Inflammatory response in obstructive jaundice and peritonitis
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Interleukin-18 improves the early antimicrobial host response to *Escherichia coli* peritonitis

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*Submitted*
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ABSTRACT

To determine the role of endogenous IL-18 during peritonitis, IL-18 gene-deficient (IL-18 KO) mice and wild type (IL-18 WT) mice were intraperitoneally (i.p.) infected with E. coli, the most common causative agent found in septic peritonitis. Peritonitis was associated with a bacterial-dose dependent increase in IL-18 concentrations in peritoneal fluid and plasma. After infection, IL-18 KO mice had significantly more bacteria in the peritoneal lavage fluid and were more susceptible for progressing to systemic infection at 6 and 20h post-inoculation.

The relative inability of IL-18 KO mice to clear E. coli from the abdominal cavity was not due to an intrinsic defect in the phagocytosing capacity of IL-18 KO peritoneal macrophages or neutrophils. IL-18 KO mice displayed an increased neutrophil influx into the peritoneal cavity, but these migrated neutrophils were less activated as reflected by a reduced CD11b surface expression. These data suggest that endogenous IL-18 plays an important role in the early antibacterial host response during E. coli induced peritonitis.

INTRODUCTION

Despite advances in diagnosis, surgery, anti-microbial therapy and intensive care support, the mortality rate associated with severe peritonitis remains high (1, 2). In particular sepsis that originates from the peritoneal cavity has a grim prognosis with mortality rates up to 80% (3). Escherichia coli (E. coli) is one of the most common causative pathogens (up to 60%) in intraperitoneal (i.p.) infections (4).

Cytokines play an important role in the pathogenesis of bacterial infections (5). The local activity of proinflammatory cytokines is required for an adequate antimicrobial defense against localized bacterial infections. On the other hand, systemic activity of proinflammatory cytokines such as observed during fulminant sepsis, can be toxic to the host and contribute to multiple organ failure and death. Our laboratory recently provided evidence for this dual effect of cytokine activity in a murine model of E. coli peritonitis (6). Indeed, mice deficient for the anti-inflammatory cytokine interleukin (IL)-10 (IL-10 knockout or KO mice) demonstrated an enhanced bacterial clearance from the abdominal cavity and a diminished dissemination of the infection to distant organs after i.p. injection of live E. coli. In spite of these findings, systemic inflammation and multiple organ failure were more prominent in IL-10 KO mice, and lethality was increased.

IL-18 was originally identified as an interferon (IFN)-γ inducing factor (IGIF), but now is generally recognized as a proinflammatory cytokine (7, 8). IL-18 can induce a wide array of inflammatory responses in different cell types, including activation of nuclear factor-kB, Fas ligand expression and induction of chemokines. IL-18 plays an important role in the host response to lipopolysaccharide (LPS), the toxic component of the Gram-negative bacterial cell membrane. IL-18 KO mice tolerated a 50% higher
LPS dose than wild type (WT) mice (9), and treatment with an anti-IL-18 antiserum protected mice against the lethal effects of both E. coli and Salmonella LPS (10). Moreover, treatment of mice with a fusion construct consisting of recombinant human IL-18 binding protein and human IgG1 Fc also conferred a strong protective effect against lethality after administration of LPS (11). Only few investigations have addressed the role of IL-18 in host defense against Gram-negative infection in vivo, demonstrating that passive immunization of mice against IL-18 impaired host defense against Salmonella typhimurium or Yersinia enterocolitica (12-14). The role of endogenous IL-18 in host defense against peritonitis is unknown. Therefore, in the present study we sought to determine the role of IL-18 in the local and systemic host response to abdominal sepsis caused by E.coli, making use of IL-18 KO mice.

MATERIALS AND METHODS

Animals
The Institutional Animal Care and Use Committee approved all experiments. IL-18 KO mice were generated as described previously (15). IL-18 KO mice were on the C57BL/6 background. Normal C57BL/6 WT mice were obtained from Harlan Sprague-Dawley (Horst, The Netherlands). Sex- and age-matched (8- to 12-wk old) mice were used in all experiments.

Induction of peritonitis
Peritonitis was induced as described previously (16). In brief, Escherichia coli (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 to 10⁶ E.coli O18.K1 colony-forming units (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.

RT-PCR
Livers were harvested at 20h after administration of E.coli or sterile saline (controls), snap-frozen in liquid nitrogen, and stored at -70°C. To extract total cellular RNA, livers from three mice per group were pooled and homogenized in 1 ml of TRIzol Reagent (Life Technologies, Grand Island, NY). Then total RNA was isolated using chloroform extraction and isopropanol precipitation. The RNA pellet was dissolved in 100 µl of diethylpyrocarbonate-treated water and quantified by spectrophotometry. Reverse transcription was performed by mixing 2 µg of total cellular RNA with 0.5 µg of oligo(dT) (Life Technologies) in a total volume of 12 µl. The mixture was incubated at 72°C for 10 min. Thereafter, 8 µl of a solution containing 4 µl of 5× First Strand buffer (Life Technologies), 10 mM DTT (Life Technologies), 1.25 mM dNTPs (Amersham Pharmacia Biotech, Little Chalfont, U.K.), and 100 U of Superscript Reverse Transcriptase (Life Technologies) was added and the mixture was incubated at 42°C for 1 h. Finally, the tubes were heated to
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72°C for 10 min after which 180 µl of H₂O was added to the reaction mixture. Samples were stored at -20°C until further use. For PCR, 5 µl of cDNA solution was mixed with 20 µl of a solution containing 1x PCR buffer (67 mM Tris-HCl (pH 8.8), 6.7 mM MgCl₂, 10 mM 2-ME, 0.67 µg of EDTA, 16.6 mM (NH₄)₂SO₄, 2% DMSO (Merck, Munchen, Germany), 1.25 µg of BSA (New England Biolabs, Beverly, MA), 0.5 U of AmpliTag DNA polymerase (Perkin-Elmer, Branchburg, NJ), and 75 ng of sense- and antisense oligonucleotide primers specific for IL-18 and β-actin (internal standard). The PCR were performed in a thermocycler (Gene Amp, PCR System 9700; PerkinElmer) using the following conditions: 94°C for 5 min (1 cycle) followed immediately by 95°C for 1 min, 58°C for 1 min, 72°C for 1 min (with variable numbers of cycles), and a final extension phase of 72°C for 10 min. For semiquantitative assessment of IL-18 mRNA, variable numbers of cycles were used to ensure that amplification occurred in the linear phase. To exclude the possibility of finding differences between tubes due to unequal concentrations of cDNA in the PCR, a PCR using β-actin as the internal standard was performed on each sample. β-actin was found to be linear at 27 amplification cycles and IL-18 was found to be linear at 29 amplification cycles. The primers used for IL-18 (433 bp) were 5'-ACTGTACAACCGCAGTAATACGG-3' (sense) and 5'-AGTGAACATCTCTATGTGG-3' (antisense), and for β-actin (617 bp) 5'-GTCAGAAGGACTCCTATGTG-3' (sense) and 5'-GCTCGTGCCGATTAGTGATG-3' (antisense). PCR products were visualized by agarose gel electrophoresis.

Monitoring of mortality and sample harvesting

Our laboratory previously established that in this model mortality occurred predominantly between 18h and 24h after E. coli challenge (6); therefore, mortality was assessed every hour in this time period. Mice surviving more than 3 days appeared to be permanent survivors. At the time of sacrifice, mice were first anesthetized by inhalation of isoflurane (Abbott Laboratories Ltd., Kent, UK)/O₂ (2%/2%). A peritoneal lavage was then performed with 5 ml sterile isotonic saline using an 18-gauge needle, and peritoneal lavage fluid was collected in sterile tubes (Plastipack; Becton-Dickinson, 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 HAM mixture (Fentanyl 0.315 mg/ml-Hamam-sone 10 mg/ml) (Janssen, Beerse, 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 heparin, and immediately placed on ice. Plasma for these determinations was prepared by centrifugation at 1400 x g for 10 min at 4°C, after which aliquots were stored at -20°C.

Determination of bacterial outgrowth

Serial 10-fold dilutions of blood, homogenized liver, and peritoneal lavage fluid were made in sterile saline and 50-µl volumes were plated onto blood agar plates. Plates were incubated at 37°C at 5% CO₂, and CFU's were counted after 16h.

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Cell counts and differentials

Cell counts were determined using a hemacytometer. Subsequently peritoneal fluid was centrifuged at 1400 x 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 10^4 cells/ml and differential cell counts were done on cytospin preparations stained with a modified Giemsa stain (Diff-Quick; Dade Behring AG, Didingen, Switzerland) according to the manufacturer's instructions.

**FACS analysis**

FACS analysis was used to measure granulocyte activation in peritoneal lavage. FACS analysis was done by using a FACSScan (Becton Dickinson). Every analysis was done by counting 5000 cells. Erythrocytes were lysed with ice-cold isotonic NH4CL solution (155 mmol/l NH4CL, 10 mmol/l KHCO3, 0.1 mmol/l EDTA, pH 7.4) for 10 minutes. Incubations for FACS analysis were performed in 96-well V-shaped micro plates (Greiner BV, Alphen a/d Rijn, the Netherlands). For staining, 5x10^5 cells/well were incubated with the following rat-monoclonal antibodies: FITC-labeled anti-Ly-6G (Gr-1) and PE labeled anti-CD11b (1:100; all Pharmingen, San Diego, CA). The appropriate isotype controls (Pharmingen) were included in all experiments. Cells were incubated on ice for 30 min, and washed twice with cold FACS buffer (PBS supplemented with 0.01% NaN3, 0.5% Bovine Serum Albumin (BSA) and 0.3 mM EDTA) and re-suspended in FACS buffer. Granulocyte CD11b expression was determined by forward scatter and side scatter gating and by gating Ly-6G positive cells. Results are expressed in mean fluorescence of Ly-6G positive cells.

**Phagocytosis and assays**

The analysis of uptake of E.coli by peritoneal macrophages and granulocytes was performed essentially as described (16). Macrophages were isolated from the peritoneal cavity of untreated mice and 0.5x10^7 cells/well were cultured overnight in RPMI containing 10% fetal calf serum at 37°C to allow adherence. Macrophages were washed twice with Hanks' balanced salt solution (HBSS) before the addition of labeled bacteria. Peritoneal granulocytes were harvested 5 hours after i.p. injection of 10^5 (w/v) protease peptone (Becton Dickinson, Sparks, MD) (17). Peritoneal exudate cells were treated with 100 mM NH4CL and 10 mM KHCO3 to lyse erythrocytes, washed with HBSS and plated at 0.5x10^7 cells/well. E.coli was heat-killed by incubation at 65°C for 1 hour and labeled with 0.2 mg/ml FITC (Sigma-Aldrich, St. Louis, MO) in 0.1 M NaHCO3 pH 9.0 for 1 hour at 37°C. FITC-labeled E.coli (equivalent to 50x10^6 CFU) were added to the cells (bacteria:cell ratio of 100:1) and incubated for 1 hour (granulocytes) or 2 hours (macrophages) at 37°C or 4°C. Phagocytosis was stopped by immediate transfer of the cells to 4°C and washing with ice-cold FACS buffer (PBS containing 0.5% bovine serum albumin, 1.5 mM NaN3, and 0.35 mM EDTA). Cells were treated with vital blue stain (Orpegen, Heidelberg, Germany) to quench extracellular fluorescence, washed with FACS buffer, and analyzed using a flowcytometer (Becton Dickinson FACS calibur). Macrophages and granulocytes were gated based on forward and side light scatter. Results are expressed as phagocytosis index, quantified as the percentage of cells with internalized E.coli times the mean fluorescence intensity.

IL-1β, IL-12, IFN-γ, macrophage inflammatory protein-2 (MIP-2), and KC were measured by ELISA's according to the instructions of the manufacturer (all R&D Systems, Abingdon, United Kingdom).
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Statistical analysis
All data are expressed as mean ± SE. Differences between groups were analyzed by Mann-Whitney U test. Survival was analyzed with Kaplan-Meier. Values of P < 0.05 were considered to represent a statistically significant difference.

RESULTS

Production of IL-18
To determine whether IL-18 is produced during peritonitis, WT mice received an i.p. injection with 200 µl NaCl containing $10^2$, $10^3$, or $10^4$ E.coli CFU or 200 µl NaCl as a control. Peritonitis was associated with elevated IL-18 concentrations in both peritoneal fluid and plasma at 6h and 20h after infection (Figure 1A). IL-18 levels increased with increasing doses of E.coli and were higher in peritoneal fluid than in plasma. After inoculation with $10^3$ E.coli CFU, IL-18 peaked at 6h, while after infection with $10^4$ CFU E.coli, IL-18 levels peaked after 20h. Mice i.p. injected with sterile saline did not have detectable IL-18 in peritoneal lavage fluid or in plasma (data not shown). In IL-18 KO mice, IL-18 was undetectable at all time points. To determine whether IL-18 mRNA is also produced during E.coli peritonitis, RT-PCR was performed on liver samples obtained from mice 20h after i.p. administration of saline or E.coli. A faint band of IL-18 mRNA was found in livers of mice that received saline, indicating that some IL-18 mRNA is constitutively expressed (Figure 1B). I.p. infection with E.coli was associated with enhanced expression of IL-18 mRNA, as indicated by equal intensity of β-actin bands and clear differences in band intensity between control and peritonitis samples for IL-18 RT-PCR products.

Figure 1A. IL-18 levels, measured by ELISA, in peritoneal fluid and plasma. WT mice were injected i.p. with 200 µl containing $10^2$, $10^3$ and $10^4$ CFU E.coli and sacrificed after 6h and 20h. Results are expressed as mean ± SEM of 8 mice per group. Filled bars represent IL-18 concentrations in peritoneal lavage fluid (PLF); open bars indicate IL-18 concentrations in plasma.
IL-18 KO mice have an increased bacterial outgrowth.

To determine the role of IL-18 in early host defense against peritonitis, we compared the bacterial outgrowth after i.p. inoculation with $10^4$ CFU of *E. coli* in peritoneal lavage fluid (the site of the infection), blood (to evaluate to which extent the infection became systemic), and in liver of WT and IL-18 KO mice (Figure 2). At both 6h and 20h post-infection, IL-18 KO mice had more bacteria in the peritoneal lavage fluid and blood than WT mice (P < 0.05). Also, livers of IL-18 KO mice tended to contain more bacteria compared to WT mice, although these differences did not reach statistical significance. Hence, IL-18 KO mice demonstrated a reduced capacity to clear *E. coli* from the primary infectious site in association with an enhanced dissemination of the infection.

![Graph showing bacterial outgrowth](image)

**Figure 1B.** IL-18 mRNA and β-actin mRNA expression in liver as determined by RT-PCR at 20h after i.p. injection of sterile saline or $10^4$ *E. coli* CFU. Livers from 3 mice per group were pooled.

**Figure 2.** Bacterial outgrowth in peritoneal lavage fluid (top panel), blood (middle panel), and liver (lower panel) in IL-18 KO and WT mice 6 and 20 h after i.p. administration of 104 CFU *E. coli*. Filled symbols represent WT mice; open symbols indicate IL-18 KO mice. Horizontal lines represent medians. * P < 0.05 for the difference between groups.
IL-18 KO mice have an increased neutrophil recruitment to the peritoneal cavity
Since leukocytes play an important role in the local host defense against invading bacteria, we next determined leukocyte counts and differentials in peritoneal lavage fluid during peritonitis. Peritonitis was associated with a profound influx of cells into the peritoneal cavity, which was mainly the result of neutrophil influx (Table 1). IL-18 KO mice displayed more neutrophils in their peritoneal fluid than WT mice (P < 0.05).

**Table 1.** Increased granulocyte influx in IL-18 KO mice into the peritoneal cavity during peritonitis

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<tr>
<td></td>
<td>IL-18 WT</td>
<td>IL-18 KO</td>
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<tr>
<td>Total cells (x10⁶/ml)</td>
<td>7.6 ± 1.4</td>
<td>11.2 ± 2.4*</td>
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<tr>
<td>Neutrophils</td>
<td>5.8 ± 1.5</td>
<td>7.9 ± 1.4*</td>
</tr>
<tr>
<td>Macrophages</td>
<td>1.3 ± 0.6</td>
<td>2.6 ± 0.7</td>
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<td>Lymphocytes</td>
<td>0.5 ± 0.3</td>
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Mice were sacrificed at 6h and 20h after i.p. administration of 10⁶ CFU E.coli to obtain peritoneal lavage fluid. Data are mean ± SEM. Each group consisted of 8 mice for each time point. * P<0.05 vs. WT mice

IL-18 KO mice have a reduced CD11b expression on peritoneal neutrophils
The extent of CD11b expression at the surface of neutrophils has been used as an activation marker of this cell type (18-20). Since IL-18 is able to activate neutrophils in vitro, which includes upregulation of CD11b (18), we considered of interest to compare neutrophil CD11b expression at the site of the infection in IL-18 KO and WT mice (Figure 3). Neutrophils derived from peritoneal fluid of IL-18 KO mice obtained 20h after infection expressed less CD11b at their surface than peritoneal neutrophils from WT mice (P < 0.05).

![Figure 3. CD11b expression was determined by FACS analysis on neutrophils harvested from peritoneal fluid at 20h postinfection as described in the Methods section. Filled bars represent WT mice; open bars represent IL-18 KO mice. Results (means ± SE) are expressed in mean fluorescence intensity of Ly-6G positive cells. * P < 0.05 vs. WT mice.](image)

Unchanged phagocytosis of E.coli by IL-18 deficient peritoneal neutrophils and macrophages
The increased bacterial load in IL-18 KO mice could be caused by an intrinsic defect of IL-18 deficient cells to phagocytose E.coli. To investigate a possibility we
harvested neutrophils and macrophages from uninfected IL-18 KO and WT mice, and compared their capacity to phagocytose FITC labeled *E. coli* (Figure 4). Both peritoneal neutrophils and macrophages displayed a normal ability to phagocytose *E. coli*.

**Figure 4.** Phagocytosis of FITC-labeled *E. coli* by macrophages and granulocytes harvested from peritoneal fluid from uninfected mice was determined by FACS analysis as described in the Methods section. Filled bars represent WT mice; open bars represent IL-18 KO mice. Results (means ± SE) are expressed as phagocytosis index, quantified as the percentage of cells with internalized *E. coli* times the mean fluorescence intensity.

**Chemokine and cytokine response**

The mouse CXC chemokines MIP-2 and KC have been implicated in the attraction of neutrophils to the site of an infection (21, 22). Therefore, the concentrations of these mediators were measured in peritoneal fluid (Figure 5). IL-18 KO mice had higher concentrations of both MIP-2 and KC at the site of the infection than WT mice (P <

**Figure 5.** MIP-2, KC, IL-12p40, and IFN-γ concentrations in peritoneal lavage fluid obtained 20h postinfection. Filled bars represent WT mice; open bars represent IL-18 KO mice. Data are mean ± SE of eight mice per strain. * P<0.05 vs. WT mice.
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In addition, since IL-18 together with IL-12 is required for optimal induction of IFN-γ (7, 8), the levels of IL-12 and IFN-γ were measured in peritoneal fluid (Figure 5). Whereas IL-12 concentrations were higher in IL-18 KO mice (P < 0.05), IFN-γ levels did not differ between IL-18 KO and WT mice.

Survival
To determine the influence of IL-18 on survival, IL-18 KO and WT mice were infected with $10^4$ E.coli CFU, and followed for 4 days (Figure 6). Survival did not differ between the two mouse strains.

![Survival graph](image)

**Figure 6.** Survival of IL-18 WT and IL-18 KO mice infected with E.coli (n=10 per group). No significant difference was found in lethality between the two strains of mice.

DISCUSSION

Severe bacterial infection is associated with enhanced production of IL-18, as reflected by elevated circulating levels of this proinflammatory cytokine in patients with sepsis (23-25). In experimental animals, several organs have been found to express IL-18 constitutively, especially liver and lung, and an increase in the plasma concentrations of IL-18 was detected after administration of high doses of LPS (10, 26, 27). In the present investigation, we sought to determine the extent of IL-18 production during abdominal sepsis caused by E. coli, and to determine the role of this endogenously produced IL-18 in the host response to this infection. We here confirm constitutive expression of IL-18 mRNA in liver, and demonstrate bacterial dose dependent release of IL-18 in peritoneal lavage fluid and plasma. In addition, IL-18 KO mice were less
able to clear bacteria from the site of infection and showed an increased dissemination of the infection to the blood compartment, indicating that IL-18 plays an important regulatory role in the early local antimicrobial host defense against *E. coli*.

Peritonitis is characterized by the recruitment of phagocytic cells, especially neutrophils, to the site of infection (6, 21). Neutrophil influx into the peritoneal cavity was markedly increased in IL-18 KO mice 6h and 20h after *E. coli* administration. This finding was unexpected in light of a previous investigation that showed enhanced influx of neutrophils into peritoneal fluid of mice i.p. injected with recombinant IL-18 (18). The most likely explanation for the increased peritoneal neutrophil numbers in IL-18 KO mice is that they are the consequence of the increased proinflammatory stimulus provided by the higher bacterial load. Theoretically, the increased recruitment of neutrophils to the peritoneal fluid of IL-18 KO mice may in part also have been mediated by the elevated local concentrations of the CXC chemokines MIP-2 and KC, which are known to contribute to neutrophil attraction to sites of the bacterial infection (21, 22). Likely, the elevated MIP-2 and KC concentrations in peritoneal fluid of IL-18 KO mice were also the consequence of the enhanced outgrowth of *E. coli*, especially considering that in contrast to our study with living bacteria, treatment of mice with anti-IL-18 Abs before challenge with *E. coli* LPS was accompanied by a significant decrease in MIP-2 levels, and a diminished neutrophil accumulation in lungs and liver (10). Furthermore, IL-18 has been reported to increase rather than to inhibit the production of IL-8, the prototypic human CXC chemokine, in vitro (18, 28).

A recent study identified a role for IL-18 in the activation of neutrophils (18). IL-18 induced cytokine and chemokine release from human peripheral blood derived neutrophils, induced degranulation, enhanced respiratory burst upon stimulation with FMLP and upregulated the surface expression of the activation marker CD11b. We here demonstrate that endogenous IL-18 plays a role in the activation of neutrophils recruited to the peritoneal cavity during *E. coli* peritonitis. Indeed, IL-18 KO mice displayed a reduced expression of CD11b at the surface of neutrophils recovered from their peritoneal fluid. These data are the first to indicate that IL-18 may contribute to neutrophil activation during infection in vivo.

IL-18 has been implicated, together with IL-12, in optimal production of IFN-γ (7, 8). IL-18 in particular seems important for IFN-γ production induced by a Gram-negative stimulus such as LPS. Indeed, elimination of endogenous IL-18 decreased IFN-γ release in mice during endotoxemia (9-11), whereas IL-18 did not contribute to IFN-γ release after administration of the superantigen staphylocoecal enterotoxin B to mice (9, 29). Although IFN-γ concentrations in peritoneal fluid did not differ between IL-18 KO and WT mice, it is conceivable that the higher bacterial load in the former mouse strain compensated for the IL-18 deficiency, and that IL-18 does play a role in IFN-γ production during Gram-negative infection.
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IL-18 exerts cellular effects by a specific interaction with the IL-18 receptor complex, consisting of a high affinity ligand binding chain (IL-18R or IL-18Rα) and a signal transducing element (IL-18R accessory protein or IL-18Rβ) (30). The intracellular signaling cascade induced after triggering of the IL-18R complex is highly similar to the signaling cascades induced after stimulation of the Toll-like receptor (TLR) family. Indeed, these distinct receptors all use the same intracellular adapter molecules (MyD88, IRAK and TRAF6) and elicit similar responses (activation of NFKB, JNK and p38 MAP kinase) (30-32). In this respect it is interesting to note that MyD88 gene deficient mice were reported to have a normal capacity to clear bacteria from their peritoneal cavity after induction of peritonitis induced by placing a stent in the colon ascendens (33). Yet, MyD88 KO mice displayed an enhanced survival in this abdominal sepsis model, presumably due to a relatively attenuated systemic inflammatory response. In contrast, mice lacking TLR4, the receptor considered to be essential for the recognition of LPS and Gram-negative bacteria, did not demonstrate an altered survival (33). Several mutually nonexclusive possibilities may explain these observations on the role of IL-18. TLR4 and MyD88 in abdominal sepsis, including differences in the models used, in the extent and localization of IL-18 production, and in the cellular and tissue distribution of the IL-18R, TLR4 and MyD88.

Previous investigations have addressed the role of IL-18 in host defense against Gram-negative bacterial infection in vivo. Administration of anti-IL-18 to mice intravenously infected with S. typhimurium was associated with a relatively enhanced outgrowth of bacteria in liver and spleen seven days after the infection (12). In line with these findings, anti-IL-18 treatment increased bacterial growth in spleens of mice intravenously infected with Yersinia enterocolitica and IL-18−/− mice demonstrated a higher bacterial load in their lungs after intranasal infection with Shigella flexneri (14, 34). IL-18 also contributes to an effective host defense against Gram-positive infection, including systemic infection with Listeria monocytogenes and pneumonia caused by Streptococcus pneumoniae (27, 35). The present investigation provides the first evidence for a role of IL-18 in the early antibacterial defense against i.p. infection, as indicated by the fact that IL-18 KO mice had a reduced ability to clear E. coli from their peritoneal cavity and were less capable of preventing dissemination of the infection.

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