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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...
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 3 ml 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 was >90% in each experiment and did not differ between groups. After collection of peritoneal fluid, deeper anesthesia was induced by i.p. injection of 0.07 ml/g FFM mixture (Pentylan, 0.315 mg/ml)-Flumisone (10 mg/ml) (Janssen, Beersenum, Belgium), Midazolam (5 mg/ml; Roche, Mijdrecht, The Netherlands). Next, the abdomen was opened and blood was drawn from the lower caval vein into a sterile syringe, transferred to tubes containing EDTA (K3) (15%), and immediately placed on ice. Blood was used for hemologic and chemical analyses and for measurement of cytokine levels. Plasma for these determinations was prepared by centrifugation at 3000 × g for 10 min at 4°C, after which aliquots were stored at −20°C.

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 (ASAT), alanine aminotransferase (ALAT), 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.

Enumeration of bacteria
Ten-fold serial dilutions of peritoneal fluid, whole blood, and lung homogenates were plated on blood agar plates and incubated at 37°C and 5% CO2. CFU were counted after 24 h. Lung homogenates were prepared by homogenization at 4°C in four volumes of sterile saline using a tissue homogenizer (Biospec Products, Bartlesville, OK).

Cell counts and differentials
Cell counts, determined in triplicate on each peritoneal fluid sample, were quantitated using a hemacytometer. Subsequently peritoneal fluid was centrifuged at 1400 × g for 10 min; the supernatant was collected in sterile tubes and stored at −20°C until determination of cytokines. The pellet was diluted with PBS until a final concentration of 105 cells/ml and differential cell counts were performed on cytospin preparations stained with a modified Giemsa stain (Diff-Quick; Dade Behring AG, Düdingen, Switzerland) according to the manufacturer’s instructions. Cell differentials were determined in duplicate by two independent investigators.

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 10³ 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 10³ 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).
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). Intrapertoneal 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 10^3 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 parenchymal 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, thrombosis occlusion in portal areas, enlargement of portal tracts by mononuclear cells, micro and macrovascular 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 gargand-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.

Table I. Leukocyte counts in peritoneal lavage fluid

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<tr>
<td></td>
<td>IL-10+/+</td>
<td>IL-10−/−</td>
<td>IL-10+/+</td>
<td>IL-10−/−</td>
<td>IL-10+/+</td>
<td>IL-10−/−</td>
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<tr>
<td>Total cells (×10^6/ml)</td>
<td>5.48 ± 0.42</td>
<td>8.31 ± 2.32*</td>
<td>1.23 ± 0.51</td>
<td>3.32 ± 0.59*</td>
<td></td>
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<tr>
<td>Neutrophils</td>
<td>3.56 ± 0.65</td>
<td>6.57 ± 0.99*</td>
<td>0.72 ± 0.59</td>
<td>2.16 ± 0.65*</td>
<td></td>
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<tr>
<td>Macrophages</td>
<td>1.32 ± 0.23</td>
<td>1.16 ± 0.25</td>
<td>0.43 ± 0.13</td>
<td>0.93 ± 0.48</td>
<td></td>
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<tr>
<td>Lymphocytes</td>
<td>0.60 ± 0.11</td>
<td>0.58 ± 0.07</td>
<td>0.08 ± 0.05</td>
<td>0.23 ± 0.12</td>
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* 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 (10^3 CFU).

**p < 0.05 vs IL-10+/+.
Therefore, we pretreated IL-10\(^{+/+}\) and IL-10\(^{-/-}\) mice with a neutralizing anti-mouse TNF mAb or an irrelevant control mAb 2 h before infection with 10\(^{9}\) 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\(^{-/-}\) 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\(^{+/+}\) mice treated with the control.

**FIGURE 4.** IL-10\(^{-/-}\) mice demonstrate enhanced multiple organ damage as reflected by clinical chemistry. IL-10\(^{+/+}\) and IL-10\(^{-/-}\) mice received an i.p. injection with 10\(^{9}\) CFU E. coli, and ALAT, ASAT (liver injury), amylase (pancreas injury), and creatinin (renal injury) concentrations were measured in plasma after 24 h. Filled bars represent IL-10\(^{+/+}\) mice; open bars indicate IL-10\(^{-/-}\) 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.** Histopathology. Representative histological pictures of liver, lung, and spleen of IL-10\(^{+/+}\) (A, C, and E) and IL-10\(^{-/-}\) (B, D, and F) mice at 24 h after i.p. injection of E. coli (10\(^{9}\) CFU). Liver tissue in IL-10\(^{+/+}\) mice showed small necrotic areas (arrowheads) compared with the garland-like necrotic areas in IL-10\(^{-/-}\) mice. More obvious thrombotic lesions (arrows) and a higher influx of polymorphonuclear granulocytes in portal areas were found in IL-10\(^{-/-}\) mice compared with IL-10\(^{+/+}\) mice. CV, central vein; PV, portal vein. Lungs revealed mild alveolar congestion and cellular infiltrates in both mouse strains; however, lungs of IL-10\(^{-/-}\) mice displayed more extensive thrombotic lesions (arrows) compared with IL-10\(^{+/+}\) mice. Spleens of IL-10\(^{+/+}\) 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\(^{-/-}\) 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\(^{-/-}\) mice compared with IL-10\(^{-/-}\) 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\(^+/+\) or IL-10\(^{-/-}\) mice (Fig. 6). In addition, in IL-10\(^+/+\) 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\(^{-/-}\) 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\(^{-/-}\) 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\(^+/+\) and IL-10\(^{-/-}\) mice were pretreated (2 h) with either anti-TNF or control mAb, inoculated i.p. with 10^5 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\(^{-/-}\) mice treated with control mAb died earlier and to a greater extent than IL-10\(^{-/-}\) mice treated with control mAb (13/19 or 68% vs 6/18 or 33%, \(p < 0.05\)). This survival disadvantage of IL-10\(^{-/-}\) mice treated with control mAb disappeared after treatment with anti-TNF (mortality 7/18 or 39%, nonsignificant vs IL-10\(^+/+\) mice treated with control mAb). Anti-TNF tended to increase survival in IL-10\(^+/+\) 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. Intra-peritoneal 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 an improved bacterial clearance from the lungs and an increased survival rate (32, 33). On the contrary, anti-IL-10 treatment of mice with peritonitis caused by CLP was associated with enhanced lethality (34, 35). Whether endogenous IL-10 influenced bacterial clearance from the peritoneal cavity or the development of multiple organ damage was not investigated in these previous studies. We now show that, similar to its effect in the pulmonary compartment, endogenous IL-10 impaired bacterial clearance from the peritoneal cavity during E. coli peritonitis, which was associated with dissemination of bacteria to other sites in the body. However, 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 inflammation in these mice was less severe than that in IL-10+/+ mice.
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 the net effect of the increased in-flux of neutrophils in peritoneal fluid of IL-10−/− mice found by others. IL-10 may exert direct anti-inflammatory effects on cells involved in the inflammatory response to infection.

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