Multiple Roles for IL-12 in a Model of Acute Septic Peritonitis

Matthew L. Steinhauser, Cory M. Hogaboam, Nickolas W. Lukacs, Robert M. Strieter and Steven L. Kunkel

*J Immunol* 1999; 162:5437-5443; http://www.jimmunol.org/content/162/9/5437

**References** This article cites 44 articles, 26 of which you can access for free at:
http://www.jimmunol.org/content/162/9/5437.full#ref-list-1

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Multiple Roles for IL-12 in a Model of Acute Septic Peritonitis¹

Matthew L. Steinhauser,* Cory M. Hogaboam,* Nickolas W. Lukacs,* Robert M. Strieter,† and Steven L. Kunkel²

The present study addressed the role of IL-12 in a murine model of septic peritonitis, induced by cecal ligation and puncture (CLP). Although CLP surgery induced IL-12 production at 6 and 24 h after surgery, IL-12 immunoneutralization was clearly deleterious in this model: 54% of CLP mice receiving preimmune serum survived, whereas mice administered IL-12 antisera prior to CLP experienced a 25% survival rate. IL-12 immunoneutralization not only led to increased mortality, but also appeared to promote a shift away from IL-12 and IFN-γ, in favor of IL-10. This cytokine shift corresponded to changes in bacterial load, as CLP mice receiving IL-12 antisera yielded more CFUs from the peritoneal cavity at 24 h after CLP. To address the role of bacterial infection in IL-12 antisera-induced mortality following CLP, antibiotics were administered for 4 days after surgery. Despite regular antibiotic administration, IL-12 immunoneutralization still reduced survival in CLP mice. Furthermore, histology of the ceca revealed that mice administered IL-12 antisera failed to show typical organization of the damaged cecum wall. Accordingly, Gram staining revealed bacteria within peritoneal fluids from these mice, while peritoneal fluids from CLP mice that received preimmune serum and antibiotics were free of bacteria. Altogether, these data suggested multiple important roles for IL-12 in the evolution of murine septic peritonitis. The Journal of Immunology, 1999, 162: 5437–5443.

Sepsis is characterized by an acute, systemic immune response to a variety of noxious insults, particularly bacterial infections. The principle physiological symptoms often associated with sepsis syndrome are shock, coagulopathy, fever or hypothermia, tachycardia, tachypnea, and multiorgan dysfunction. Ultimately, 25–35% of all septic episodes end in death (1, 2), while those patients suffering from septic peritonitis experience a much higher mortality rate of 60–80% (3). Bacterial sepsis commences with the initiation of the host’s innate immune response to bacterial infection. The Gram-negative bacterial cell wall component, LPS, mediates many of the pathophysiological symptoms of sepsis by inducing the release of TNF-α and IL-1. These host-derived, early response cytokines initiate a cascade of inflammatory cytokines, which together mediate the hemodynamic changes and inflammatory events that typify sepsis (4). IL-12 plays a critical role in this early inflammatory process by augmenting the production of IFN-γ and other cytokines from NK and T cells, by enhancing the cytolytic activity of CTLs and NK cells, and by stimulating the proliferation of activated T and NK cells (5). Furthermore, IL-12 appears to be a vital component of the host defense against both Gram-positive and Gram-negative bacterial organisms, as evidenced by the heightened host resistance conferred by IL-12 administration in several bacterial infection models (6–10). However, the proinflammatory effects of IL-12 can become detrimental during sepsis. The exaggerated proinflammatory response often associated with IL-12 production during sepsis may result in many of the injurious and sometimes fatal physiological symptoms of the disease (11, 12).

Host-derived, anti-inflammatory mediators like IL-10 counterbalance the IL-12-mediated inflammatory responses to bacterial pathogens. For example, IL-10 exerts potent in vitro and in vivo anti-inflammatory effects, in part, by suppressing the production of proinflammatory cytokines, including TNF-α, IL-1, and IFN-γ, and by repressing macrophage and neutrophil phagocytic and bactericidal activities (13–15). Various studies have shown that a septic response dominated by proinflammatory cytokines is toxic. Conversely, tipping the cytokine balance in favor of an anti-inflammatory response with either IL-10 treatment or IL-12 neutralization has proved beneficial in various sepsis models (13, 16–18); however, in a murine model of neonatal, bacterial sepsis, IL-12 neutralization was clearly detrimental, suggesting that the severe suppression of proinflammatory mediators may also harm septic humans (19).

In this study, we explored the role of IL-12 in a clinically relevant model of bacterial sepsis, namely cecal ligation and puncture (CLP). The CLP mouse serves as a clinically relevant disease model, because it mimics the septic response sometimes associated with postsurgical or accidental trauma. Furthermore, this model meets the two general diagnostic criteria for sepsis, as the CLP mice display systemic inflammatory akin to the human systemic inflammatory response syndrome and experience an active bacterial infection (1). In the CLP model, IL-12 neutralization was clearly detrimental. Thus, subsequent experiments were designed in an attempt to delineate a mechanistic role for IL-12 in this model. Previously it has been shown that IL-12 and IL-10 antagonize each other in Th cell-mediated immune responses (20, 21). In this study, we clearly show that IL-12 can also balance IL-10 in the context of an innate immune response, as IL-12 immunoneutralization results in a cytokine profile dominated by IL-10 with insufficient IL-12 and IFN-γ. Furthermore, disrupting IL-12 levels

---

1 This work was supported by National Institutes of Health Grants IP50HL56402, HL35276, HL31963, 136302, IP50HL60289, and CA66180.
2 Address correspondence and reprint requests to Dr. Steven L. Kunkel, Department of Pathology, University of Michigan Medical School, 1301 Catherine Rd., Ann Arbor, MI 48109-0602. E-mail address: slkunkel@umich.edu
3 Abbreviation used in this paper: CLP, cecal ligation and puncture.

Department of *Pathology and † Internal Medicine, Division of Pulmonary and Critical Care, University of Michigan Medical School, Ann Arbor, MI 48109

Copyright © 1999 by The American Association of Immunologists

0022-1767/99/$02.00
produces a cascade of events that ultimately blocks bacterial clearance and tissue repair. These studies support the vital role of IL-12 in the host response during experimental sepsis in which the host faces the dual threat of pathophysiological imbalances and bacterial colonization.

**Materials and Methods**

**Animals**

Specific pathogen-free CD-1 mice (6- to 12-wk-old females, Charles River Breeding Laboratories, Wilmington, MA) were used in all experiments. All mice were housed in specific pathogen-free conditions within the animal care facility at the University of Michigan Unit for Laboratory Animal Medicine (ULAM, Ann Arbor, MI) until the day of sacrifice.

**Animal model**

The CLP model was used as previously described (17). The mice were anesthetized by i.p. injection with 3–3.5 mg of ketamine HCl (Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) followed by inhaled methoxyflurane (Metafane, Pitman-Moore, Mundelein, IL) as needed. The mice were given a 1- to 2-cm longitudinal incision to the lower left quadrant of the abdomen. The cecum was exposed and the distal one-third was ligated with 3-0 silk suture, and punctured through and through with a 21-gauge needle. The cecum was then replaced in the peritoneal cavity and the incision was closed with surgical staples. In sham controls, the cecum was not ligated or punctured, then returned to the abdominal cavity. All mice were administered 1 ml of sterile saline s.c. as a fluid resuscitation measure immediately following surgery.

**Marine cytokine ELISA**

Murine IFN-γ, IL-12, and IL-10 were quantitated using a modification of a double-ligand method as previously described (22). Briefly, flat-bottom 96-well microtiter plates (Immuno-Plate I 96-F; Nunc, Glostrup, Denmark) were coated with 50 μg/well of rabbit Ab against the various cytokines (1 μg/ml in 0.6 M NaCl, 0.26 M H3BO3, and 0.08 M NaOH, pH 9.6) for 16 h at 4°C and then washed with PBS, pH 7.5, 0.05% Tween-20 (wash buffer). Microtiter plate nonspecific-binding sites were blocked with 2% BSA in PBS and incubated for 90 min at 37°C. Plates were rinsed four times with wash buffer and diluted (neat and 1:10) cell-free supernatants (50 μl) in duplicate were added, followed by incubation for 1 h at 37°C. Plates were washed four times, followed by the addition of 50 μl/well biotinylated rabbit Abs against the specific cytokines (3.5 μg/ml in PBS (pH 7.5), 0.05% Tween-20, and 2% FCS), and plates incubated for 30 min at 37°C. Plates were washed four times, Streptavidin-peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA) was added, and the plates were incubated for 30 min at 37°C. Plates were washed again four times and chromogen substrate (Bio-Rad Laboratories) was added. The plates were incubated at room temperature to the desired extinction, and the reaction was terminated with 50 μl/well of 3 M H2SO4 solution. Plates were read at 490 nm in an ELISA reader. Standards were 1/2 log dilutions of recombinant murine cytokines from 1 pg/ml to 100 ng/ml. This ELISA method consistently detected murine cytokine concentrations above 25 pg/ml, and ELISA specificity was confirmed for each cytokine and chemokine measured.

**Determination of peritoneal CFU**

At the time of sacrifice, the abdominal wall was exposed. Two milliliters of sterile saline was injected into the peritoneal cavity. The peritoneal wash was then opened, and peritoneal wash fluids were aseptically collected and placed on ice. Serial 1:10 dilutions of peritoneal lavage samples were made. Ten microliters of each dilution was plated on soy base agar plates (Difco, Detroit, MI). Plates were incubated for 18 h at 37°C, after which colonies were counted.

**Gram staining of peritoneal washings**

Peritoneal wash samples were cytopun onto microscopy slides, and the slides were allowed to air dry. Prior to staining, the samples were heat fixed for approximately 10 s. All staining reagents were contained in a Gram Stain Kit (Fisher Scientific, Orangeburg, NY).

**Reagents**

As previously described, cytokine-specific Abs were generated in our laboratory for use in ELISAs and in immunneutralization experiments (23). Briefly, polyclonal IL-12-, IL-10-, and IFN-γ-specific antisera were gen-

**FIGURE 1.** CLP induces IL-12 production. CLP or sham surgeries were performed. Peritoneal lavage and plasma samples were collected at 6 h (A) and 24 h (B) after surgery. IL-12 concentrations were evaluated by ELISA. Experimental $n = 6–8$ per group.

uated in New Zealand White rabbits by multiple site immunization with the appropriate recombinant murine cytokine (R&D Systems, Minneapolis, MN) and adjuvant. Polyclonal Abs were titered by direct ELISA, and Ab specificity was verified for each ELISA. In the immunneutralization studies, each mouse received 0.5 ml of IL-12 specific antiserum. Primaxin I.V. (Merck, West Point, PA) was utilized in all experiments in which antibiotic treatments were administered. Primaxin contains equal proportions of imipenem and cilastatin. This antibiotic preparation was chosen, because imipenem is clinically effective in treating a wide variety of bacterial infections, including many caused by Gram-negative or Gram-positive aerobes and anaerobes. Furthermore, imipenem/cilastatin is often recommended for the treatment of polymicrobial, intraabdominal infections (24).

**Statistical analysis**

ANOVA followed by two-tailed $t$ testing was utilized to compare mean cytokine concentrations. Survival curves were analyzed by the log-rank test. The one-tailed Mann-Whitney test was used to compare bacterial CFU scattergrams. All calculations were performed on a Power Macintosh 7200 computer using Prism 2.0 (Graphpad Software, San Diego, CA). Significance was assigned for $p$ values $< 0.05$.

**Results**

**CLP induces IL-12 production**

IL-12 production in CLP animals was initially examined because of the well-documented proinflammatory properties of this cytokine and its previously identified role in various other models of sepsis (5, 19, 25–27). Mice underwent CLP or sham surgeries, and peritoneal and plasma IL-12 concentrations were analyzed by ELISA at 6 and 24 h after surgery (Fig. 1). IL-12 levels were undetectable in samples from the peritoneum and plasma of sham-operated mice at 6 and 24 h. However, animals experiencing CLP-induced sepsis produced significant levels of immunoreactive IL-12 at both time points.
Passive immunization of IL-12 in CLP mice increases mortality

IL-12 immunoneutralization was utilized to elucidate a functional role for IL-12 in the septic response. Rabbit anti-IL-12 immune serum or preimmune serum was injected i.p. 2 h prior to CLP surgery. This method of immunoneutralization proved to be clearly detrimental in the context of the CLP model, as the 54% survival rate observed in the control group (CLP mice given preimmune serum) diminished to 25% in CLP mice receiving IL-12 antisera (Fig. 2). The detrimental effects of anti-IL-12 treatment were most apparent in the first 48 h after CLP, as the survival rate dropped from 60 to 25%.

Passive immunization of IL-12 in CLP mice results in reduced production of IL-12 and IFN-γ concomitant with an increase in IL-10 production

To examine potential mechanisms leading to increased mortality in CLP mice treated with anti-IL-12 Abs, specific cytokine levels in peritoneal washings and plasma were next determined (Fig. 3). No IL-12 was detected in the serum or peritoneal washings of anti-IL-12 Ab-treated mice at 6 h after CLP surgery, while mice treated with preimmune serum contained IL-12 in both the serum and the peritoneum. Furthermore, at 6 h after CLP surgery, IL-12 immunoneutralization prevented the production of measurable amounts of IFN-γ in the peritoneum. This reduction in IFN-γ corroborates previous findings showing that IL-12 drives IFN-γ production (5). Interestingly, at 6 h after surgery, IL-10 was strikingly elevated in both the peritoneum and serum of CLP animals administered IL-12-specific Abs. A trend toward increased IL-10 production was also evident at the 24-h time point (Fig. 3). By 48 h after CLP, however, we did not observe any differences in the production of these key regulatory cytokines (data not shown). These data suggested that IL-12 immunoneutralization in the context of murine sepsis abrogates IL-12 and IFN-γ, possibly permitting the unregulated production of IL-10.

IL-12 neutralization decreases bacterial clearance in the peritoneum of CLP mice

It has previously been shown that IL-10 inhibits the antimicrobial host response (28), while IL-12 and IFN-γ promote innate immunity (8, 29). Thus, this study explored the possibility that anti-IL-12-treated CLP mice experienced an uncontrolled bacterial infection due to a lack of IL-12 and IFN-γ and a concomitant overproduction of IL-10. We specifically examined the presence of bacteria in the peritoneum at 24 h after CLP, because survival data (Fig. 2) suggested that IL-12 immunoneutralization altered CLP-induced mortality between the 24- and 48-h time points. At 24 h after CLP, mice treated with Abs to IL-12 displayed reduced clearance of bacteria, as indicated by greater numbers of cultured CFUs in the peritoneum (Fig. 4). Furthermore, 58% of CLP mice administered preimmune serum had no evidence of viable bacteria in the peritoneum, whereas only 33% of CLP mice treated with anti-IL-12 Ab appeared to have cleared all viable bacteria from the peritoneum. These data suggested that the inability to clear bacteria might be an important contributing factor to the increase in mortality found in those mice administered IL-12 Abs prior to CLP.

**FIGURE 2.** Passive immunization of CLP mice to IL-12 increases mortality. IL-12 antisera (αIL-12) or preimmune serum (control) was administered 2 h prior to CLP surgery. Time zero corresponds to time of CLP surgery. Experimental n = 45-47 per group; p < 0.05.

**IL-12 neutralization does not improve survival in antibiotic-treated CLP mice**

We next explored the specific role of IL-12 in the CLP model by coupling the administration of IL-12-specific Abs with a full course of antibiotic therapy. As previously described, mice were administered IL-12-specific antisera or preimmune serum i.p. 2 h prior to CLP surgery, after which the mice were dosed with Primaxin (200 μg/dose) at the time of surgery and every 8 h thereafter. In CLP mice administered preimmune serum, the antibiotic treatment improved survival from 46 to 66%, suggesting that Primaxin partially prevented the detrimental effects of bacterial leakage into the peritoneum (Fig. 5). Nonetheless, IL-12 neutralization remained deleterious in the context of antibiotic therapy, as the survival rate decreased below 50% in CLP mice treated with both anti-IL-12 serum and antibiotics. These results suggested that the role of IL-12 during CLP-induced sepsis may extend beyond the activation of the innate immune response.

**Passive immunization of IL-12 in the CLP model impairs the fibrinous wound-healing response in and around the injured cecum**

To examine why IL-12 neutralization continued to be detrimental in the context of antibiotic treatment, Gram stains were performed on cytospins from peritoneal lavages of CLP mice treated with antibiotics and anti-IL-12 or preimmune serum. At 4 days after CLP surgery, bacteria were present in peritoneal fluids from mice administered both IL-12 antisera and antibiotics (Fig. 6B). In contrast, Gram stains of peritoneal fluids from control mice receiving preimmune serum and antibiotics revealed no evidence of bacteria (Fig. 6A). Upon gross morphological examination of the abdominal cavities of CLP mice, those mice administered preimmune serum and antibiotics displayed extensive fibrotic adhesions arising from the cecum. Whereas CLP mice given IL-12 antisera and antibiotics showed no similar response. Furthermore, histological examination of cross-sections of the cecum revealed the formation of a fibrin membrane or “pseudomembrane” on the lumen side of the cecum in control mice treated with preimmune serum and antibiotics, whereas this response was not evident in CLP mice treated with IL-12 Abs (Fig. 7). This pseudomembrane appeared to be composed of cellular debris, inflammatory cells, and extracellular matrix. Although differences in cellular recruitment were not directly assessed, at 4 days after CLP surgery ELISAs revealed no differences in several important chemotactic cytokines. However, increased levels of IFN-γ in the peritoneum 4 days after CLP surgery corresponded with the lack of pseudomembrane formation in the anti-IL-12 Ab-treated mice (data not shown). Increased IFN-γ production may have contributed to the delay in pseudomembrane formation or the fibrinous response, and as a consequence may have
contributed to the increased mortality observed in CLP mice passively immunized to IL-12 and treated with antibiotics.

Discussion
The present study queried the following possibilities. 1) Could IL-12 neutralization render a cytokine profile that was less favorable to the innate immune response during sepsis? 2) Since Th cell-mediated responses have been shown to be reciprocally modulated by IL-12 and IL-10 (20, 21), could IL-10 and IL-12 antagonistically regulate each other in an acute inflammatory disease, such as CLP-induced murine sepsis? Our observation that CLP induced IL-12 production provided the impetus to assess the contribution of this cytokine by systemically neutralizing IL-12 in the context of CLP-induced sepsis. The administration of

FIGURE 3. Immunoneutralization of IL-12 in CLP mice results in reduced IFN-γ concomitant with increased IL-10 production at 6 h and 24 h. IL-12 antisera or preimmune serum was administered at 2 h prior to CLP surgery. Peritoneal and plasma samples were collected at 6 h and 24 h after CLP surgery. Cytokine concentrations were measured by ELISA. Experimental n = 6–9 per group. * p < 0.05 as compared with preimmune serum control.
anti-IL-12 polyclonal Abs 2 h prior to CLP surgery resulted in a significant decrease in survival. One possible reason for this decreased survival could have been some treatment-induced physiological imbalance. However, this explanation seemed unlikely as IL-12 has been identified as a deleterious byproduct in various physiological models of septic shock, and IL-12 neutralization has proved beneficial in an endotoxin-induced inflammatory response (26, 30). Conversely, it seemed probable that CLP mice required IL-12 production to contain the bacteria, as evidenced by several studies with various infection models, which have shown that an IL-12-mediated innate immune response is vital for the resolution of infection (6, 8, 9, 31). Accordingly, we quantitated total bacterial CFU in the peritoneal cavities of CLP mice treated with preimmune or anti-IL-12 serum, and found that the immunoneutralization of IL-12 in this model reduced bacterial clearance in the peritoneum. The results from the present study agree with previous observations that used a neonatal, murine sepsis model caused by group B streptococci in which IL-12 neutralization induced lethality by facilitating bacterial infection (19). We subsequently sought to examine how IL-12 contributed to the containment of the bacterial peritonitis associated with the CLP model.

While it has previously been shown that recurring peritoneal infection is not directly responsible for the poor outcome associated with peritonitis (32), the manipulation of the balance between pro- and anti-inflammatory cytokines following the immunoneutralization of IL-12 may permit lethal bacterial colonization of the

FIGURE 4. The immunoneutralization of IL-12 reduces peritoneal bacterial containment. Mice were treated with either IL-12-specific antisera (αIL-12) or preimmune serum (control) 2 h prior to CLP surgery, and peritoneal lavages were performed at 6 (A) or 24 h (B) after surgery. Experimental n = 9–21. Line represents median CFU count, ∗, p < 0.05 as compared with preimmune serum control.

FIGURE 5. The immunoneutralization of IL-12 in antibiotic-treated CLP mice reduces survival compared with CLP mice treated with preimmune serum and antibiotics. IL-12-specific antisera or control serum was administered 2 h prior to CLP surgery. Antibiotic treatments (Primaxin: 200 µg/dose) were administered immediately after CLP surgery and every 8 h thereafter. Experimental n = 15–47 per group.

FIGURE 6. Gram staining of peritoneal wash cytospins at 4 days after CLP surgery. Control (A): pretreatment with preimmune serum followed by full course of antibiotic therapy. Experimental (B): pretreatment with IL-12-specific antisera followed by full course of antibiotic therapy. IL-12-specific antiserum or control serum was administered 2 h prior to CLP surgery. Antibiotic treatments (Primaxin: 200 µg/dose) were administered immediately after CLP surgery and every 8 h thereafter. Representative of 7–10 samples per group.
peritoneum. An examination of cytokine concentrations substantiated this hypothesis, as the diminished presence of immunoreactive IL-12 resulted in decreased IFN-γ in favor of elevated IL-10. Although IL-12 was classically described as an important mediator of Th1 phenotypic cell-mediated immune responses, recently more diverse roles have been ascribed to this cytokine. The contribution of IL-12 to innate immune activation appears especially relevant in the context of an active bacterial infection, as host responses to a gamut of intracellular and extracellular pathogens necessitates IL-12 activity. Furthermore, monocyte-derived IL-12 production has been shown to be vital in human patients suffering from postoperative sepsis (33). Although, IL-12 can directly augment host innate immunity by increasing cytolytic activity of CD8+ and NK cell populations, its most important contribution to host defense in the CLP model may be indirect inflammatory cell activation via up-regulation of IFN-γ production by NK and T cells (5, 11, 34). As a hallmark cytokine of the innate immune response, IFN-γ up-regulates HLA-DR expression, primes macrophages for enhanced TNF and IL-1β synthesis, and functions to stimulate PMN and macrophage microbicidal activity. Conversely, IL-10 serves an important immunoregulatory role during an inflammatory response by modulating potent inflammatory processes and by preventing tissue injury and/or shock. In addition, there is well-documented evidence for the production of important activating and/or chemotactic cytokines and by inhibiting neutrophil and macrophage phagocytic and bactericidal activity (14, 37–41). The CLP model mirrors many of the common clinical symptoms of a septic response to polymicrobial infection. Thus, it is likely that excessive IL-10 production without a sufficient IL-12- and IFN-γ-mediated response may prevent disease resolution. It appears that in the CLP model, IL-12 neutralization effects an imbalanced cytokine profile in the peritoneal cavity, resulting in profound, early suppression of the innate immune response leading to increased lethality in this bacterial sepsis model.

To further determine whether bacterial colonization associated with IL-12 neutralization increased sepsis-induced lethality, anti-IL-12 polyclonal Abs were administered, followed by antibiotic treatment. CLP mice receiving preimmune serum or IL-12 benefited from antibiotic therapy, yet IL-12 immunoneutralization resulted in a worse prognosis for CLP mice treated with antibiotics. Despite aggressive antibiotic treatments in the anti-IL-12 Ab treatment group, we explored the possibility that the septic mice undergoing IL-12 immunoneutralization still suffered from bacterial contamination. In order to explore this hypothesis, mice were sacrificed at 4 days after CLP surgery. Gram stains of peritoneal wash cytospins revealed no obvious bacterial presence in those mice administered antibiotics in addition to preimmune serum, whereas CLP mice treated with anti-IL-12 serum and antibiotics showed clear evidence of bacteria in the peritoneum. This positive identification of bacteria in the peritoneum at 4 days after CLP suggested either 1) a persistent bacterial colonization within the peritoneal cavity or 2) some deficiency in the containment of intestinal microbes within the bowel. An examination of the injured ceca suggested the latter, as CLP mice receiving preimmune serum and antibiotic therapy displayed fibrotic encasement of the cecum, with fibrous adhesions to other portions of the bowel. Histologic examination of cross-sections of the damaged ceca revealed that CLP mice undergoing antibiotic treatment had developed a protective membrane on the lumen side of the cecum wall. In contrast, CLP mice administered IL-12-specific Abs and antibiotics were deficient in both the fibrotic response around the cecum and lacked evidence of pseudomembrane formation inside the cecum wall. While inflammation of the intestinal wall leading to fibrosis is a well-recognized response in chronic inflammatory bowel diseases (42), the present study suggests that the fibrotic containment of the damaged cecum is an important protective mechanism in the acute response to CLP induced sepsis, serving to separate the lumen and its contents from the damaged cecum wall. These data suggest that the immunoneutralization of IL-12 either prevented or delayed the development of a protective barrier to the further infiltration of the peritoneum by intestinal microbes. Without this mechanism of containing the damaged cecum from the rest of the body, neither the bacterial killing response of the host nor frequent antibiotic treatments quelled the bacterial infection that appeared to contribute to the observed lethality. While the lack of a healing or fibrotic response in the anti-IL-12 Ab-treated mice is currently being explored, it is postulated that the repair of the damaged cecum is analogous to wound repair in other parts of the body. This process

**FIGURE 7.** Representative composite cecum histologic sections from CLP control (preimmune serum + antibiotics) or experimental (IL-12 antisera + antibiotics) mice. IL-12-specific antisera or control serum was administered 2 h prior to CLP surgery. Antibiotic treatments (Primaxin: 200 μg/dose) were administered immediately after CLP surgery and every 8 h thereafter. Cecum samples were collected 4 days after CLP surgery. A and B represent the preimmune/antibiotics group at ×100 and ×400 respective magnifications. C and D represent the IL-12 antisera/antibiotics group at ×100 and ×400 respective magnifications. L = lumen; M = membrane. Representative of 7–10 samples per group.
requires a sequence of events that begins with inflammation and is followed by tissue restoration and resolution (43). The early damping of the inflammatory response by IL-12 immunoneutralization may prevent normal progression to subsequent stages of repair. The twofold increase in IFN-γ production at 96 h by CLP mice administered both IL-12 antisera and antibiotics is suggestive of prolonged inflammation, without progress to tissue restoration and resolution. While increased IFN-γ production may be the natural effect of a continued cellular response to uncontrolled bacterial infection, this cytokine also functions as a potent inhibitor of wound repair and fibrotic processes (44–46).

In the CLP model of bacterial sepsis, IL-12 function is complex, and includes the following aspects: 1) IL-12 is a vital cofactor in the innate immunity against bacteria contained in spilled fecal matter and 2) IL-12 is involved in the containment of bacteria within the damaged cecum by directly (or indirectly) stimulating the fibrotic organization of the cecum wall. Furthermore, the latter roles for IL-12 in the septic response appeared to be critical to the survival of CLP mice, as antibiotic treatment failed to spare anti-IL-12 Ab-treated mice. Treatment of sepsis in humans has proved highly ineffective in preventing mortality, possibly casting importance upon the concept of therapeutic manipulation of cytokine cascades in septic patients. Accordingly, the present study warrants additional investigation into the role of cytokine balance during sepsis, while perhaps validating a reexamination of the clinical treatment avenue of single cytokine modulation.

Acknowledgment

We thank Robin G. Kunkel for her artistic help.

References