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Matrix Metalloproteinase-9 Deficiency Impairs Host Defense against Abdominal Sepsis[1]

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Matrix metalloproteinase (MMP)-9 is involved in extracellular matrix degradation and leukocyte migration. To determine the role of MMP-9 in the innate immune response to peritonitis, MMP-9 gene-deficient (MMP-9$^{-/-}$) and normal wild-type mice were i.p. infected with *Escherichia coli*. MMP-9 mRNA and pro-MMP-9 protein levels increased rapidly upon induction of peritonitis. Although MMP-9$^{-/-}$ neutrophils showed a normal phagocytosis of *E. coli* in vitro, MMP-9$^{-/-}$ mice displayed a reduced resistance against *E. coli* peritonitis, as indicated by an enhanced bacterial outgrowth in the peritoneal cavity and increased dissemination of the infection. Furthermore, the cytokine response to LPS was not influenced by MMP-9 deficiency. However, during *E. coli* peritonitis, MMP-9$^{-/-}$ mice showed much higher peritoneal chemokine and cytokine levels compared with wild-type mice. Despite the increased local chemokine concentrations, MMP-9$^{-/-}$ mice displayed a diminished recruitment of leukocytes to the site of infection, indicating that cellular migration was impaired. Moreover, MMP-9$^{-/-}$ mice developed more severe distant organ damage during infection. These data suggest that MMP-9 is an essential component of an effective host response to *E. coli* peritonitis. ***The Journal of Immunology,*** **2006, 176: 3735–3741.**

Sepsis is the most common cause of death in noncoronary critical care units in the United States with >750,000 cases/year (1). Peritonitis is the second most common cause of sepsis (2), with *Escherichia coli* as one of the major pathogens involved (3). Because *E. coli* peritonitis is a life-threatening disease, an immediate and adequate host defense is necessary to contain and kill the pathogen. Proteases released by activated leukocytes and degradation of connective tissue structures have been implicated to play an important role in inflammatory host responses. Matrix metalloproteinases (MMP),[3] such as MMP-9, are involved in the migration of inflammatory cells across the extracellular matrix, as well as tissue remodeling (4, 5). MMP-9 was first identified in neutrophils, but can also be expressed by various other cell types, like monocytes/macrophages, lymphocytes, and endothelial cells (5, 6). MMP-9 is not produced constitutively, but needs a trigger to be expressed (5). LPS, the major constituent of the outer cell wall of Gram-negative bacteria and the principal mediator of inflammatory responses to these pathogens, induces the release of MMP-9 by neutrophils and monocytes in vitro (7, 8). Moreover, in mice, *E. coli* LPS administration led to a quick release of MMP-9 into the circulation, with peak values as soon as 1 h after injection (9). In line, during experimental endotoxemia in healthy human volunteers, plasma levels of MMP-9 increased strongly, peaking at 1.5–3 h after LPS injection (10). Furthermore, in a sublethal and lethal *E. coli* sepsis model in baboons, MMP-9 was found to increase in serum, again with peak levels early after the induction of the infection (11). Elevated MMP-9 levels were also found in human sepsis patients and correlated with the severity and mortality of the disease (10, 12, 13).

Previously, it was demonstrated that MMP-9 deficiency protects against mortality in an endotoxic shock model in mice, and selective MMP-9 blocking was suggested as a possible new therapeutic approach for sepsis (9). However, although this study clearly established the anti-inflammatory potential of MMP-9 inhibition, the endotoxin model does not adequately mimic clinical sepsis because it lacks an infectious source from which bacteria invade the host and cause a systemic inflammatory response syndrome. To our knowledge, our study is the first to investigate the role of MMP-9 in host defense against intra-abdominal infection, induced in this study by i.p. injection of *E. coli*, resulting in peritonitis with rapid dissemination of the infection and sepsis. Our findings show that MMP-9 plays a pivotal protective role in the host defense against *E. coli*-induced peritonitis and indicate that blocking of MMP-9 may be harmful during abdominal sepsis.

Materials and Methods

Animals and design

The Institutional Animal Care and Use Committee approved all experiments. MMP-9$^{-/-}$ mice with a FVB/N background and normal FVB/N wild-type (Wt) mice were obtained from The Jackson Laboratory. Eight-week-old female mice were used in all experiments. Peritonitis was induced by i.p. injection of 10^4 CFU *E. coli* O18:K1, as described previously (14, 15). In one experiment, 200 μg of LPS (*E. coli* 0111:B4; Sigma-Aldrich) was injected i.p. Peritoneal lavage fluid, blood, and organs were harvested and processed for measurements of CFU, leukocyte counts, cytokines, and chemokines, as described (14, 15).

Evaluation of MMP-9 mRNA levels by quantitative RT-PCR

Total RNA was isolated using the RNeasy Mini Kit system (Qiagen) and treated, as described before (16). RT-PCR were performed on cDNA using FastStart DNA Master SYBR Green I (Roche) with 2.5 mM $MgCl_2$ in a LightCycler (Roche) apparatus. PCR conditions were 5 min, 95°C, followed by 95°C for 15 s, 60°C for 5 s, and 72°C for 20 s, during 40 cycles. Standard

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[3] Abbreviations used in this paper: MMP, matrix metalloproteinase; HK, heat killed; KC, keratinocyte-derived cytokine; Wt, wild type.

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curves were constructed by PCR on serial dilutions of a concentrated cDNA and analyzed using the LightCycler software. Gene expression is presented as a ratio of the expression of the housekeeping gene β_2-microglobulin (17). Primers for the MMP-9 gene were 5′-TGCATTTCTTCAAGGACGGT-3′ (sense) and 5′-CTGACGTGGGTTACCTCTG-3′ (antisense), and for the β_2-microglobulin gene 5′-TGGTCTTTCTGGTGCTTGTCT-3′ (sense) and 5′-ATTTTTTTCCCGTTCTTCAGC-3′ (antisense) (all Eurogentec).

In situ hybridization

MMP-9-specific digoxigenin-labeled riboprobes were prepared by the T7 RNA polymerase-driven in vitro transcription from clone-specific PCR products as template. Primers used were 5′-ATT TAG GTG ACA CTA TAG CAG ATG ATG GGA GAG AAG CAG-3′ and 5′-TAA TAC GAC TCA CTA TAG GGG CA CCA TTT GAG TTT CAC TA-3′, yielding a 563-bp product. The underlined primer regions encode the T7-promotor element. Using the probe, in situ hybridization was performed, as described previously (18), using the digoxigenin-labeled riboprobes at a concentration of 300 ng/ml. After hybridization, slides were washed, and bound alkaline phosphatase activity was visualized with NBT chloride and 5-bromo-4-chloro-3-indolylphosphate, toluidine salt (Roche).

Assays

Pro-MMP-9, MIP-2, and keratinocyte-derived cytokine (KC) were measured by ELISAs (R&D Systems). TNF-α, IL-6, IFN-γ, and IL-10 were measured by cytometric bead array multiplex assay (BD Biosciences). Aspartate aminotransferase and alanine aminotransferase were determined with commercially available kits (Sigma-Aldrich), using a Hittachi analyzer (Boehringer Mannheim). Myeloperoxidase was measured by ELISA (Hycult Biotechnology BV).

Phagocytosis of E. coli *by bone marrow-derived neutrophils*

Phagocytosis of *E. coli* by neutrophils was determined, as previously described (19), with minor modifications. Neutrophils were isolated from bone marrow of Wt and MMP-9$^{-/-}$ mice using a discontinuous Percoll gradient, as described (20). The neutrophil-enriched fractions consisted of 55.2 ± 5.5% Gr1-positive cells. Cells were suspended in HBSS, plated at 0.5×10^6 cells/well, and subsequently incubated with 12.5×10^6 heat-killed (HK) FITC-labeled *E. coli* (bacterium:cell ratio of 25:1). After incubation for 1 h 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 supplemented with 0.01% NaN_3, 0.5% BSA, and 0.35 mM EDTA). Cells were treated with vital blue stain (Orpegen Pharma) to quench extracellular fluorescence, labeled with Gr1-PE (BD Biosciences), washed with FACS buffer, and analyzed using a flow cytometer (FACSCalibur; BD Biosciences). Neutrophils were gated based on forward light scatter and Gr1 positivity. Results are expressed as phagocytosis index, defined as the percentage of cells with internalized *E. coli* times the mean fluorescence intensity.

Peritoneal macrophages and whole blood ex vivo stimulation

Peritoneal macrophages from untreated MMP-9$^{-/-}$ and Wt mice were harvested by peritoneal lavage and washed and resuspended in medium (RPMI 1640 containing 10% FCS, 1 mM pyruvate, 2 mM L-glutamine, penicillin, and streptomycin). Cells were then incubated in 96-well flat-bottom microtiter plates (Greiner Bioscience) (1×10^5 cells in 100 μl/well) for 2 h at 37°C, 5% CO_2, and then washed with medium to remove nonadherent cells. Next, the adherent monolayer cells were stimulated for 20 h in 100 μl of medium alone or with LPS from *E. coli* (500 ng/ml; Sigma-Aldrich) or HK-*E. coli* (1×10^7 CFU/ml) at 37°C, 5% CO_2. Supernatants were stored at −20°C until assayed. Whole blood was collected from untreated MMP-9$^{-/-}$ and Wt mice in heparinized tubes and diluted 1/5 with medium alone or with LPS (200 ng/ml) or HK-*E. coli* (1×10^7 CFU/ml) and incubated in polypropylene tubes for 20 h at 37°C, 5% CO_2. Supernatants were stored at −20°C until assayed.

Histology

Liver and lung samples were fixed with 4% Formalin and embedded in paraffin, and 4-μm sections were stained with H&E. All slides were scored by a pathologist without knowledge of the strain of mice, according to the following parameters: 1) number of thrombi in five random fields; 2) presence and degree of inflammation; and 3) for liver, presence and degree of necrosis. Inflammation and hepatic necrosis were rated from 0 to 3: 0 = absent, 1 = occasionally, 2 = regularly, 3 = massively. Granulocyte staining was performed, as described previously (21).

Statistical analysis

All data are expressed as mean ± SE. Differences between groups were analyzed by the Mann-Whitney *U* test. Values of $p < 0.05$ were considered to represent a statistically significant difference.

Results

MMP-9 is up-regulated during E. coli*-induced abdominal sepsis*

To evaluate the role of MMP-9 during Gram-negative abdominal sepsis, we used a murine *E. coli* peritonitis model and compared host responses in MMP-9$^{-/-}$ and Wt mice. To confirm MMP-9 production in this model, we measured MMP-9 mRNA levels in liver and lung tissue, and pro-MMP-9 protein concentrations in plasma before and at 6 and 20 h after the induction of peritonitis. Intraperitoneal injection of 10^4 CFU *E. coli* strongly induced MMP-9 mRNA and protein levels (all $p < 0.05$ vs baseline; Fig. 1). To obtain insight into the cellular source of MMP-9 during abdominal sepsis, in situ hybridization was performed on liver and

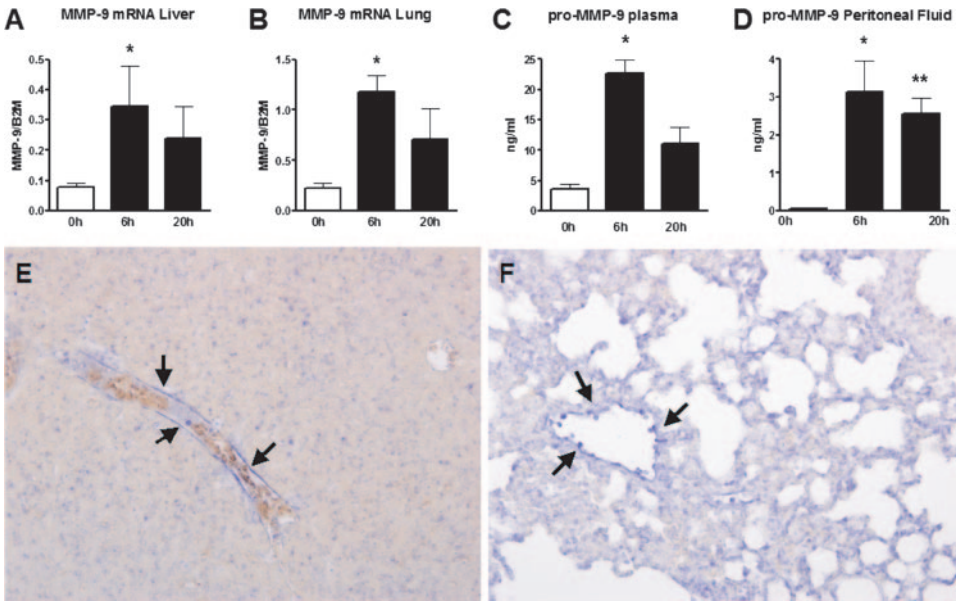

FIGURE 1. Enhanced MMP-9 production during septic peritonitis. Liver (*A*) and lung (*B*) MMP-9 mRNA expression and pro-MMP-9 protein levels in plasma (*C*) and peritoneal lavage fluid (*D*). Mice were injected i.p. with 10^4 CFU *E. coli* at 0 h and were sacrificed before and 6 and 20 h postinfection. Results are expressed as means ± SE of six mice per time point. *, $p < 0.05$; **, $p < 0.01$ vs 0 h. In situ hybridization for MMP-9 was performed in liver (*E*) and lung (*F*) tissues at 6 h after i.p. injection with 10^4 CFU *E. coli*. Wt mice showed MMP-9 mRNA localization (positive signal in blue) in leukocytes and vascular endothelium. Magnification, ×200.

lung tissue at 6 h after *E. coli* injection (at the time peak pro-MMP-9 levels were detected). Liver and lung tissues both showed MMP-9 mRNA expression, which was colocalized mainly with the endothelium as well as the leukocytes adhering to the inner vessel wall (Fig. 1).

MMP-9$^{-/-}$ mice have an enhanced bacterial outgrowth

To examine whether MMP-9 deficiency influenced the bacterial outgrowth, we established the number of *E. coli* CFU at 6 and 20 h postinfection in peritoneal lavage fluid, blood, and liver of MMP-9$^{-/-}$ and Wt mice. After 6 h, no differences in bacterial outgrowth were found between both genotypes. However, at 20 h after infection, MMP-9$^{-/-}$ mice had a significantly higher bacterial load in their peritoneal lavage fluid than Wt mice (both $p < 0.05$; Fig. 2). In addition, blood and liver homogenates of MMP-9$^{-/-}$ also contained more bacteria compared with Wt mice ($p < 0.05$; Fig. 2). Hence, MMP-9$^{-/-}$ mice showed a clearly increased outgrowth of *E. coli* at the primary site of infection, which was associated with an enhanced dissemination of bacteria.

Unchanged phagocytosis of E. coli *by MMP-9$^{-/-}$ neutrophils in vitro*

To investigate whether the increased bacterial outgrowth in MMP-9$^{-/-}$ mice could be the result of an intrinsic defect in the ability of MMP-9$^{-/-}$ neutrophils to phagocytose *E. coli*, we isolated bone marrow-derived neutrophils from uninfected MMP-9$^{-/-}$ and Wt mice and compared their capacity to phagocytose HK-*E. coli* ex vivo. Neutrophils from the MMP-9$^{-/-}$ mice displayed a normal ability to phagocytose *E. coli* (Fig. 3).

Reduced cell influx into the peritoneal cavity in MMP-9$^{-/-}$ mice

The recruitment of leukocytes to the site of an infection is an essential part of the host defense to invading bacteria. Because MMP-9 has been implicated to play a role in cellular migration (5), probably by degradation of extracellular matrix components, we evaluated the inflammatory cell influx into the peritoneal cavity at 6 and 20 h after injection of *E. coli* in MMP-9$^{-/-}$ and Wt mice. *E. coli* injection resulted in a profound increase in total leukocyte numbers in the peritoneal lavage fluid, mainly consisting of neutrophils and macrophages (Table I). MMP-9$^{-/-}$ mice showed a significantly reduced leukocyte influx at 20 h postinfection ($p < 0.05$; Table I). These data indicate that MMP-9 deficiency is associated with impaired inflammatory cell recruitment to the site of infection.

Higher chemokine and cytokine levels in MMP-9$^{-/-}$ mice

The mouse CXC chemokines KC and MIP-2 have been implicated in the attraction of neutrophils to the site of an infection (22, 23). To investigate whether a difference in local chemokine levels could have influenced the neutrophil influx, we determined MIP-2 and KC levels in peritoneal lavage fluid at 6 and 20 h after *E. coli* injection in MMP-9$^{-/-}$ and Wt mice. The levels of both chemokines were markedly increased in both mouse strains (Table I). At 6 h after infection, no differences were found between both mouse strains. However, MMP-9$^{-/-}$ mice showed significantly higher levels of MIP-2 and KC than Wt mice at 20 h postinfection (both $p < 0.05$; Table I), excluding a reduced production of neutrophil-attracting chemokines as a possible explanation of the impaired neutrophil influx in MMP-9$^{-/-}$ mice. Furthermore, to determine whether MMP-9 influenced the production of cytokines during septic peritonitis, local and systemic levels of pro- and anti-inflammatory cytokines were measured (Fig. 4). At 6 h postinfection, TNF-α, IL-6, IFN-γ, and IL-10 levels were similar in peritoneal lavage fluid and plasma of both genotypes (data not shown for IL-10); however, after 20 h, TNF-α, IL-6, and IFN-γ were significantly higher in MMP-9$^{-/-}$ mice than in Wt mice (all $p < 0.05$; Fig. 4). At this time point, the concentrations of IL-10 in peritoneal lavage fluid and plasma were very low and undetectable in most mice (data not shown).

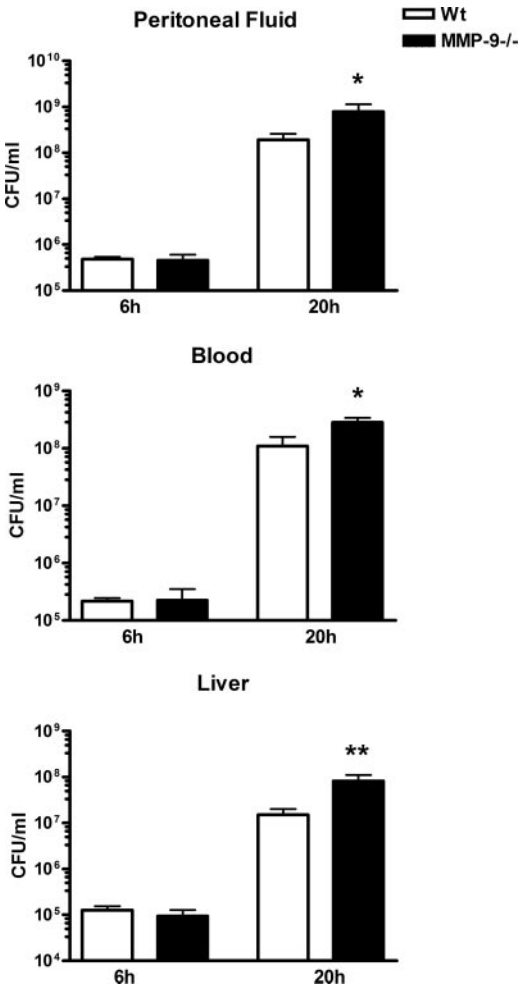

FIGURE 2. MMP-9$^{-/-}$ mice have an enhanced bacterial outgrowth. Bacterial outgrowth in peritoneal lavage fluid, blood, and liver in Wt (□) and MMP-9$^{-/-}$ (■) mice, at 6 and 20 h after i.p. injection with 10^4 CFU *E. coli*. Data are means ± SE of eight mice per genotype per time point. *, $p < 0.05$; **, $p < 0.01$ vs Wt mice at the same time point.

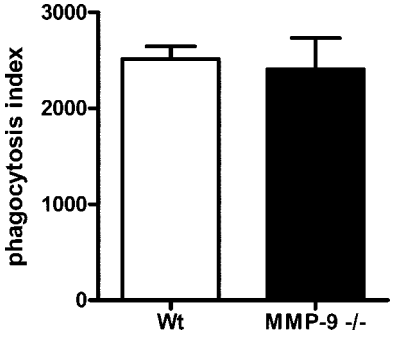

FIGURE 3. Unchanged phagocytosis of *E. coli* by MMP-9$^{-/-}$ neutrophils. Phagocytosis of HK-*E. coli* by MMP-9$^{-/-}$ and Wt bone marrow-derived neutrophils was compared ex vivo. There was no difference in the mean phagocytosis index between both genotypes, as defined by the percentage of cells with internalized *E. coli* times the mean fluorescence intensity. Data are means ± SE of six mice per genotype.

Table I. *Cell counts and chemokine levels in peritoneal fluid*[a]

	6 h		20 h	
	Wt	MMP-9$^{-/-}$	Wt	MMP-9$^{-/-}$
Cells ($\times 10^4$/ml)				
Total cells	17 ± 2.4	16.9 ± 1.9	149 ± 35	65.7 ± 9.5*
Neutrophils	5.3 ± 1.2	5.2 ± 0.7	66.9 ± 26	25.6 ± 12
Macrophages	11.2 ± 1.3	11.3 ± 1.2	82.5 ± 22	40.1 ± 6.7
Chemokines (ng/ml)				
KC	2.0 ± 0.2	1.9 ± 0.4	4.2 ± 1.5	12.7 ± 1.4**
MIP-2	0.12 ± 0.02	0.16 ± 0.04	1.2 ± 0.4	2.5 ± 0.3*

[a] Data are means ± SEM at 6 and 20 h after i.p. injection of 10^4 CFU *E. coli*. $n = 8$ mice/time point. *, $p < 0.05$; **, $p < 0.01$ vs Wt mice at the same time point.

MMP-9$^{-/-}$ mice show no difference in LPS-induced cytokine release

To investigate whether MMP-9 deficiency directly influences the induction of cytokines in vivo, we compared the cytokine response to *E. coli* LPS in MMP-9$^{-/-}$ and Wt mice. LPS was injected i.p., and cytokine release was measured at 2 h postinjection. LPS-induced local (peritoneal lavage fluid) and systemic (plasma) levels of TNF-α, IL-6, IFN-γ, and IL-10 injection did not differ between MMP-9$^{-/-}$ and Wt mice (as shown for TNF-α in Fig. 5). In addition, we examined the cytokine release by MMP-9$^{-/-}$ and Wt peritoneal macrophages and whole blood upon stimulation with LPS and HK-*E. coli* ex vivo. In line, there were no significant differences in cytokine production by MMP-9$^{-/-}$ and Wt peritoneal macrophages or whole blood cultures after 20 h of stimulation with LPS or HK-*E. coli* (shown for TNF-α in Fig. 5).

More severe organ damage in MMP-9$^{-/-}$ mice

Our model of *E. coli* peritonitis is associated with profound liver injury (14, 15, 24). To evaluate the role of endogenous MMP-9 in liver injury during abdominal sepsis, we determined liver damage in MMP-9$^{-/-}$ and Wt mice 20 h after infection. Upon histopathological examination (Fig. 6, *A* and *B*), 50% of the Wt mice showed signs of hepatic inflammation. In contrast, all MMP-9$^{-/-}$ mice showed inflammation of the hepatic parenchyma, as characterized by the influx of leukocytes into the interstitium. The mean total histology score of the liver (quantified according to the scoring system described in *Materials and Methods*) was significantly higher in the MMP-9$^{-/-}$ mice compared with the Wt mice ($p < 0.01$; Fig. 6*E*). The histological findings of more severe liver inflammation in MMP-9$^{-/-}$ mice were confirmed by granulocyte stainings of liver

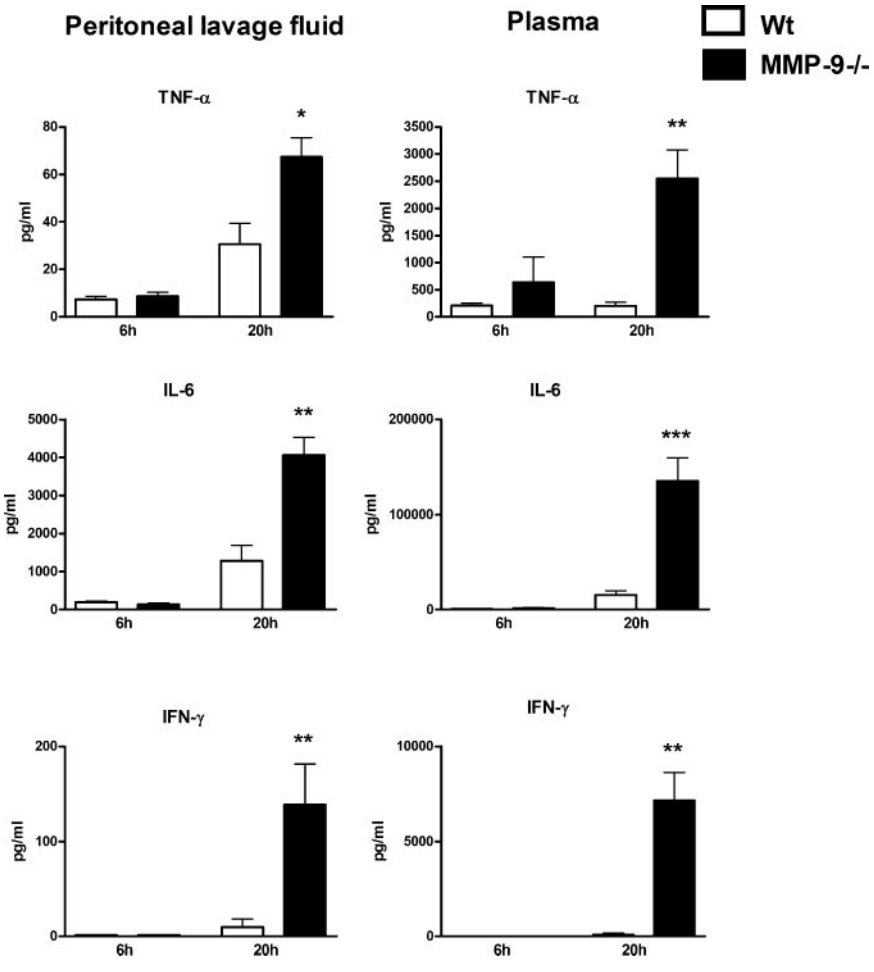

FIGURE 4. Higher cytokine levels in MMP-9$^{-/-}$ mice during infection. TNF-α, IL-6, and IFN-γ were measured in peritoneal lavage fluid (*left panels*) and plasma (*right panels*) at 6 and 20 h after i.p. injection of 10^4 CFU *E. coli*. Data are means ± SE of eight mice per genotype per time point. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs Wt mice at the same time point.

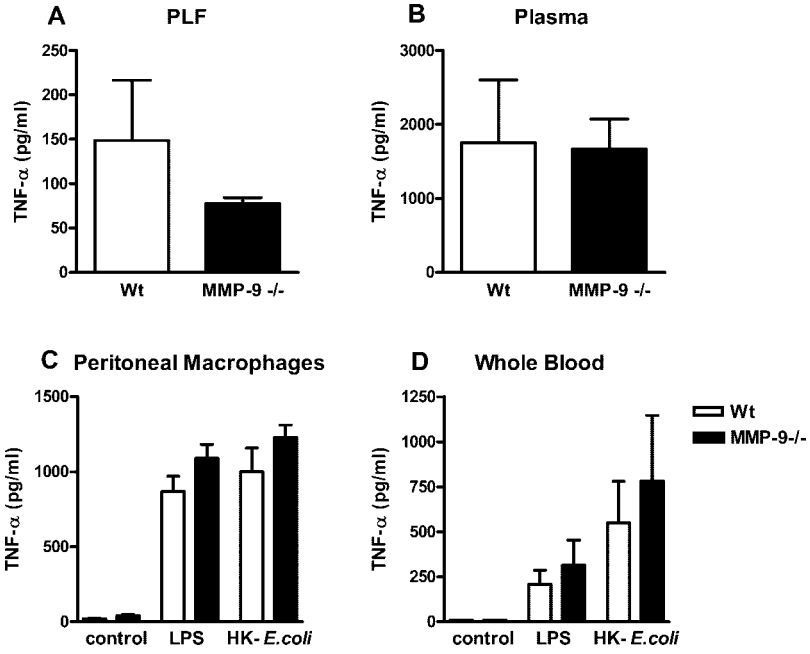

FIGURE 5. LPS-induced TNF-α release is unchanged in MMP-9$^{-/-}$ mice. TNF-α was measured in peritoneal lavage fluid (*A*) and plasma (*B*) at 2 h after i.p. injection of 200 μm of LPS. Ex vivo TNF-α production by peritoneal macrophages (*C*) and whole blood (*D*) from untreated MMP-9$^{-/-}$ and Wt mice was also measured at 20 h after stimulation with LPS and HK-*E. coli*. Data are means ± SE of six mice per genotype.

sections (Fig. 6, *C* and *D*), showing clearly more neutrophil infiltration in MMP-9$^{-/-}$ mice. In line, MMP-9$^{-/-}$ mice had significantly higher myeloperoxidase levels (reflecting the neutrophil content of an organ) in liver homogenates (Fig. 6*F*). Furthermore, the extent of liver injury was also determined by clinical chemistry, i.e., MMP-9$^{-/-}$ mice had significantly higher plasma levels of aspartate aminotransferase compared with Wt mice ($p < 0.01$; Fig. 6*G*). Alanine aminotransferase levels were also higher in MMP-9$^{-/-}$ mice, but the difference with Wt mice did not reach statistical significance (Fig. 6*H*). In conclusion, MMP-9 deficiency was clearly associated with more liver inflammation and damage.

To obtain insight into the role of MMP-9 in the development of inflammation in a more distant organ, lungs were harvested at 20 h after the induction of *E. coli* infection. Sixty-two percent of the lungs of Wt mice showed signs of inflammation compared with all MMP-9$^{-/-}$ mice, as reflected by accumulation of leukocytes in the interstitium and along vessel walls (Fig. 7, *A* and *B*). Again, the total histological scores were higher in the MMP-9$^{-/-}$ mice compared with the Wt mice ($p < 0.05$; Fig. 7*E*). Neutrophil stainings showed more neutrophil influx in the lungs of MMP-9$^{-/-}$ mice (Fig. 7, *C* and *D*). Also, myeloperoxidase levels were higher in MMP-9$^{-/-}$ mice than in Wt mice, but the difference did not reach statistical significance (Fig. 7*F*).

Discussion

MMP-9 has been implicated as an important enzyme during inflammation because of its ability to assist leukocyte trafficking through the extracellular matrix (5). Indeed, MMP-9 was found to stimulate leukocyte migration under the influence of chemotactic factors in vivo (25). During local and systemic bacterial infections, inflammatory responses play a very important role in fighting the pathogens, but, in contrast, can also cause tissue damage. Previously, it was demonstrated that MMP-9 deficiency protects against mortality in an endotoxic shock model in mice, and selective MMP-9 blocking was suggested as a possible new therapeutic approach for sepsis (9). However, although this study clearly established the anti-inflammatory potential of MMP-9 inhibition, the endotoxin model does not adequately mimic clinical sepsis because it lacks an infectious source from which bacteria invade the host and cause a systemic inflammatory response syndrome. To our knowledge, our study is the first to investigate the role of MMP-9 in host defense against intra-abdominal infection, induced

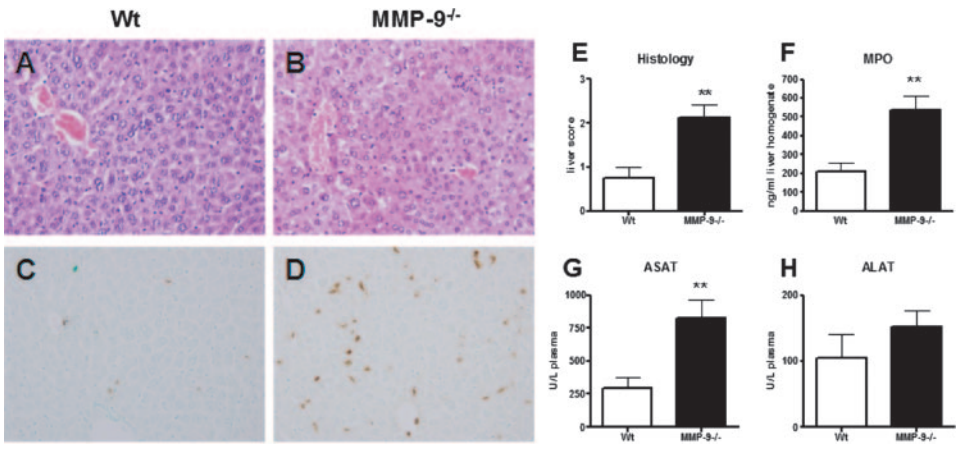

FIGURE 6. More severe liver damage in MMP-9$^{-/-}$ mice. Mice were sacrificed at 20 h after i.p. injection of 10^4 CFU *E. coli*. Representative H&E (*A* and *B*) and neutrophil stainings (*C* and *D*) of liver tissue of Wt (*left panels*) and MMP-9$^{-/-}$ (*right panels*) mice. Magnification ×200. *E*, Graphical representation of the histology scores in Wt (□) and MMP-9$^{-/-}$ (■) determined according to the scoring system described in *Materials and Methods*. *F*, Myeloperoxidase (MPO) levels in liver homogenates. Plasma concentrations of aspartate aminotransferase (ASAT) (*G*) and alanine aminotransferase (ALAT) (*H*). Data are means ± SE of eight mice per genotype. **, $p < 0.01$ vs Wt mice.

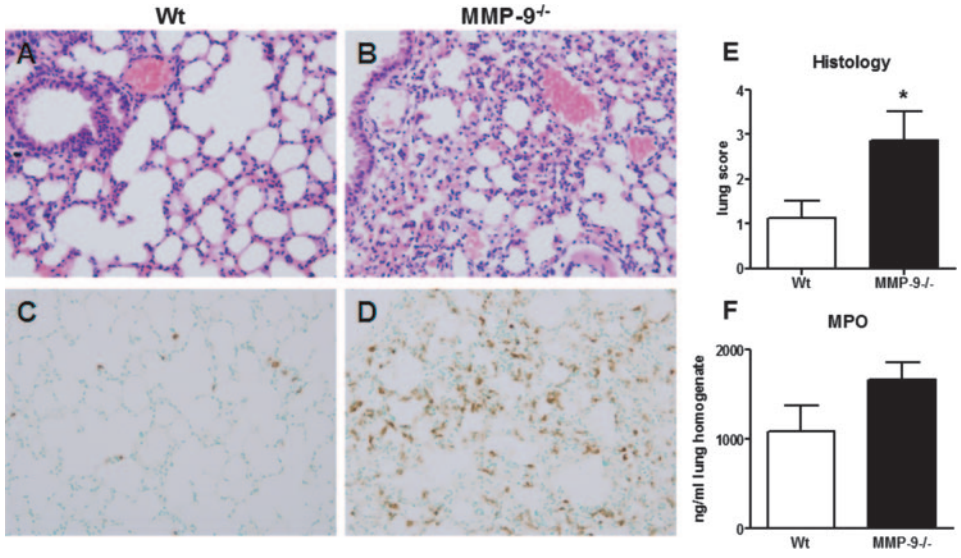

FIGURE 7. Increased pulmonary inflammation in MMP-$9^{-/-}$ mice. Mice were sacrificed at 20 h after i.p. injection of 10^4 CFU *E. coli*. Representative H&E (*A* and *B*) and neutrophil stainings (*C* and *D*) of lung tissue of Wt (*left panels*) and MMP-$9^{-/-}$ (*right panels*) mice. Magnification, ×200. *E*, Graphic representation of the degree of pulmonary inflammation and injury in Wt (□) and MMP-$9^{-/-}$ (■) mice, determined according to the scoring system described in *Materials and Methods*. *F*, Myeloperoxidase (MPO) levels were measured in lung homogenates by ELISA. Data are means ± SE of eight mice per genotype. *, $p < 0.05$ vs Wt mice.

in this study by i.p. injection of *E. coli*, resulting in peritonitis with rapid dissemination of the infection and sepsis. The main findings of our study are that MMP-9 deficiency causes an enhanced outgrowth of *E. coli* at the primary site of infection together with increased spreading of bacteria to other body compartments and more severe organ damage. The reduced resistance against *E. coli* in MMP-$9^{-/-}$ mice did not seem to result from a reduced phagocytosis capacity of the MMP-$9^{-/-}$ neutrophils. However, the impaired recruitment of leukocytes to the peritoneal cavity can, at least in part, explain the reduced host defense against *E. coli* in this model, exemplifying the important role of MMP-9 in leukocyte migration. These findings show that MMP-9 plays a pivotal protective role in the host defense against *E. coli*-induced peritonitis and indicate that blocking of MMP-9 may be harmful during abdominal sepsis. Of note, the earlier finding that MMP-9 deficiency protects against mortality in an endotoxic shock model (9) does not contradict the current finding. Indeed, several studies have indicated that whereas excessive systemic inflammation, such as elicited by administration of high dose endotoxin, can be harmful to the host, an adequate inflammatory response is required to eliminate bacteria in live infection models (26). Similarly, earlier findings that MMP-$9^{-/-}$ mice displayed a better outcome in other models of sterile inflammation (27, 28) do not contradict our current findings, although differences in the genetic background of mice used in different studies might have influenced the results.

MMP form a family of enzymes whose function is mainly the remodeling of extracellular matrix components. MMP-9 is a secreted multidomain enzyme, which cleaves denatured collagens (gelatins) and type IV collagen, which are present in basement membranes. This cleavage helps leukocytes to enter and leave the blood and lymph circulation (5). By the use of MMP-$9^{-/-}$ mice, more evidence has been provided for an in vivo role of MMP-9 in the induction of immunopathology in various noninfectious inflammation models. For instance, MMP-9 deficiency had a protective effect in mouse models of autoimmune diseases, like experimental autoimmune encephalomyelitis (27) and complement-mediated inflammation (29). In addition, MMP-9 deficiency impaired cellular infiltration during allergen-induced airway inflammation (28). Furthermore, LPS-induced lethality was reduced by MMP-9 deficiency (9). However, after intratracheal installation of LPS, MMP-$9^{-/-}$ mice showed no difference in neutrophil migration to the lungs compared with Wt mice (30). Furthermore, at 4 h after thioglycolate-induced peritonitis, MMP-$9^{-/-}$ mice had similar neutrophil counts in their peritoneal cavity (31). These data are in contrast to our current finding that MMP-$9^{-/-}$ mice have a reduced capacity to recruit inflammatory cells to the peritoneal cavity during *E. coli* peritonitis. This discrepancy might be due to the differences between thioglycolate- and *E. coli*-induced peritonitis. The thioglycolate-induced peritonitis model is a very strong acute inflammatory model, whereas the inflammatory cell influx during *E. coli* peritonitis develops much slower. In addition, there might be a difference in cytokine responses and systemic activation of MMP-9 release between the two models. The exact mechanism by which MMP-9 is activated during peritonitis and how it mediates the cellular migration remain to be investigated.

MMP-9 mRNA and pro-MMP-9 protein levels peaked at 6 h after infection. These findings are in line with other studies showing early up-regulation of MMP-9 during experimental sepsis models (9–11). In situ hybridization showed colocalization of MMP-9 mRNA with endothelium and adherent leukocytes to the inner wall of greater vessels. This is in line with previous studies that identified mRNA expression of MMP-9 in leukocytes and endothelial cells (5). Notably, we were unable to detect MMP-9 activity in peritoneal lavage fluid, liver, or lung using a zymographic assay, which successfully detected MMP-9 activity in other models (32, 33). Possibly, the dilution in saline of the samples obtained (in particular peritoneal lavage fluid) was too high to enable detection. Considering the evident phenotype of MMP-$9^{-/-}$ mice in *E. coli* peritonitis, these results imply that MMP-9 activity is very low in this model, too low to allow detection by zymography, yet clearly sufficient to be biologically active.

Neutrophils constitute an important component of early host defense against bacterial infection. The reduced capacity of MMP-$9^{-/-}$ neutrophils to migrate to the site of the infection was associated with an impaired local antibacterial defense and an enhanced dissemination of the infection. Earlier reports on peritonitis have documented a similar association (34–36), further indicating a protective role of migrating neutrophils. It is quite conceivable that locally produced glutamic acid-leucine-arginine-positive CXC chemokines, such as MIP-2 and KC, contribute to this characteristic innate immune response (22, 23). In the present study, MMP-$9^{-/-}$ mice had higher local MIP-2 and KC levels during peritonitis, suggesting that even in the presence of a stronger chemotactic gradient MMP-$9^{-/-}$ neutrophils are less capable to migrate toward an intra-abdominal infection. Most likely, the increased MIP-2 and KC concentrations in MMP-$9^{-/-}$ mice were the result of the higher bacterial load, providing a more potent proinflammatory stimulus. Dubois et al. (9) found that at

16 h after LPS injection, mRNA expression of cytokines in liver, spleen, and kidney was similar between MMP-9$^{-/-}$ and Wt mice. In line with this study, we showed that the LPS-induced cytokine release in peritoneal lavage fluid and plasma was similar between MMP-9$^{-/-}$ and Wt mice in vivo. In addition, there were no differences in LPS- and HK-*E. coli*-induced cytokine production by MMP-9$^{-/-}$ and Wt peritoneal macrophages or whole blood in vitro. In contrast, in our *E. coli* peritonitis model, MMP-9$^{-/-}$ mice had much higher levels of cytokines compared with Wt mice. These differences were most likely due to the increased bacterial load in the MMP-9$^{-/-}$ mice. Furthermore, the increased bacterial load in MMP-9$^{-/-}$ mice resulted in enhanced tissue inflammation and injury, as indicated by histopathology, neutrophil stainings, MPO levels, and clinical chemistry. Thus, although MMP-9 deficiency protected against LPS-induced mortality probably by reducing systemic inflammatory cell responses and subsequent tissue damage, MMP-9$^{-/-}$ mice had much more severe tissue damage during *E. coli*-induced abdominal sepsis compared with Wt mice. Most likely, the differences in the bacterial load overruled the influence of MMP-9$^{-/-}$ deficiency on inflammation in liver and lungs. This may also explain why MMP-9$^{-/-}$ mice had more neutrophils in these organs despite a relatively insufficient neutrophil migration to the primary site of infection. It remains to be established why neutrophil migration into the peritoneal cavity was reduced in MMP-9$^{-/-}$ mice, whereas in liver and lungs from these animals more neutrophils accumulated. Little is known about the mechanisms involved in vivo, but our current data suggest that MMP-9 may play a role in this study.

The present study is the first to document a protective role for MMP-9 in the host defense against intra-abdominal infection. Taken together with the previously reported detrimental role of MMP-9 in endotoxic shock (9), these data further illustrate the existence of a delicate balance between inflammation and anti-inflammation, in which a certain degree of inflammation is required to combat invading pathogens and exaggerated inflammation can result in severe tissue injury.

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Disclosures

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