Differential effect of imipenem treatment on injury caused by cecal ligation and puncture in wild-type and NK cell-deficient β2-microglobulin knockout mice

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Enoh, Victor T., Cheng Y. Lin, Tushar K. Varma, and Edward R. Sherwood. Differential effect of imipenem treatment on injury caused by cecal ligation and puncture in wild-type and NK cell-deficient β2-microglobulin knockout mice. Am J Physiol Gastrointest Liver Physiol 290: G277–G284, 2006. First published September 15, 2005; doi:10.1152/ajpgi.00338.2005.—Our previous studies showed that β2-microglobulin knockout mice treated with anti-asialoGM1 (β2MKO/αAsGM1 mice) are resistant to injury caused by cecal ligation and puncture (CLP). However, CLP-induced injury is complex. Potential mechanisms of injury include systemic infection, cecal ischemia, and translocation of bacterial toxins such as endotoxin and superantigens. Currently, it is unclear which of these mechanisms of injury contributes to mortality in wild-type mice and whether β2MKO/αAsGM1 mice are resistant to any particular mechanisms of injury. In the present study, we hypothesized that systemic infection is the major cause of injury after CLP in wild-type mice and that β2MKO/αAsGM1 mice are resistant to infection-induced injury. To test this hypothesis, wild-type and β2MKO/αAsGM1 mice were treated with the broad-spectrum antibiotic imipenem immediately after CLP to decrease the impact of systemic infection in our model. Treatment of wild-type and β2MKO/αAsGM1 mice with imipenem 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 had significant hypothermia, metabolic acidosis, and high plasma concentrations of the cytokines interleukin-6, macrophage inflammatory protein-2, and keratinocyte-derived chemokine. β2MKO/αAsGM1 mice showed 40% long-term survival, which was increased to 90% by imipenem treatment. β2MKO/αAsGM1 mice had less hypothermia, decreased metabolic acidosis, and lower cytokine concentrations at 18 h after CLP compared with wild-type mice. These results suggest that infection is not the major cause of mortality for wild-type mice in our model of CLP. Other mechanisms of injury such as cecal ischemia or translocation of microbial toxins may be more important. β2MKO/αAsGM1 mice appear resistant to these early, non-infection-related causes of CLP-induced injury but showed delayed mortality associated with bacterial dissemination, which was ablated by treatment with imipenem.

We have previously demonstrated that β2-microglobulin knockout mice that are treated with anti-asialoGM1 (β2MKO/αAsGM1 mice) exhibit decreased systemic inflammation and improved survival compared with wild-type mice after cecal ligation and puncture (CLP; see Refs. 19 and 27). β2MKO/αAsGM1 mice have multiple immunological defects, including the absence of CD8+ T, natural killer (NK), and NK T cells as well as deficient expression of the class I major histocompatibility complex and CD1 molecules (14, 15, 17). Our studies showed that depletion of CD8+ T and NK cells is the major factor conferring resistance to CLP-induced injury in β2MKO/αAsGM1 mice (18, 19). These findings indicate that CD8+ T and NK cells either directly mediate or facilitate the lethal inflammatory response induced by CLP. However, a complete understanding of the mechanisms by which these cell types mediate CLP-induced injury is complicated by the complexity of the CLP model. 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. In the present study, we hypothesized that systemic infection is a major cause of injury and mortality in wild-type mice after CLP and that β2MKO/αAsGM1 mice are resistant to this mechanism of injury. To test these hypotheses, wild-type and β2MKO/αAsGM1 mice were subjected to CLP and treated with the broad-spectrum antibiotic imipenem to minimize local bacterial growth and systemic dissemination of gut-derived microorganisms. Survival and bacterial counts were measured to assess the impact of imipenem on bacterial burden and infection-associated mortality after CLP. Temperature, acid-base balance, and cytokine production were evaluated as indexes of physiological function and inflammation.

 MATERIALS AND METHODS

Mice. Female 6- to 8-wk-old C57BL/6J wild-type and β2-microglobulin knockout (β2MKO, strain B6 129P-B2m1Unc) mice were purchased from Jackson Laboratory (Bar Harbor, ME). Selective depletion of NK cells was performed by injection of anti-asialoGM1 (50 μg ip; Cedarlane Laboratories, Hornby, Ontario, Canada) 24 h before CLP (19). 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.

CLP. Mice were anesthetized with 2% isoflurane in oxygen via a facemask. A 1- to 2-cm midline incision was made through the abdominal wall; the cecum was identified and ligated with a 3-0 silk tie 1 cm from the tip. Care was taken not to cause bowel obstruction. A single puncture of the cecal wall was performed with a 20-gauge needle. The cecum was lightly squeezed to express a small amount of stool from the puncture site to assure a full-thickness perforation. Great care was taken to preserve continuity of flow between the small...
and large bowels. Inspection of mice at various intervals after CLP did not reveal evidence of bowel obstruction. The cecum was returned to the abdominal cavity, and the incision was closed with surgical clips. Mice were presented to the surgeon in a blinded fashion to minimize experimental bias. Sham mice underwent anesthesia and midline laparotomy; the cecum was exteriorized and returned to the abdomen, and the wound was closed with surgical clips. Mice received intraperitoneal injection of imipenem-cilistatin (25 mg/kg Primaxin; Merck, Whitehouse Station, NJ) in 1 ml normal saline beginning immediately after CLP and every 8 h thereafter. Control mice received 1 ml normal saline in the same regimen. Measurement of arterial blood gases, temperature, cytokine levels, and bacterial colony counts was performed at 18 h after CLP. The 18-h time point was chosen because wild-type mice exhibit significant morbidity at that time, and mortality begins at 20–24 h after CLP in the wild-type group.

ELISA. Peritoneal fluid was harvested from mice by lavage of the peritoneal cavity with 5 ml sterile saline, and heparinized blood was obtained by carotid laceration in mice anesthetized with 2% isoflurane. Plasma was harvested by centrifuging blood (1,200 g for 10 min). Interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived chemokine (KC) 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 antibodies to the cytokine of interest and incubated for 2 h. After washing, horseradish peroxidase-conjugated, cytokine-specific antibody was added to each well, incubated for 2 h, and washed. Substrate solution was added and incubated for 30 min, and the reaction was terminated by the addition of stop solution. Cytokine levels were determined by measuring optical density at 450 nm using a microtiter plate reader (Dynatech Laboratories, Chantilly, VA).

Measurement of temperature and arterial blood gases. Body temperature was measured by insertion of a rectal temperature probe before induction of anesthesia with 1.5–2.5% isoflurane in 100% oxygen via a facemask. 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 tissue harvesting was performed under 2% isoflurane anesthesia. Blood was obtained by carotid laceration after aseptic preparation of the neck. Peritoneal fluid was harvested by an injection of 5 ml sterile saline in the peritoneal cavity after aseptic preparation of the abdominal wall followed by aspiration of peritoneal fluid. 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. All data were analyzed using GraphPad Prism software (GraphPad Software, San Diego, CA). Survival curves were compared...
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 a post hoc Tukey test to compare groups. In studies in which two groups were compared, a nonpaired Student’s t-test was performed. For measurements of bacterial colony-forming units (CFU), all data points are presented, and the median was determined. Groups were compared using a nonparametric Kruskal-Wallis test followed by a post hoc Dunn’s test. A value of $P < 0.05$ was considered statistically significant for all experiments.

RESULTS

Effect of imipenem treatment on bacterial counts and endotoxin concentrations in blood and peritoneal fluid following CLP. The numbers of aerobic and anaerobic bacteria in blood and peritoneal fluid were measured at 18 h after CLP in wild-type and β2MKO/αAsGM1 mice (Fig. 1). Imipenem treatment decreased the number aerobic bacteria in blood and peritoneal fluid by at least two orders of magnitude in both wild-type and β2MKO/αAsGM1 mice. Treatment of wild-type and β2MKO/αAsGM1 mice with imipenem eliminated anaerobes from blood and decreased counts of anaerobic bacteria in peritoneal fluid by more than two orders of magnitude.

Endotoxin concentrations in plasma and peritoneal fluid were also measured at 18 h after CLP in wild-type and β2MKO/αAsGM1 mice (Fig. 2). Imipenem treatment did not significantly change endotoxin concentrations in plasma or peritoneal fluid from wild-type or β2MKO/αAsGM1 mice.

Effect of imipenem treatment on survival in wild-type and β2MKO/αAsGM1 mice after CLP. Mice underwent CLP and were treated with either saline or imipenem (25 mg/kg ip). Survival was monitored every 8 h. $P < 0.05$, significantly different from saline-treated WT mice (*) and from saline-treated β2MKO/αAsGM1 mice (●); $n = 10$ mice/group.

Effect of imipenem treatment on survival in wild-type and β2MKO/αAsGM1 mice after CLP. WT and β2MKO/αAsGM1 mice underwent CLP and were treated with either saline or imipenem (25 mg/kg ip). Survival was monitored every 8 h. $P < 0.05$, significantly different from saline-treated WT mice (*) and from saline-treated β2MKO/αAsGM1 mice (●); $n = 10$ mice/group.

Effect of imipenem treatment on survival in wild-type and β2MKO/αAsGM1 mice after CLP. WT and β2MKO/αAsGM1 mice underwent CLP and were treated with either saline or imipenem (25 mg/kg ip). Survival was monitored every 8 h. $P < 0.05$, significantly different from saline-treated WT mice (*) and from saline-treated β2MKO/αAsGM1 mice (●); $n = 10$ mice/group.

Effect of imipenem treatment on temperature and acid-base balance in wild-type and β2MKO/αAsGM1 mice. Core body temperature and arterial blood gases were measured at 18 h after CLP in wild-type and β2MKO/αAsGM1 mice that received saline or imipenem treatment (Fig. 4). Wild-type mice treated with saline or imipenem had significant hypothermia and acidosis compared with sham mice. 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 mice (Fig. 4). Temperature and acid-base balance were not significantly different between saline and imipenem-treated wild-type mice at 18 h after CLP. CLP-induced hypothermia and metabolic acidosis were significantly attenuated in β2MKO/αAsGM1 mice compared with wild-type mice. Temperature and the measured acid-base parameters were not significantly different when comparing saline- and imipenem-treated β2MKO/αAsGM1 mice (Fig. 4).

Effect of imipenem treatment on cytokine concentrations in wild-type and β2MKO/αAsGM1 mice at 18 h after CLP. Concentrations of IL-6, MIP-2, and KC in plasma and peritoneal fluid were measured at 18 h after CLP in wild-type and β2MKO/αAsGM1 mice (Table 1). Wild-type mice had high levels of IL-6, MIP-2, and KC in plasma and peritoneal fluid at
18 h after CLP. Concentrations of IL-6, MIP-2, and KC in plasma and peritoneal fluid were significantly lower in β2MKO/αAsGM1 mice compared with wild-type mice. Imipenem treatment did not significantly change concentrations of IL-6, MIP-2, or KC in plasma or peritoneal fluid in wild-type or β2MKO/αAsGM1 mice at 18 h after CLP.

Effect of imipenem treatment on temperature, acid-base balance, and cytokine production at 36 h after CLP in β2MKO/αAsGM1 mice. Results of the present study showed that imipenem treatment improves survival in β2MKO/αAsGM1 mice. However, differences in rectal temperature, acid-base balance, and cytokine production were not observed at 18 h after CLP when saline- and imipenem-treated β2MKO/αAsGM1 mice were compared. Therefore, studies were undertaken to assess these parameters at 36 h after CLP. Saline-treated β2MKO/αAsGM1 mice had >10^5 aerobic and anaerobic bacterial CFU in blood and peritoneal fluid at 36 h after CLP (Fig. 5). Aerobic bacteria were not detectable in blood or peritoneal fluid of imipenem-treated β2MKO/αAsGM1 mice at 36 h after CLP, and only one of six cultures from blood and peritoneal fluid had detectable anaerobic bacteria. Plasma endotoxin concentrations were not significantly different between saline- and imipenem-treated β2MKO/αAsGM1 mice at 36 h after CLP (Fig. 6). Rectal temperature, blood pH, and blood bicarbonate concentrations were significantly decreased in saline-treated β2MKO/αAsGM1 mice compared with those treated with imipenem (Fig. 7). Base deficit was significantly increased in saline-treated β2MKO/αAsGM1 mice compared with imipenem-treated β2MKO/αAsGM1 mice at 36 h after CLP. Plasma and peritoneal fluid concentrations of IL-6 and MIP-2 were significantly lower in imipenem-treated β2MKO/αAsGM1 mice compared with saline-treated mice at 36 h after CLP (Fig. 8).

Table 1. Cytokine concentrations at 18 h after CLP

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Plasma</th>
<th>Peritoneal Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>β2MKO/αAsGM1</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>Imipenem</td>
</tr>
<tr>
<td>IL-6</td>
<td>5,640±1,967</td>
<td>5,283±1,993</td>
</tr>
<tr>
<td>MIP-2</td>
<td>2,291±299</td>
<td>1,720±76</td>
</tr>
<tr>
<td>KC</td>
<td>3,188±612</td>
<td>2,859±431</td>
</tr>
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</table>

Values represent the means ± SE; n = 5–7 mice/group. IL-6, interleukin-6; MIP-2, macrophage inhibitory 2; KC, keratinocyte-derived chemokine; CLP, cecal ligation and puncture. Plasma and peritoneal fluid were harvested at 18 h after CLP. Cytokine concentrations were measured using an ELISA. *Significantly (P < 0.05) less than saline-treated wild-type mice.
DISCUSSION

The present study clearly demonstrates high bacterial counts in blood and peritoneal fluid from non-antibiotic-treated mice after CLP. This finding is consistent with numerous other reports that have shown bacterial dissemination in mice after CLP (2, 18, 34). One goal of our study was to determine whether systemic infection is a major cause of injury and mortality in wild-type mice after CLP. Results of this study show that treatment of wild-type mice with imipenem, a broad-spectrum antibiotic, does not improve survival despite decreasing bacterial burden in blood and peritoneal fluid by more than two orders of magnitude. This observation suggests that systemic infection is not the primary cause of mortality for wild-type mice in our model of CLP. Other investigators have also examined the effect of antibiotic treatment on inflammation and mortality in mice after CLP (2, 28, 30, 32, 33). Most prior studies show only limited improvement in survival after antibiotic treatment despite marked decreases in bacterial burden. Taken together, these studies indicate that bacterial dissemination is not the only factor contributing to CLP-induced morbidity and mortality.

We also hypothesized that β2MKO/αAsGM1 mice are resistant to the infectious component of CLP. β2MKO/αAsGM1 mice that did not receive imipenem treatment had improved survival after CLP compared with wild-type mice. Saline-treated β2MKO/αAsGM1 mice that died after CLP also had a delayed mortality pattern compared with wild-type mice. Treatment of β2MKO/αAsGM1 mice with imipenem conferred near-complete resistance to CLP-induced mortality. One interpretation of these findings is that β2MKO/αAsGM1 mice are resistant to early, non-infection-related mechanisms of CLP-induced injury but eventually succumb to bacterial dissemination. Treatment of β2MKO/αAsGM1 mice with imipenem appears to eliminate infection as a mechanism of injury and provides near-complete resistance to CLP-induced mortality in these mice. These findings suggest that early injury in our model of CLP is caused by non-infection-related mechanisms. Other potential mechanisms of CLP-induced injury include cecal ischemia and dissemination of bacterial products such as endotoxin and superantigens. There is a high probability that these early mechanisms of injury are mediated or facilitated by CD8⁺ T and NK cells, because our previous studies showed that deletion of CD8⁺ T and NK cells is the primary alteration conferring the resistance of β2MKO/αAsGM1 mice to CLP-induced injury (18, 26). Mice, such as β2MKO/αAsGM1 mice, which are resistant to these early mechanisms of CLP-induced injury, appear susceptible to infection-associated morbidity and mortality. Treatment of β2MKO/αAsGM1 mice with imipenem removed infection as a source of injury and markedly improved long-term survival in β2MKO/αAsGM1 mice after CLP.

In the present study, the full length of the cecum was ligated. Great care was taken to prevent small bowel obstruction, and postmortem examination did not reveal evidence of bowel obstruction in any mice used in this investigation. Ligation of...
the full length of the cecum resulted in severe injury and mortality within 48 h after CLP in wild-type mice. This finding supports the contention that cecal ischemia is an important mechanism of injury after CLP. 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 (8). Therefore, ligation of the cecum at its base will disrupt blood flow to areas distal to the ligation site, resulting in tissue ischemia. The functional importance of cecal ischemia in CLP-induced morbidity and mortality is supported by the studies of Singleton and Wischmeyer (21), in which the length of cecum ligated, rather than puncture size, was the major predictor of inflammation and mortality in rats after CLP. Other studies have shown that resection of the ischemic cecum will reverse CLP-induced mortality (16). Whether CD8\(^+\) T and NK cells mediate ischemia-induced injury after CLP remains to be fully established. However, studies have shown that T cells participate in ischemia-associated injury in a variety of tissues. Granger and colleagues (6, 20) showed that CD8\(^+\) T cells modulate local inflammation and facilitate vascular permeability within hours after intestinal ischemia-reperfusion injury. Rabb and coworkers (1, 35) showed that T cell depletion provides protection from ischemia-induced renal injury. CD4\(^+\) and CD8\(^+\) T cells have also been shown to facilitate innate mechanisms of neutrophil recruitment and endothelial injury during ischemic injury of the kidneys (3, 7, 36). Further studies will be required to fully elucidate the cellular interactions that potentiate systemic injury during gut ischemia.

Another important consideration in the pathogenesis of inflammation caused by gut ischemia is interaction of T and NK cells with bacterial toxins such as endotoxin and bacterial superantigens that may disseminate after cecal injury. Gut ischemia can lead to deterioration of mucosal integrity with subsequent translocation of bacterial products in the systemic circulation (9, 24). In the CLP model, there is also direct spillage of bacteria and their by-products into the peritoneal cavity. It has been postulated that endotoxin translocation is a major stimulus for postischemic inflammation. Some reports showed a clear correlation between plasma endotoxin concentrations, lactate production, and proinflammatory cytokine re-

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**Fig. 8.** Plasma and peritoneal fluid cytokine levels in saline- and imipenem-treated \(\beta_2\)MKO/\(\alpha\)AsGM1 mice. Plasma and peritoneal fluid were harvested at 36 h after CLP. Cytokine concentrations were measured using an ELISA. A: interleukin (IL)-6 levels; B: macrophage inflammatory protein (MIP)-2 levels. Values represent means ± SE; n = 6–9 mice/group. *Significantly (\(P < 0.05\)) less than saline-treated \(\beta_2\)MKO/\(\alpha\)AsGM1 mice.

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**Fig. 7.** Temperature and acid-base balance in saline- and imipenem-treated \(\beta_2\)MKO/\(\alpha\)AsGM1 mice. Rectal temperature (A) and arterial blood gases (B) were analyzed at 36 h after CLP in \(\beta_2\)MKO/\(\alpha\)AsGM1 mice treated with saline or imipenem. Sham WT mice served as controls. C: blood bicarbonate levels; D: base deficit. *\(P < 0.05\), significantly different from sham (*) and from saline-treated \(\beta_2\)MKO/\(\alpha\)AsGM1 mice (●). Values represent means ± SE; n = 6–9 mice/group.
In the present study, endotoxin concentrations in blood and important factors causing T and NK cell activation after CLP. Other investigators have postulated that bacterial superantigens play a role in stimulating local and systemic inflammation associated with gut injury (11, 12). Bacterial superantigens such as toxic shock syndrome toxin and staphylococcal enterotoxins stimulate an inflammatory response that is mediated primarily by T cells (10). As described above, we have reported that combined NK and T cell depletion provides a level of survival benefit after CLP that is greater than depletion of either cell type individually (18, 19). This finding suggests that both cell types become activated after CLP to mediate or facilitate a lethal inflammatory response. The mechanisms of T and NK cell activation remain to be fully elucidated. However, translocation of both endotoxin and bacterial superantigens may be important factors causing T and NK cell activation after CLP. In the present study, endotoxin concentrations in blood and peritoneal fluid were not altered by imipenem treatment despite a marked decrease in bacterial counts. Further studies must be performed to establish the importance of microbial products as a stimulus for CD8+ T and NK cell-mediated inflammation after CLP.

Results of the present study show that CLP-induced mortality correlates with hypothermia, metabolic acidosis, and increased production of proinflammatory cytokines and chemokines. These findings confirm and extend our previous findings on temperature regulation, acid-base balance, and cytokine production in wild-type and β2MKO/αAsGM1 mice after CLP. Hypothermia and severe metabolic acidosis are predictors of mortality in mice after CLP (18, 19). Metabolic acidosis is a surrogate marker of tissue hypoperfusion and is commonly seen during gut ischemia and in shock states (22). The presence of severe metabolic acidosis indicates that a state of tissue hypoperfusion exists in wild-type mice after CLP. This is consistent with our previous reports showing that the ultimate mechanism of mortality in wild-type mice after CLP is cardiovascular collapse and shock (25–27). Treatment of wild-type mice with imipenem did not improve CLP-induced hypothermia and severe metabolic acidosis, which indicates that systemic infection is not the underlying cause of these alterations. Our studies on cytokine production showed that IL-6, MIP-2, and KC were significantly lower in β2MKO/αAsGM1 mice compared with wild-type controls. MIP-2 and KC were measured because a previous study by Heuer et al. (5) showed that increased concentrations of these chemokines are one of the best predictors of mortality in rodent CLP. Several studies have shown a strong correlation between IL-6 production and CLP-induced mortality (16, 28). Our previous studies have shown IL-6, MIP-2, and KC to be accurate markers of ultimate mortality in mice after CLP (18, 19). The present study confirms that these cytokines and chemokines are accurate markers of mortality in mice exposed to CLP. These observations also indicate that inflammation is a major factor contributing to CLP-induced morbidity and mortality. Interestingly, treatment of wild-type mice with imipenem did not significantly decrease cytokine production. This finding indicates that local and systemic infection are not the major factors contributing to inflammation in the first 48 h after CLP and provides further evidence that infection is not an important cause of morbidity and mortality in our model of CLP.

In conclusion, the present study shows that treatment of β2MKO/αAsGM1, but not wild-type C57BL/6J, mice with imipenem improves survival after CLP. This improved outcome was associated with attenuation of hypothermia, metabolic acidosis, and systemic inflammation. These findings indicate that infection is not the major cause of inflammation, morbidity, and mortality in wild-type mice after CLP. Noninfection-related causes of injury, such as cecal ischemia or translocation of bacterial toxins, may be important. Furthermore, β2MKO/αAsGM1 mice appear resistant to these noninfectious mechanisms of CLP-induced injury but exhibit delayed mortality associated with bacterial dissemination. The latter mechanism of injury was greatly attenuated by treatment of β2MKO/αAsGM1 mice with imipenem.