition of stop solution. Cytokine levels were determined by measurement of optical density at 450 nm using a microtiter plate reader (Dynatech Laboratories, Chantilly, VA).

Measurement of temperature and arterial blood gases. Before induction of anesthesia with 1.5–2.5% isoflurane in 100% oxygen via facemask, a rectal temperature probe was inserted for measurement of body temperature. After induction of anesthesia, arterial blood for blood gas measurements was obtained by laceration of the carotid artery under direct visualization using a surgical microscope. Blood was harvested using heparinized syringes, and blood gas measurements were performed using iStat cartridges (iStat, East Windsor, NJ).

Microbiology. Bacterial counts were performed on aseptically harvested blood and peritoneal fluid. All fluid and tissues were harvested under 2% isoflurane anesthesia. Blood was aseptically obtained by carotid laceration. Sterile saline (5 ml) was injected into the peritoneal cavity after aseptic preparation of the abdominal wall, and peritoneal fluid was obtained by aspiration. Samples were serially diluted in sterile saline and cultured on tryptic soy agar plates. Plates were incubated (37°C) for 48–72 h, and colony counts were performed. Anaerobic conditions were achieved using an anaerobic chamber and the BBL GasPak Plus Anaerobic System (Becton Dickinson, Sparks, MD). Endotoxin concentrations in plasma and peritoneal fluid were measured using a Limulus amoebocyte lysate assay (Cambrex BioScience, Walkersville, MD).

Statistics. Data were analyzed using GraphPad Prism software (GraphPad Software, San Diego, CA). Survival curves were compared

Fig. 1. Survival of saline- and imipenem-treated (25 mg/kg every 8 h ip) wild-type (WT) and CD8KO/αAsGM1 mice after cecal ligation and puncture (CLP) or cecal ligation without puncture. Survival was monitored every 12 h. Values are means ± SE (n = 10). *Significantly (P < 0.05) different from WT. **Significantly (P < 0.05) different from CD8KO/αAsGM1.
using the log-rank test. For temperature, acid-base, cytokine, and endotoxin measurements, means ± SE were calculated. Data from multiple group experiments were analyzed using one-way ANOVA followed by Tukey’s post hoc test to compare groups. In studies in which two groups were compared, an unpaired Student’s t-test was performed. For measurements of bacterial colony-forming units (cfu), all data points were presented and the median was determined. Groups were compared using a nonparametric Kruskal-Wallis test followed by Dunn’s post hoc test. \( P < 0.05 \) was considered statistically significant for all experiments.

RESULTS

Effect of imipenem on survival in wild-type and CD8KO/αAsGM1 mice after cecal injury. Mice underwent CLP or cecal ligation without puncture followed by treatment with saline or imipenem, and survival was monitored for 2 wk (Fig. 1). In saline- and imipenem-treated wild-type mice, median survival time was 24 h, and mortality was 100% by 36 and 48 h, respectively, after CLP. Survival rates were not significantly

Fig. 2. Bacterial counts 18 h after CLP or cecal ligation without puncture (CL) in saline- and imipenem-treated (25 mg/kg every 8 h ip) wild-type and CD8KO/αAsGM1 mice. Aerobic and anaerobic bacteria were counted in serially diluted samples cultured on tryptic soy agar plates. Ratios indicate number of mice with positive cultures as a proportion of total mice in each group. Median bacterial count is shown for each group. CFU, colony-forming units. *Significantly \( (P < 0.05) \) less than saline-treated wild-type mice after CLP. *Significantly \( (P < 0.05) \) less than saline-treated wild-type mice after CL.

Fig. 3. Plasma endotoxin levels 18 h after CLP or CL. Endotoxin concentrations were measured using Limulus amoebocyte lysate assay. Values are means ± SE \( (n = 5–7) \). EU, endotoxin units.
different between these groups. In saline-treated CD8KO/αAsGM1 mice, survival after CLP was 50%, which was significantly (P < 0.05) improved compared with both wild-type groups. In CD8KO/αAsGM1 mice, imipenem improved survival to 100%, which was significantly greater than survival in saline-treated CD8KO/αAsGM1 mice. All mice that survived to 120 h after CLP were alive 2 wk after CLP with no evidence of ongoing morbidity. One potential complication of ligation of a large segment of the cecum is the development of bowel obstruction. Numerous mice were examined postmortem in our study, and none showed evidence of bowel obstruction.

After cecal ligation without puncture in saline-treated wild-type mice, median survival time was 36 h, and 1 in 10 mice survived for 2 wk after cecal ligation (Fig. 1). In wild-type mice treated with imipenem, median survival time was 48 h and time to 100% mortality was 96 h. Survival was not significantly different between saline- and imipenem-treated wild-type mice after cecal ligation. For CD8KO/αAsGM1 mice treated with saline and imipenem, survival rate was 80 or 90%, respectively, which was significantly better than that for wild-type mice. Survival was not significantly different between saline- and imipenem-treated CD8KO/αAsGM1 mice. For wild-type mice subjected to cecal ligation and washout with antibiotic irrigation (n = 5), survival was 100% over the 2-wk observation period.

Effect of imipenem on bacterial counts and endotoxin concentrations in blood and peritoneal fluid after cecal injury. Aerobic and anaerobic bacteria in blood and peritoneal fluid were counted 18 h after CLP or cecal ligation without puncture in wild-type and CD8KO/αAsGM1 mice (Fig. 2). After CLP, median aerobic and anaerobic bacterial counts in blood and peritoneal fluid were ~10^4 and 10^5 cfu in saline-treated wild-type and CD8KO/αAsGM1 mice, respectively. Bacterial counts in blood and peritoneal fluid were not significantly different between wild-type and CD8KO/αAsGM1 mice after CLP. Imipenem treatment significantly decreased the aerobic bacterial count in blood and peritoneal fluid after CLP by approximately two orders of magnitude in wild-type and CD8KO/αAsGM1 mice. Treatment of wild-type and CD8KO/αAsGM1 mice with imipenem after CLP eliminated anaerobes from blood and peritoneal fluid.

Aerobic and anaerobic bacteria were not detectable in blood from saline- or imipenem-treated wild-type or CD8KO/αAsGM1 mice after cecal ligation without puncture (Fig. 2). The median aerobic bacterial count in peritoneal fluid was significantly lower in saline-treated wild-type and CD8KO/αAsGM1 mice after cecal ligation without puncture than in saline-treated mice subjected to CLP. Anaerobic bacteria level was also lower in peritoneal fluid of saline-treated mice after cecal ligation without puncture than after CLP, but the difference was not statistically significant. Treatment of wild-type...
and CD8KO/αAsGM1 mice with imipenem after cecal ligation without puncture eliminated aerobic and anaerobic bacteria from peritoneal fluid. In mice in which the cecum was ligated and cecal contents were removed by washout with antibiotic irrigation (n = 5), aerobic and anaerobic bacteria were not detectable in blood or peritoneal fluid 18 h after the ligation-and-irrigation procedure.

Endotoxin concentrations in plasma were also measured 18 h after CLP or cecal ligation without puncture in wild-type and CD8KO/αAsGM1 mice (Fig. 3). Although a trend toward decreased endotoxin concentrations was observed in imipenem-treated mice compared with saline-treated mice after CLP, the difference was not statistically significant. In addition, plasma endotoxin concentrations were not significantly different between saline- and imipenem-treated wild-type mice 18 h after CLP. Comparison of wild-type mice subjected to CLP or cecal ligation without puncture also did not reveal significant differences in temperature or acid-base balance. Hypothermia and metabolic acidosis were significantly attenuated in CD8KO/αAsGM1 mice compared with wild-type mice after CLP or cecal ligation without puncture. Temperature and acid-base parameters were not significantly different between saline- and imipenem-treated CD8KO/αAsGM1 mice or between CD8KO/αAsGM1 mice subjected CLP and those subjected to cecal ligation without puncture (Fig. 4).

Effect of imipenem on temperature and acid-base balance in wild-type and CD8KO/αAsGM1 mice after cecal injury. Core body temperature and arterial blood gases were measured 18 h after CLP or cecal ligation without puncture in saline- and imipenem-treated wild-type and CD8KO/αAsGM1 mice (Fig. 4). Wild-type mice treated with saline or imipenem had significant hypothermia and acidosis compared with sham-operated mice after CLP or cecal ligation without puncture. The acidosis was primarily metabolic, as indicated by significantly decreased blood bicarbonate concentrations and increased base deficits in saline- and imipenem-treated wild-type mice compared with sham-operated mice (Fig. 4). Temperature and acid-base parameters were not significantly different between saline- and imipenem-treated wild-type mice 18 h after CLP.

Effect of imipenem on cytokine production in wild-type and CD8KO/αAsGM1 mice 18 h after cecal injury. Concentrations of IL-6 and MIP-2 in plasma were measured 18 h after CLP or CD8KO/αAsGM1 mice with imipenem after cecal ligation without puncture eliminated aerobic and anaerobic bacteria from peritoneal fluid. In mice in which the cecum was ligated and cecal contents were removed by washout with antibiotic irrigation (n = 5), aerobic and anaerobic bacteria were not detectable in blood or peritoneal fluid 18 h after the ligation-and-irrigation procedure.

Endotoxin concentrations in plasma were also measured 18 h after CLP or cecal ligation without puncture in wild-type and CD8KO/αAsGM1 mice (Fig. 3). Although a trend toward decreased endotoxin concentrations was observed in imipenem-treated mice compared with saline-treated mice after CLP, the difference was not statistically significant. In addition, plasma endotoxin concentrations were not significantly different between mice subjected to cecal ligation without puncture and those subjected to CLP. Plasma concentration of endotoxin was <2 endotoxin units/ml 18 h (n = 5) in mice subjected to cecal ligation and washout with antibiotic irrigation.
cecal ligation without puncture in wild-type and CD8KO/αAsGM1 mice (Fig. 5). Plasma levels of IL-6 and MIP-2 were high 18 h after CLP or cecal ligation without puncture in wild-type mice. Imipenem treatment did not significantly decrease plasma cytokine concentrations after CLP or cecal ligation without puncture. Plasma cytokine concentrations in wild-type mice after CLP were not significantly different from those in mice subjected to cecal ligation without puncture. IL-6 and MIP-2 concentrations were significantly lower in CD8KO/αAsGM1 than in wild-type mice after CLP or cecal ligation without puncture. Imipenem treatment did not significantly change concentrations of IL-6 or MIP-2 in plasma of CD8KO/αAsGM1 mice 18 h after CLP or cecal ligation without puncture. Plasma cytokine concentrations in CD8KO/αAsGM1 mice subjected to CLP were not significantly different from those in CD8KO/αAsGM1 mice subjected to cecal ligation without puncture.

In further studies, cytokine mRNA expression in heart and lung were measured 18 h after CLP or cecal ligation without puncture (Fig. 6). CLP-induced IL-6 and MIP-2 mRNA expression in wild-type mice was not changed by imipenem treatment. IL-6 and MIP-2 expression in heart and lung were
decreased in CD8KO/αAsGM1 mice compared with wild-type mice treated with saline or imipenem. Cecal ligation without puncture also stimulated IL-6 mRNA expression in hearts of saline- and imipenem-treated wild-type mice (Fig. 6). IL-6 mRNA expression was decreased in CD8KO/αAsGM1 mice compared with wild-type mice after cecal ligation.

**Effect of imipenem on bacterial burden, temperature, acid-base balance, and cytokine production 36 h after CLP in CD8KO/αAsGM1 mice.** Imipenem treatment improved survival in CD8KO/αAsGM1 mice after CLP. However, there were no differences in rectal temperature, acid-base balance, and cytokine production 18 h after CLP between saline- and imipenem-treated CD8KO/αAsGM1 mice. Therefore, studies were undertaken to assess these parameters 36 h after CLP. In saline-treated CD8KO/αAsGM1 mice, the aerobic and anaerobic bacterial count was \( \sim 10^4 \) cfu in blood and peritoneal fluid 36 h after CLP, and imipenem treatment significantly decreased aerobic and anaerobic bacteria in blood and peritoneal fluid (Fig. 7). Rectal temperature, blood pH, and blood bicarbonate concentrations were significantly decreased and base deficit was increased in saline-treated CD8KO/αAsGM1 compared with sham-operated mice (Fig. 8). Rectal temperature, blood pH, and blood bicarbonate were significantly higher and base deficit was lower in imipenem- than in saline-treated CD8KO/αAsGM1 mice. Plasma and peritoneal fluid concentrations of IL-6 and MIP-2 were significantly lower in imipenem- than in saline-treated CD8KO/αAsGM1 mice 36 h after CLP (Fig. 9).

**DISCUSSION**

In the present study, high bacterial counts were observed in blood and peritoneal fluid from antibiotic-untreated mice after CLP. This observation is consistent with previous reports (3, 15) and has led us, and others, to assume that systemic dissemination of bacteria is the primary mechanism of injury after CLP. However, our study shows that treatment of wild-type mice with imipenem did not improve survival, despite a more than twofold decrease in the bacterial burden in blood and peritoneal fluid. In further studies, ligation of the cecum without puncture of the cecal wall and CLP caused comparable inflammation, physiological dysfunction, and mortality in wild-type mice. However, bacterial counts in blood and peritoneal fluid were markedly lower in mice subjected to cecal ligation without puncture than in those subjected to CLP. These observations indicate that bacterial dissemination is not the primary cause of CLP-induced injury in wild-type mice if a large length of the cecum is ligated. Therefore, other mechanisms of injury may be important. The potential significance of noninfection-induced mechanisms of injury is supported by other studies that have examined the effects of antibiotics on CLP-induced injury (3, 24, 25, 27, 28). Survival benefits of 0–56% have been reported, and most studies show ≤20% improvement in survival after antibiotic treatment. Furthermore, in mice that develop a hyperinflammatory state after CLP, as indicated by high plasma IL-6 concentrations, mortality is 75–100%, despite antibiotic treatment (14, 24, 28). Taken together, these studies imply that bacterial dissemination is not the only factor contributing to CLP-induced morbidity and mortality.

One potential mechanism of CLP-induced injury is cecal ischemia. The blood supply to the rodent cecum arises primarily from the cranial mesenteric artery and runs from the base of the cecum to the apex (6). Therefore, ligation of the cecum at its base will disrupt blood flow to areas distal to the ligation site, resulting in tissue ischemia. Our finding of comparable inflammation and mortality in mice subjected to cecal ligation without puncture of the cecal wall and those subjected to CLP supports the contention that cecal ischemia is a potential mechanism of injury in our study. The importance of cecal ischemia in CLP-induced morbidity and mortality is further supported by the studies of Singleton and Wischmeyer (17), in which the length of cecum that was ligated was the major predictor of inflammation and mortality in rats after CLP. A recent study by Torres and De Maio (23) also showed that the length of cecum that was ligated was an important determinant of the severity of CLP-induced inflammation. Therefore, cecal ischemia may be an important mechanism of injury after CLP, especially if a large portion of the cecum is ligated. In general, the relative contributions of cecal ischemia and bacterial dissemination to CLP-induced inflammation are likely to be influenced by the length of cecum ligated and the level of cecal disruption caused by multiple punctures or lacerations of the cecum. The amount of mortality observed after CLP is highly dependent on these factors. In the present study, a large length of cecum was ligated. This model appears to induce mechanisms of inflammatory injury that are independent of bacterial dissemination. Cecal ischemia may not be as prevalent in models of CLP in which a shorter length of cecum is ligated.
The presence of gastrointestinal flora in the cecal lumen also appears to be essential for induction of systemic injury after cecal ligation. In the present study, removal of cecal contents by irrigation with antibiotic-containing saline markedly decreased inflammation and eliminated mortality caused by cecal ligation. These findings suggest that translocation of bacterial toxins may be an important mechanism for induction of systemic inflammation after cecal injury. Gut ischemia can lead to deterioration of mucosal integrity, with subsequent translocation of bacterial toxins into the systemic circulation (8, 19). Some reports show a clear correlation between plasma endotoxin concentrations, lactate production, and proinflammatory cytokine release in models of postburn and hemorrhagic shock (18). This has prompted some investigators to postulate that the gut is the “engine” driving inflammation and organ dysfunction in critically ill patients (7, 8). Bacterial superantigens may also translocate across damaged cecal mucosa and stimulate systemic inflammation (9, 10). Superantigens, such as toxic shock syndrome toxin and staphylococcal enterotoxins, induce an inflammatory response that is mediated primarily through polyclonal activation of T lymphocytes (13). Superantigen-induced activation of T cells causes systemic release of proinflammatory cytokines, resulting in hemodynamic instability and a shock syndrome that is similar to endotoxin-induced shock (10). Furthermore, superantigens can synergize with endotoxin to induce systemic inflammation, shock, and death. The lethal doses for each agent can be decreased by two orders of magnitude if endotoxin and superantigens are administered simultaneously (2). Taken together, these findings indicate that translocation of bacterial contents is a plausible mechanism of injury after CLP.

In the present study, we show that mice depleted of CD8-positive T and NK cells, particularly those treated with antibiotics, are resistant to systemic injury caused by CLP. Nonantibiotic-treated CD8KO/αAsGM1 mice exhibit a mortality pattern that is delayed compared with that of wild-type mice, and treatment of CD8KO/αAsGM1 mice with antibiotics ablated CLP-induced mortality. These findings suggest that CD8-positive T and NK cell-deficient mice are resistant to mechanisms of injury that are independent of bacterial dissemination but exhibit delayed mortality associated with systemic infection. Translocation of gut-derived bacterial endotoxin and superantigens provides a plausible mechanism of simultaneous CD8-positive T and NK cell activation after CLP. We previously showed greater decreases in physiological dysfunction and mortality after CLP induced by depletion on NK and CD8-positive T cells than by depletion of either cell type individually (15, 16, 20, 22). These findings suggest that both cell types become activated after CLP to mediate or facilitate a lethal inflammatory response. NK cells play a pivotal role in endotoxin-induced inflammation, and T cells are critical for the effects of superantigens (1, 4, 5, 11–13, 26, 29). The mechanisms of T and NK cell activation after cecal injury remain to be fully elucidated. However, the respective activation of these cell types by gut-derived endotoxin and superantigens provides a plausible mechanism of CLP-induced inflammation.

In summary, the present study shows that imipenem treatment, despite markedly decreasing bacterial burden in blood and peritoneal fluid, does not improve survival in wild-type mice when the full length of the cecum is ligated during CLP. Furthermore, in wild-type mice, the pattern of inflammation and mortality after cecal ligation without puncture of the cecal wall was similar to that observed after CLP, despite a marked decrease in systemic bacterial counts. Taken together, these findings indicate that bacterial dissemination is not the major cause of inflammation and mortality in wild-type mice after CLP in our study and that other mechanisms, such as cecal ischemia, may be important. The presence of gastrointestinal flora in the cecal lumen appears necessary for the development of systemic injury after cecal ligation, because mice subjected to washout of cecal contents did not show mortality after cecal ligation. This finding supports the contention that translocation of bacterial toxins may be an important source of inflammation. CD8KO/αAsGM1 mice show resistance to CLP-induced mortality and exhibit a pattern of mortality that is delayed compared with that of wild-type mice. Furthermore, treatment of CD8KO/αAsGM1 mice with imipenem completely ablated CLP-induced mortality. Therefore, CD8KO/αAsGM1 mice appear resistant to early, noninfection-mediated mechanisms of CLP-induced injury but may be susceptible to injury caused by bacterial dissemination. Activation of CD8-positive T and NK cells by translocation of bacterial toxins may be an important source of inflammation after CLP.

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