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IL-10-Deficient Mice Demonstrate Multiple Organ Failure and Increased Mortality During Escherichia coli Peritonitis Despite an Accelerated Bacterial Clearance

Miguel E. Sewnath,* Dariusz P. Olszyna,‡‡ Rakesh Birjmohun,* Fiebo J. W. ten Kate,§ Dirk J. Gouma,* and Tom van der Poll†‡

To determine the role of endogenous IL-10 in local antibacterial host defense and in the development of a systemic inflammatory response syndrome during abdominal sepsis, IL-10 gene-deficient (IL-10−/−) and wild-type (IL-10+/+) mice received an i.p. injection with Escherichia coli. Peritonitis was associated with a bacterial dose-dependent increase in IL-10 concentrations in peritoneal fluid and plasma. The recovery of E. coli from the peritoneal fluid, blood, and lungs was diminished in IL-10−/− mice, indicating that endogenous IL-10 impaired bacterial clearance. Despite a lower bacterial load, IL-10−/− mice had higher concentrations of TNF, macrophage inflammatory protein-2 and keratinocyte in peritoneal fluid and plasma, and demonstrated more severe multiple organ damage as indicated by clinical chemistry and histopathology. Furthermore, IL-10−/− mice showed an increased neutrophil recruitment to the peritoneal cavity. To examine the role of elevated TNF levels in the altered host response in IL-10−/− mice, the effect of a neutralizing anti-TNF mAb was determined. Anti-TNF did not influence the clearance of E. coli in either IL-10+/+ or IL-10−/− mice. Furthermore, anti-TNF did not affect leukocyte influx in the peritoneal fluid, multiple organ damage, or survival in IL-10+/+ mice. In IL-10−/− mice, anti-TNF partially attenuated neutrophil recruitment and multiple organ damage, and prevented the increased lethality. These data suggest that although endogenous IL-10 facilitates the outgrowth and dissemination of bacteria during E. coli peritonitis, it protects mice from lethality by attenuating the development of a systemic inflammatory response syndrome by a mechanism that involves inhibition of TNF release. The Journal of Immunology, 2001, 166: 6323–6331.

Peritonitis continues to be one of the major abdominal emergencies causing high in-hospital morbidity and mortality rates up to 38% (1–3). Although the overall mortality of sepsis is ∼35% (4), abdominal sepsis is associated with mortality rates up to 80% (5). Although different bacteria have been identified as causative organisms in peritonitis, Escherichia coli remains one of the most common pathogens (up to 60%) in intra-peritoneal infections (6, 7). Surgical and supportive treatment of peritonitis often do not suffice, and an increase in resistance to many antibiotic compounds has developed (8, 9), especially among the Enterobacteriaceae, where some isolates have acquired extended spectrum β-lactamases (10, 11). More knowledge of the regulation of inflammatory responses during peritonitis is warranted.

Cytokines play an important role in the pathogenesis of bacterial infections (12). In models of severe systemic infection or inflammation produced by i.v. administration of high doses of bacteria or bacterial products such as endotoxin, excessive production of proinflammatory cytokines significantly contributes to organ failure and death, as reflected by findings that neutralization of either TNF or IL-1 activity markedly reduced mortality in these systemic challenge models (13–15). However, in experiments in which an at least initially localized infection was induced, including pneumonia and peritonitis, the local activity of proinflammatory cytokines appeared important for antibacterial host defense at the site of the infection (16–19). Together these data suggest that proinflammatory cytokines function as “double-edged swords,” on the one hand required locally for effective antibacterial effector mechanisms, in contrast potentially toxic when secreted into the circulation.

IL-10 is an 18-kDa cytokine produced under different conditions of immune activation by a variety of cell types, including T cells, B cells, monocytes, and macrophages (20, 21). IL-10 is considered a prototypic anti-inflammatory cytokine and potently inhibits the production of proinflammatory cytokines in vitro and in vivo (22–26). Several animal studies have pointed to an important role of IL-10 in the pathogenesis of bacterial infection. Elevated plasma concentrations of IL-10 have been found in patients with sepsis (27, 28). In mouse models of systemic inflammation induced by injection of endotoxin, IL-10 serves a protective role. Indeed, elimination of endogenous IL-10 resulted in an increased production of several proinflammatory cytokines, including TNF, and an enhanced mortality (29, 30). Similarly, IL-10 gene-deficient (IL-10−/−) mice demonstrated an enhanced mortality after endotoxin injection, which was associated with elevated levels of TNF and several other proinflammatory mediators (31). The role of endogenous IL-10 in localized bacterial infection is less unequivocal. During murine pneumonia, IL-10 produced within the pulmonary compartment impaired host defense against invading bacteria, as reflected by findings that treatment with anti-IL-10 Abs inhibited

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bacterial outgrowth in lungs and improved survival (32, 33). However, during septic peritonitis induced by cecal ligation and puncture (CLP) elimination of IL-10 was associated with an increased mortality (34, 35). The mechanisms by which anti-IL-10 treatment increased mortality during peritonitis were not elucidated in these previous studies. In particular, no attempts were undertaken to determine the effect of IL-10 on bacterial clearance from the peritoneal cavity and on the development of a systemic inflammatory response syndrome. Therefore, in this study we sought to determine the influence of endogenous IL-10 on host defense mechanisms during *E. coli* peritonitis, making use of IL-10−/− mice.

**Materials and Methods**

**Animals**

Male C57BL/6 wild-type (IL-10+/+) mice were purchased from Harlan CPB (Zeist, The Netherlands). C57BL/6 IL-10−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were housed (five per cage) in the same temperature-controlled room with alternating 12-h light/dark cycles, and were allowed to equilibrate for at least 5 days before the study. Animals were provided regular mice chow (SRM-A; Hope Farms, Woerden, The Netherlands) and water ad libitum. Age (8–10 wk) and sex-matched IL-10+/+ and IL-10−/− mice were used in all experiments. The experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, Amsterdam, The Netherlands.

**Antibodies**

Rat anti-mouse TNF neutralizing mAb was provided by David Shealy (Centocor, Malvern, PA). Rat IgG2a (clone R7D4) was used as control Ab. Abs were given i.p. in a dose of 0.5 mg 2 h before induction of peritonitis.

**Induction of peritonitis**

*E. coli* O18:K1 was cultured in Luria Bertani medium (LB; Difco, Detroit, MI) at 37°C, harvested at mid-log phase, and washed twice with sterile saline before injection to clear the bacteria of medium. Mice were injected i.p. with 105, 103, or 104 viable *E. coli* O18:K1 CFU in 200 μl sterile isotonic saline. The inoculum was plated immediately after inoculation on blood agar plates to determine viable counts. Control mice received 200 μl normal saline.

**Monitoring of mortality and organ and blood sampling**

In survival studies, mortality was assessed every 12 h during the first 4 days after *E. coli* challenge. In preliminary studies, mortality occurred predominantly between 24 and 36 h after *E. coli* challenge; therefore, mortality was assessed every hour in this period. Mice that survived >3 days appeared to be permanent survivors.

At time of sacrifice, mice were first anesthetized by inhalation of isoflurane (Abbott Laboratories, Kent, U.K.), 2% (2/2 liters). A peritoneal lavage was then performed with sterile isotonic saline using an 18-gauge needle, and peritoneal lavage fluid was collected in sterile tubes (Plastipack; BD Biosciences, Mountain View, CA). The recovery of peritoneal fluid after 103 CFU, IL-10−/− mice could be measured at any time point.

**Histologic analysis**

Shortly after killing, samples from all liver lobes and other parenchymal organs were removed, fixed in 4% formalin, and embedded in paraffin for routine histology. Sections of 2–5 μm thickness were stained with hematoxylin and eosin. Histologic examination was performed on coded samples by two independent investigators, blinded for treatment groups.

**Assays**

Cytokines and chemokines were measured by ELISAs according to the recommendations of the manufacturer (with detection limits in pg/ml), i.e., TNF (31.2) (Genzyme, Cambridge, MA), IL-10 (24.7) (PharMingen, San Diego, CA), macrophage inflammatory protein-2 (MIP-2) (9.6), and keratinocyte (KC) (4) (both obtained from R&D Systems, Minneapolis, MN). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and amylase were determined with commercially available kits (Sigma, St. Louis, MO), using a Hitachi analyzer (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s instructions.

**Statistical analysis**

All values are given as means ± SE. Comparisons were done by unpaired Student’s t tests with Bonferroni correction where appropriate. Survival curves were compared with the log-rank test. Values of *p* < 0.05 were considered to represent a significant difference.

**Results**

**Induction of IL-10**

In normal wild-type mice, peritonitis was associated with elevated IL-10 concentrations in both plasma and peritoneal fluid at 6 and 24 h after infection (Fig. 1). IL-10 levels increased with increasing doses of *E. coli*, and at each bacterial dose were ~5-fold higher in peritoneal fluid than in plasma. After inoculation with 102 CFU *E. coli*, IL-10 levels in peritoneal fluid and plasma peaked at 6 h, whereas after infection with higher doses, 103 and 104 CFU *E. coli*, IL-10 levels peaked after 24 h. Mice that were i.p. injected with sterile saline did not have detectable IL-10 in peritoneal fluid or blood. In addition, in IL-10−/− mice no IL-10 immunoreactivity could be measured at any time point.

**IL-10−/− mice have an enhanced bacterial clearance**

Having established that IL-10 is produced during peritonitis, we wished to determine the role of endogenous IL-10 in antibacterial defense. For this purpose bacterial outgrowth was determined at 6 and 24 h after induction of peritonitis by either 102, 103, or 104 CFU *E. coli* (Fig. 2). We counted CFU in three body compartments: 1) the peritoneal cavity (the site of the infection), 2) blood (to evaluate to which extent the infection became systemic), and 3) the lung (an organ distant from the primary site of infection). At 6 h after inoculation with any of the three doses, similar numbers of CFU were recovered from IL-10−/− and IL-10+/+ mice, except for peritoneal fluid obtained from IL-10−/−/ mice after infection with 103 CFU, which showed more CFU than peritoneal fluid obtained from IL-10−/− mice inoculated with this dose (*p* < 0.05). At 24 h after infection with 102 or 103 CFU, from only some of the IL-10−/−/ mice, but none of the IL-10−/− mice, *E. coli* could be recovered from peritoneal fluid, blood, and lungs (*p* < 0.05 for lungs after both 102 and 103 CFU, IL-10−/−/+ vs IL-10−/−; *p* < 0.05 for peritoneal fluid after 103 CFU, IL-10−/−/+ vs IL-10−/−). At 24 h after infection with 104 CFU, IL-10−/−/+ mice had significantly more CFU in their peritoneal fluid, blood, and lungs than IL-10−/− mice (all *p* < 0.05). Hence, overall outgrowth of *E.
coli was impaired in IL-10$^{-/-}$ mice when compared with IL-10$^{+/+}$ mice in all body compartments tested. Subsequent studies on host defense mechanisms during peritonitis in IL-10$^{-/-}$ and IL-10$^{+/+}$ mice were performed using a bacterial inoculum of $10^3$ CFU.

IL-10$^{-/-}$ mice have an increased neutrophil recruitment to the peritoneal cavity

Because leukocytes play an important role in the local host defense against invading bacteria, we next determined leukocyte counts and differentials in peritoneal fluid during peritonitis. Intraperitoneal administration of $10^3$ CFU E. coli resulted in an influx of leukocytes into the peritoneal fluid, which was mainly associated with an increase in neutrophil numbers and which was especially apparent at 6 h after infection (Table I). At this early time point, IL-10$^{-/-}$ mice had more neutrophils in their peritoneal fluid than IL-10$^{+/+}$ mice ($p < 0.05$). Peritoneal fluid leukocyte numbers decreased between 6 and 24 h postinfection in both mouse strains, although the number of neutrophils recovered from peritoneal fluid of IL-10$^{-/-}$ mice at 24 h remained higher ($p < 0.05$).

FIGURE 1. E. coli peritonitis is associated with a dose-dependent increase in IL-10 concentrations in peritoneal fluid and plasma. Normal wild-type mice received an i.p. injection with $10^2$, $10^3$, or $10^4$ CFU E. coli, and IL-10 levels were measured after 6 and 24 h. Data represent mean ± SE of eight mice per time point for each bacterial inoculum. Note that the y-axis scale is different for the lowest bacterial dose. IL-10 remained undetectable in mice that received an i.p. injection with sterile saline.

FIGURE 2. Diminished recovery of E. coli in IL-10$^{-/-}$ mice. IL-10$^{+/+}$ and IL-10$^{-/-}$ mice received an i.p. injection with $10^2$, $10^3$, or $10^4$ CFU E. coli, and CFUs were counted in peritoneal fluid, blood, and lungs after 6 and 24 h. Filled symbols represent IL-10$^{+/+}$ mice; open symbols indicate IL-10$^{-/-}$ mice. Horizontal lines represent medians.
**ROLE OF IL-10 DURING E. coli PERITONITIS**

**IL-10 mice have elevated TNF and chemokine concentrations in peritoneal fluid and plasma**

Because IL-10 has been found to inhibit TNF production in vitro and in vivo (20–26), and because TNF is considered an important proinflammatory mediator in bacterial infection (12), we were interested in TNF release in peritoneal fluid and plasma during peritonitis in IL-10+/+ and IL-10−/− mice. Peritonitis was associated with an increase in TNF concentrations in both peritoneal fluid and plasma (Fig. 3, upper panels). IL-10−/− mice had higher TNF concentrations in peritoneal fluid and plasma than IL-10+/+ mice (p < 0.05).

CXC chemokines have been implicated in the attraction of neutrophils to the site of an infection (36). Therefore, we measured the main mouse CXC chemokines MIP-2 and KC in peritoneal fluid and plasma (Fig. 3, lower panels). Intrapерitoneal administration of *E. coli* resulted in a rise in peritoneal fluid and plasma MIP-2 and KC levels. IL-10−/− mice had higher levels of MIP-2 and KC in peritoneal fluid and plasma than IL-10+/+ mice (p < 0.05).

**IL-10−/− mice have more severe multiple organ damage**

Abdominal sepsis can be associated with multiple organ failure (37). To determine the role of endogenous IL-10 herein, we measured biochemical parameters of liver damage (ASAT, ALAT), pancreas damage (amylase), and renal failure (creatinine) at 24 h after i.p. injection of 10⁷ *E. coli* CFU (Fig. 4). IL-10−/− mice demonstrated biochemical evidence of more severe multiple organ damage than IL-10+/+ mice, as reflected by higher ALAT, ASAT, amylase, and creatinine concentrations (all p < 0.05).

Histology of parenchymatous organs of IL-10+/+ and IL-10−/− mice (age 8–10 wk) without *E. coli* infection was similar and displayed no signs of abnormalities. However, in line with the laboratory findings indicative for multiple organ damage, IL-10−/− mice displayed more severe histopathological damage of parenchymatous organs than IL-10+/+ mice at 24 h after infection (Fig. 5). Vascular congestion was generalized in all organs examined, and more obvious in IL-10−/− mice compared with IL-10+/+ mice. In the livers of IL-10+/+ mice, thrombotic occlusion in portal areas, enlargement of portal tracts by mononuclear cells, micro and macrovacuolar steatosis, and scattered foci of hepatocellular degeneration and necrosis were noted. These changes were more pronounced in livers of IL-10−/− mice, especially with respect to the extent of hepatocellular necrosis, characterized by garland-shaped necrotic areas, covering almost half of the liver parenchyma, whereas in IL-10+/+ this was far less. When inspecting the lungs macroscopically, the lungs of IL-10+/+ mice were less hyperemic and showed fewer hemorrhagic spots compared with IL-10−/− mice. In addition, lungs of both mouse strains displayed macroscopic hemorrhage and generalized endovasculitis. IL-10+/+ mice showed fewer thrombotic lesions and inflammatory infiltrates in the alveolar septa than IL-10−/− mice. Noteworthy, IL-10+/+ mice suffered more often from bacterial pleuritis with large purulent exudates. Increased levels of creatinine were predominantly due to prerenal failure and general tissue deterioration (e.g., muscle tissue) because there were only mild inflammatory changes in the kidneys with sporadic necrotic tubular cells.

**Role of elevated TNF levels in altered host responses to peritonitis in IL-10−/− mice**

TNF is considered a central mediator in the early host response to bacterial infection. On the one hand, high levels of TNF in the circulation can cause severe tissue toxicity and organ damage (38, 39). In contrast, at local tissue level, TNF contributes to an effective host defense (16–19). Because IL-10−/− mice displayed elevated levels of TNF in both peritoneal fluid and plasma (Fig. 3), we were interested to determine the role of enhanced TNF release in the altered host responses to peritonitis in IL-10−/− mice.

![Image](https://www.jimmunol.org/)

**FIGURE 3.** IL-10−/− mice demonstrate elevated TNF, MIP-2, and KC concentrations in peritoneal fluid and plasma during *E. coli* peritonitis. IL-10+/+ and IL-10−/− mice received an i.p. injection with 10⁷ CFU *E. coli*, and TNF, MIP-2, and KC concentrations were measured in peritoneal fluid and plasma after 6 and 24 h. Filled bars represent IL-10+/+ mice; open bars indicate IL-10−/− mice. Data are mean ± SE of eight mice per time point for each mouse strain.

**Table I. Leukocyte counts in peritoneal lavage fluid**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IL-10+/+</th>
<th>IL-10−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>T6h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cells (×10⁶/ml)</td>
<td>5.48 ± 0.42</td>
<td>8.31 ± 2.32*</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.56 ± 0.65</td>
<td>6.57 ± 0.99*</td>
</tr>
<tr>
<td>Macrophages</td>
<td>1.32 ± 0.23</td>
<td>1.16 ± 0.25</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.60 ± 0.11</td>
<td>0.58 ± 0.07</td>
</tr>
<tr>
<td>T24h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cells (×10⁶/ml)</td>
<td>1.23 ± 0.51</td>
<td>3.32 ± 0.59*</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.72 ± 0.59</td>
<td>2.16 ± 0.65*</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.43 ± 0.13</td>
<td>0.93 ± 0.48</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.08 ± 0.05</td>
<td>0.23 ± 0.12</td>
</tr>
</tbody>
</table>

Data are mean ± SE (n = 8 mice per group for each time point) at 6 or 24 h after i.p. administration of *E. coli* (10⁷ CFU).

* p < 0.05 vs IL-10+/+.
Therefore, we pretreated IL-10<sup>+/+</sup> and IL-10<sup>-/-</sup> mice with a neutralizing anti-mouse TNF mAb or an irrelevant control mAb 2 h before infection with 10<sup>3</sup> E. coli CFU and determined bacterial outgrowth, leukocyte counts in peritoneal fluid, and biochemical parameters of organ damage at 24 h after inoculation. In these experiments, IL-10<sup>-/-</sup> mice treated with the control mAb showed fewer E. coli CFU in peritoneal fluid, blood, and lungs, an enhanced influx of cells to the peritoneal cavity, and increased levels of biochemical parameters of liver and pancreas damage and renal failure when compared with IL-10<sup>+/+</sup> mice treated with the control

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** IL-10<sup>-/-</sup> mice demonstrate enhanced multiple organ damage as reflected by clinical chemistry. IL-10<sup>+/+</sup> and IL-10<sup>-/-</sup> mice received an i.p. injection with 10<sup>3</sup> CFU E. coli, and ALAT, ASAT, and creatinin concentrations were measured in plasma after 24 h. Filled bars represent IL-10<sup>+/+</sup> mice; open bars indicate IL-10<sup>-/-</sup> mice. Data are mean ± SE of eight mice for each mouse strain. Dotted lines represent the mean values obtained from normal plasma of mice that were i.p. injected with sterile saline (six mice).

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** Histopathology. Representative histological pictures of liver, lung, and spleen of IL-10<sup>-/-</sup> (A, C, and E) and IL-10<sup>+/+</sup> (B, D, and F) mice at 24 h after i.p. injection of E. coli (10<sup>3</sup> CFU). Liver tissue in IL-10<sup>+/+</sup> mice showed small necrotic areas (arrowheads) compared with the garland-like necrotic areas in IL-10<sup>-/-</sup> mice. More obvious thrombotic lesions (arrows) and a higher influx of polymorphonuclear granulocytes in portal areas were found in IL-10<sup>-/-</sup> mice compared with IL-10<sup>+/+</sup> mice. CV, central vein; PV, portal vein. Lungs revealed mild alveolar congestion and cellular infiltrates in both mouse strains; however, lungs of IL-10<sup>-/-</sup> mice displayed more extensive thrombotic lesions (arrows) compared with IL-10<sup>+/+</sup> mice. Spleens of IL-10<sup>+/+</sup> mice displayed increased congestion of the red pulp, which contained numerous hemopoietic cells, including megakaryocytes, extensive reactive changes of the white pulp with numerous “starry sky” macrophages with ingested debris, and thrombotic occlusion of the vessels (arrows). All these splenic pathologic changes were more profound in IL-10<sup>-/-</sup> mice. Noteworthy is the extensive purulent exudate with numerous clots of bacteria on the surface of the spleen, which was more pronounced in IL-10<sup>-/-</sup> mice compared with IL-10<sup>+/+</sup> mice. Similar inflammatory infiltrates and accumulation of bacteria were found in areas near the liver capsule (data not shown). Slides shown are representative of a total of eight mice per group. (hematoxylin and eosin staining, original magnification ×25).
mAb (Figs. 6 and 7, Table II), confirming the experiments presented in Figs. 2 and 4, and Table I. Anti-TNF did not significantly influence the number of *E. coli* CFUs recovered from peritoneal fluid, blood, or lungs in either IL-10<sup>1/1</sup> or IL-10<sup>2/2</sup> mice (Fig. 6). In addition, in IL-10<sup>1/1</sup> mice anti-TNF did not influence leukocyte recruitment to the peritoneal cavity (Table II) or biochemical evidence of multiple organ damage (Fig. 7). However, in IL-10<sup>2/2</sup> mice, anti-TNF reduced inflammatory responses to *E. coli* peritonitis, i.e., anti-TNF attenuated leukocyte influx and especially neutrophil influx in peritoneal fluid (*p*, 0.05 vs IL-10<sup>2/2</sup> mice treated with control mAb; Table II), and diminished the rises in ALAT, ASAT, amylase, and creatinine (all *p*, 0.05; Fig. 7).

Role of IL-10 and TNF in survival

To examine the role of endogenous IL-10 and TNF in lethality induced by peritonitis, IL-10<sup>1/1</sup> and IL-10<sup>2/2</sup> mice were pre-treated (2 h) with either anti-TNF or control mAb, inoculated i.p. with 10<sup>8</sup> CFU *E. coli*, and followed for 10 days (Fig. 8). All deaths occurred between days 1 and 3; mice surviving for 3 days appeared permanent survivors. IL-10<sup>2/2</sup> mice treated with control mAb died earlier and to a greater extent than IL-10<sup>1/1</sup> mice treated with control mAb (13/19 or 68% vs 6/18 or 33%; *p* < 0.05). This survival disadvantage of IL-10<sup>2/2</sup> mice treated with control mAb (13/19 or 68% vs 6/18 or 33%; *p* < 0.05) disappeared after treatment with anti-TNF (mortality 7/18 or 39%, nonsignificant vs IL-10<sup>1/1</sup> mice treated with control mAb). Anti-TNF tended to increase survival in IL-10<sup>1/1</sup> mice (nonsignificant).

### Discussion

The cytokine network plays a pivotal role in the orchestration of inflammatory responses to bacterial infection. The balance between pro- and anti-inflammatory cytokines critically influences the function of immunocompetent cells and the resistance against infection. It has been suggested that the cytokine network can act as a double-edged sword during infection, i.e., whereas in an infected organ a predominantly proinflammatory response contributes to the effective clearance of bacteria, at the systemic level such a response may be harmful to the host (12). Although this hypothesis is plausible, only few studies have simultaneously examined...
bacterial clearance from an infected body site and signs of systemic inflammation in a clinically relevant model of sepsis. Intraperitoneal administration of live *E. coli* results in a paradigm that resembles a clinical condition commonly associated with septic peritonitis, with diaphragmatic lymphatic clearance, and systemic bacteremia and endotoxemia (40). We chose this model to examine the role of IL-10, the prototypic anti-inflammatory cytokine, and TNF, the prototypic proinflammatory cytokine, in the antibacterial defense on the one hand, and in the development of a systemic inflammatory response syndrome on the other hand. The main findings of our study were that during septic peritonitis endogenous IL-10 impairs bacterial clearance from the peritoneal cavity and facilitates dissemination of bacteria to distant organs, yet attenuates the systemic inflammatory reactions and multiple organ failure associated with this abdominal sepsis syndrome by a mechanism that in part involves inhibition of TNF production. Consequently, endogenous IL-10 protected against lethality during abdominal sepsis despite hampering antibacterial effector mechanisms.

The protective role of endogenous IL-10 in systemic inflammation induced by a bolus dose of endotoxin has been established in a number of investigations (29–31). However, in these studies the potential disadvantageous effects of IL-10 on the local outgrowth of bacteria in an infected organ and the subsequent dissemination of bacteria, resulting in sepsis, could not be examined. The role of IL-10 in localized bacterial infection seems to depend on the organ that is infected. Indeed, immunoneutralization of IL-10 in mouse models of Gram-negative or Gram-positive pneumonia was associated with dissemination of bacteria to other sites in the body. How- ever, this did not result in an exaggerated systemic inflammatory response syndrome. Endogenous IL-10 was found to inhibit the local and systemic release of TNF, which at least in part appeared associated to the development of multiple organ damage and death. These data illustrate the importance of inflammation in lethality induced by abdominal bacterial infection, i.e., the inflammatory response induced by the bacteria, tightly controlled by endogenous IL-10, rather than the bacterial load itself, determined the outcome. It remains to be established why in pulmonary infection models elimination of IL-10 improved survival (32, 33). Conceivably, such pneumonia models are not associated with a systemic inflammatory response syndrome and multiple organ damage, an issue that was not investigated in these reports (32, 33). If this is true, the beneficial inhibitory effects of endogenous IL-10 on systemic inflammation would not play a significant role in the outcome of experimental pneumonia.

Treatment of normal wild-type mice with anti-TNF did not significantly influence the course of *E. coli* peritonitis. This finding is in line with previous studies that reported no effect of anti-TNF treatment on survival during peritonitis induced by i.p. administration of *E. coli* (41, 42) or CLP (34, 43). Our present study adds to these earlier reports that anti-TNF not only failed to influence survival, but also had no effect on bacterial clearance and the development of multiple organ damage. Together these data suggest that TNF likely does not play an important role in the pathogenesis of abdominal sepsis, although in mild sublethal peritonitis endogenous TNF may contribute to host defense (16). This study also indicates that during abdominal sepsis the anti-inflammatory arm of the cytokine network (i.e., IL-10) may have a more important regulatory role in the host response than the proinflammatory arm of the cytokine network (i.e., TNF). This supposition is supported by a recent study in which neutralization of another anti-inflammatory cytokine, IL-13, enhanced systemic inflammation and reduced survival during peritonitis induced by CLP (44). Anti-IL-13 therapy did not alter the bacterial load in the peritoneal cavity nor did it influence leukocyte influx or IL-10 levels, suggesting that IL-13 and IL-10 influence host defense during peritonitis by different mechanisms. Interestingly, anti-TNF did influence host responses during peritonitis in IL-10−/− mice. In particular, it diminished the development of multiple organ damage and it reduced mortality. Because anti-TNF did not affect bacterial clearance in IL-10−/− mice, these data further suggest that the systemic

### Table II. Leukocyte counts in peritoneal lavage fluid after anti-TNF pretreatment

<table>
<thead>
<tr>
<th></th>
<th>IL-10−/−</th>
<th>Control mAb</th>
<th>Anti-TNF</th>
<th>IL-10−/−</th>
<th>Control mAb</th>
<th>Anti-TNF</th>
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<td>T 24 h</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total cells (×10⁶/ml)</td>
<td>1.27 ± 0.41</td>
<td>1.14 ± 0.56</td>
<td>3.15 ± 0.49*</td>
<td>2.22 ± 0.51†</td>
<td></td>
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</tr>
<tr>
<td>Neutrophils</td>
<td>0.79 ± 0.31</td>
<td>0.66 ± 0.21</td>
<td>2.05 ± 0.30*</td>
<td>1.40 ± 0.29†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.39 ± 0.13</td>
<td>0.39 ± 0.15</td>
<td>0.89 ± 0.38</td>
<td>0.60 ± 0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.09 ± 0.04</td>
<td>0.09 ± 0.04</td>
<td>0.21 ± 0.09</td>
<td>0.22 ± 0.11</td>
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* Data are mean ± SE (n = 8 mice per group) at 24 h after i.p. administration of *E. coli* (10⁵ CFU).
* p < 0.05 vs IL-10−/− mice (+ control mAb or anti-TNF); †, p < 0.05 vs IL-10−/− mice (+ control mAb or anti-TNF); †‡, p < 0.05 vs IL-10−/− mice + control mAb.

**FIGURE 8.** Anti-TNF reverses the enhanced lethality of IL-10−/− mice during *E. coli* peritonitis. IL-10+/+ and IL-10−/− mice received an i.p. injection of either a neutralizing anti-TNF mAb (0.5 mg) or a control mAb (0.5 mg), followed by an i.p. injection with 10⁵ CFU *E. coli* after 2 h. Data are derived from 18–19 mice per treatment for each mouse strain. Mice that survived 3 days were permanent survivors.
inflammatory response syndrome rather than the bacterial dissemination determined the outcome. In addition, whereas the wild-type TNF response did not have an important impact on the course of peritonitis, the exaggerated TNF response in IL-10−/− mice apparently did contribute to multiple organ damage and death. This observation is in line with studies in which sterile systemic inflammation was induced by bolus administration of endotoxin. Indeed, anti-TNF therapy partly reversed the increased susceptibility of anti-IL-10-treated and IL-10−/− mice to endotoxin-induced lethality (30, 31).

The mechanisms involved in the improved clearance of bacteria from the peritoneal cavity of IL-10−/− mice remain to be established. IL-10 may exert direct anti-inflammatory effects on cells involved in host defense against bacteria, i.e., IL-10 can decrease neutrophil degranulation and chemotaxis, and can suppress oxygen radical and NO synthesis (21). The net effect of the increased influx of neutrophils in peritoneal fluid of IL-10−/− mice, likely at least in part mediated by locally elevated concentrations of the CXC chemokines MIP-2 and KC (45, 46), is uncertain. On the one hand, this enhanced inflammatory response may have contributed to an effective local antibacterial defense (47). On the other hand, accumulation of neutrophils in the peritoneal cavity may also injure the host, as suggested by a report in which a reduction in neutrophil influx to the abdomen by treatment with an anti-MIP-2 Ab was associated with increased survival during peritonitis induced by CLP (45). In this respect, it should be noted that anti-TNF reduced neutrophil influx in peritoneal fluid of IL-10−/− mice without influencing bacterial clearance, suggesting that neutrophils did not play a major role in local antibacterial effector mechanisms. Moreover, considering the earlier findings in anti-MIP-2-treated mice (45), this anti-TNF effect may have contributed to the protective effect of this intervention.

It should be noted that IL-10 may influence host defense mechanisms during peritonitis in other ways than by diminishing TNF production. Indeed, although this study focused on possible interactions between IL-10 and TNF, it is conceivable that other proinflammatory mediators not measured and/or antagonized may have played a role in the phenotype of IL-10−/− mice during peritonitis. For example, IL-10 has been reported to inhibit the expression of adhesion molecules on endothelial cells and to attenuate certain proinflammatory neutrophil functions (48–51).

During abdominal sepsis, proinflammatory and anti-inflammatory members of the cytokine network are considered to regulate local antibacterial effector mechanisms on the one hand, and the systemic inflammatory response syndrome ensuing from the severe bacterial infection on the other hand. If the balance in the cytokine network is lost, the inflammatory response to infection can become self-destructive. Here we demonstrate the seemingly paradoxical role of endogenous IL-10 during septic peritonitis. The absence of IL-10 was detrimental to the survival of mice, accompanied by profound multiple organ damage, despite a more effective bacterial clearance and a reduced dissemination of bacteria to distant organs. These data exemplify the complex role of IL-10 in bacterial infection, and indicate that the net effect of endogenous IL-10 on the outcome of a bacterial infection is determined by the balance between its local effects (facilitating the outgrowth of microorganisms) and its systemic effects (attenuating inflammation).


