Enterococcus faecium infections: where bacterial virulence meets innate immunity
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PERITONEAL MACROPHAGES ARE IMPORTANT FOR THE EARLY CONTAINMENT OF ENTEROCOCCUS FAECIUM PERITONITIS IN MICE

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Abstract

The increasing incidence of infections with multi-drug resistant Enterococcus faecium necessitates studies to increase knowledge on the pathogenesis of these infections. In this study, the contribution of peritoneal macrophages during E. faecium peritonitis was investigated. In an ex vivo setting, peritoneal macrophages harvested from C57BL/6 mice were responsive to, and able to phagocytose and kill E. faecium. In vivo, peritoneal macrophages were depleted by intraperitoneal injection of clodronate-encapsulated liposomes, prior to inducing E. faecium peritonitis. Depletion of resident peritoneal macrophages caused a clear delay in peritoneal clearance of E. faecium with increased systemic dissemination. Mice depleted of peritoneal macrophages were able to recruit macrophages and neutrophils to the peritoneal cavity after infection, comparable to control mice. Furthermore, increased levels of peritoneal cytokines and chemokines were found in mice depleted of peritoneal macrophages. This study indicates that peritoneal macrophages are important in the early containment of E. faecium peritonitis and for the regulation of the inflammatory response.
Introduction

Enterococci belong to the normal microflora of the gastrointestinal tract. For a long time, these bacteria were considered relatively harmless nosocomial pathogens; however, they have now emerged as a major cause of nosocomial infections worldwide.\(^1\) They are the third most common cause of hospital-acquired bacteremia in the US and the fourth in Europe (http://www.earss.rivm.nl). Enterococci are relatively harmless in healthy individuals, but become pathogenic in patients in intensive care units and in hospitalized patients with severe underlying diseases, an impaired immune system or in the elderly.\(^2\)

The emergence of infections involving enterococci can largely be attributed to their ability to acquire high-level drug resistance through horizontal gene transfer and their multi-drug resistant nature to various classes of antibiotics. In particular, *Enterococcus faecium* has adapted to the abundant antibiotic use in hospitals by acquiring resistance to high-dose aminoglycosides, β-lactam antibiotics and vancomycin.\(^3-5\) Additionally, clinical isolates frequently contain a pathogenicity island that harbors the gene for the enterococcal surface protein (Esp), that was demonstrated to enhance binding to biotic and abiotic surfaces and biofilm formation.\(^6,7\)

Innate immunity plays a crucial role in determining the outcome of bacterial infections.\(^8,9\) Despite the clinical importance of enterococci, little is known about the cellular and molecular mechanisms of host defense against invasive enterococcal infections. In previous studies, we described the normal immune response during primary *E. faecium* peritonitis in mice.\(^10\) In a non-lethal model, we found a rapid peritoneal neutrophil influx and a consecutive rapid decline in peritoneal and systemic enterococcal load. Unlike neutrophils, peritoneal macrophages (PM) are resident cells within the peritoneal cavity and, therefore, are among the first cells that *E. faecium* will encounter upon induction of peritonitis. In this study, we sought to obtain insight into the *in vivo* role of PM in host defense against *E. faecium* peritonitis. For this purpose, mice were depleted of PM by intraperitoneal administration of clodronate-containing liposomes prior to infection with *E. faecium*. Intraperitoneal treatment with clodronate-liposomes depletes peritoneal and omental macrophages without damaging other cells.\(^9\)
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Materials and Methods

Mice
Specific, pathogen-free 10-wk-old, female, C57BL/6 mice were purchased from Harlan Sprague-Dawley (Horst, The Netherlands). The animals were housed in rooms with a controlled temperature and a 12-h light-dark cycle. They were acclimatized for 1 week prior to usage, received standard rodent chow and water ad libitum. The Animal Care and Use Committee of the University of Amsterdam approved all experiments.

In vivo macrophage depletion
Selective depletion of peritoneal and omental macrophages was achieved by pretreatment of mice with dichloromethylene bisphosphonate (clodronate) loaded liposomes. Clodronate was a gift from Roche Diagnostics (Mannheim, Germany). Preparation of clodronate-liposomes was performed as described previously. Aliquots of 200 μl of clodronate-liposomes or sterile saline was administered intraperitoneally 4 days and 1 day prior to induction of *E. faecium* peritonitis on day 0. Some mice were not infected with *E. faecium* after treatment with clodronate-liposomes to document the effect of this treatment on its own.

Bacterial strain
*E. faecium* strain E155, was used in all experiments. This clinical isolate from the Cook County Hospital (Chicago, IL) is a multi-drug resistant *E. faecium* strain, expressing high-level quinolone, aminoglycoside, ampicillin and vancomycin resistance, and contains a pathogenicity island, containing the variant *esp* gene. For all experiments, the bacteria were grown for 18 h on agar sheep blood (BA) plates and then grown for ~3.5 h in Todd-Hewitt broth (Difco, Detroit, MI) to mid-logarithmic phase at 37°C, with shaking.

Induction of peritonitis
*E. faecium* was cultured as described above and resuspended in sterile isotonic saline. Mice were injected intraperitoneally with 10⁶ colony-forming units (CFU) of *E. faecium* in 200 μl of saline. This bacterial dose is gradually cleared by wild-type mice and is not associated with lethality. The inoculum was plated immediately after inoculation on BA plates to determine viable counts.
Collection of samples
Mice were anesthetized by inhalation of isoflurane (Abbot Laboratories Ltd., Kent, UK) and oxygen (2%/2 liter) and a peritoneal lavage was performed with 5 ml sterile phosphate-buffered-saline using an 18-gauge needle; peritoneal lavage fluid (PLF) was collected in polypropylene tubes (Plastipack; Beckton-Dickinson, Mountain View, CA). After collection of PLF, blood was drawn by cardiac puncture, transferred to heparin-gel vacutainer tubes and placed on ice. Next, the abdomen was opened and the liver and lungs were harvested.

Determination of bacterial outgrowth
The number of *E. faecium* CFU was determined in PLF, blood, liver and lung homogenates. Lungs and livers were homogenized at 4°C in 4 volumes of sterile saline using a tissue homogenizer (Biospec Products, Bartlesville, OK). Colony forming units were determined from serial dilutions of organ homogenates, PLF and blood, by plating 50 μl of each dilution onto BA plates and incubated at 37°C at 5% CO₂ for 20 h before colonies were counted.

Cell counts and differentials of blood and peritoneal lavage fluid
Erythrocytes were lysed with NH₄Cl solution (155 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA, pH 7.4). Remaining cells were counted using a Coulter Counter (Beckman, Fullerton, CA). Peritoneal cells were counted using a hemocytometer chamber, following Trypan Blue treatment. Differential cell counts were performed on cytospin preparations, stained with Giemsa (Diff-Quick; Dade Behring). Additionally, macrophage/monocyte and granulocyte counts in peritoneal fluid and blood were analyzed by FACS; blood and peritoneal cells were stained with rat-anti-mouse antibody on ice for 30 min, APC-labeled F4/80 mAb and FITC-labeled Ly6G mAb (BD Pharmingen, San Diego, CA). Cells were analyzed by flow cytometry using a FACScan (BD, San Jose, CA). PLF supernatant and plasma were stored at -20°C until determination of cytokines.

Preparation of peritoneal macrophages
Peritoneal macrophages from untreated mice were harvested and counted as described. Cells were resuspended in RPMI 1640 medium (GibcoBRL, Invitrogen, Breda, The Netherlands) containing 10% FCS, 1 mM pyruvate, 2 mM L-glutamine, penicillin, and streptomycin) and incubated in 96-well flat-bottom microtiter plates (1 x 10⁴ cells in 100 μl/well) at 37°C with 5% CO₂ for 2 h, and washed with medium to remove non-adherent cells. Adherent monolayer cells were stimulated with living *E. faecium* (5 x 10⁶ CFU/ml) for 6 h. Supernatants were stored at –20°C.
Phagocytosis and bacterial killing assays

Phagocytosis was evaluated as described before.15,16 Peritoneal macrophages were harvested and treated as described above and 5x10⁵ cells/ml were allowed to adhere in 48-well microtiter plates overnight. Macrophages were incubated with FITC-labeled heat-killed *E. faecium* (2.5x10⁷ CFU/ml) for 60, 90 and 120 min. Phagocytosis was stopped by placing cells on ice, cells were washed and suspended in Quenching solution (Orpegen, Heidelberg, Germany). The degree of phagocytosis was determined using the FACS Calibur (Becton Dickinson). Bacterial killing was determined as described previously.17 *E. faecium* was added at a multiplicity of infection of 50 and spun onto cells at 2000 rpm for 5 min; plates were placed at 37°C for 10 min. Each well was washed to remove extracellular bacteria. To determine bacterial uptake after 10 min, cells were lysed with sterile dH₂O and designated as t = 0. Medium was added to remaining wells and plates were placed at 37°C for 5, 15, 30 and 60 min after which cells were lysed with dH₂O. Cell-lysates were plated on BA plates and bacterial counts were enumerated after 16 h. Bacterial killing was expressed as the percentage of killed bacteria in relation to t = 0.

Histology

Lungs and liver were fixed in 4% formalin, and embedded in paraffin for routine histology. Sections of 4 μm thickness were stained with haematoxylin and eosin. All slides were coded and scored by a pathologist without knowledge of the type of mice or treatment.

Assays

Macrophage inflammatory protein (MIP)-2, cytokine-induced neutrophil chemoattractant (KC) and LPS-induced C-X-C chemokine (LIX) were measured by ELISA’s (R&D Systems, Minneapolis, MN) according to the manufacturers’ instructions. Tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, IL-12p70, interferon (IFN)-γ and monocyte chemoattractant protein (MCP)-1 were measured by using a commercially available cytometric bead array multiplex assay (BD Biosciences, San Jose, CA) in accordance with the manufacturer’s recommendations.

Statistical analysis

Data are expressed as mean ± SEM. Serial data were analyzed by two-way analysis of variance (ANOVA) followed by a post hoc Bonferroni test. Two group comparisons were performed by Mann-Whitney U test. For all analyses, GraphPad Prism version 4 (GraphPad Software, San Diego, CA) was used. A p-value < 0.05 was considered statistically significant.
Results

Peritoneal macrophages release cytokines and chemokines upon exposure to \textit{E. faecium} \textit{in vitro}

We previously showed that exposure of primary PM to \textit{E. faecium} \textit{in vitro} resulted in a rapid release of the pro-inflammatory cytokine TNF-\(\alpha\).\(^{10}\) To obtain further evidence that PM sense and respond to \textit{E. faecium} \textit{in vitro}, we here confirm and expand these results by showing that PM in addition release IL-6, IL-10, KC and MIP-2 upon stimulation with \textit{E. faecium} (Fig. 1). Interestingly, and as previously described,\(^{18}\) primary PM did not produce LIX.

Peritoneal macrophages phagocytose and kill \textit{E. faecium}

To obtain a first insight into the role of PM in the innate defense against \textit{E. faecium} peritonitis, we examined the ability of PM to phagocytose and kill \textit{E. faecium} \textit{in vitro}. Primary PM harvested from the peritoneal cavity of uninfected mice were capable of rapidly phagocytosing and killing \textit{E. faecium} (Fig. 2A and B). In addition, PM harvested from mice 1 h after intraperitoneal injection of \textit{E. faecium} \textit{in vivo} demonstrated many intracellular \textit{E. faecium} cells (Fig. 2C).
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Figure 2. Peritoneal macrophages are able to phagocytose and kill *E. faecium*. (A) Phagocytosis of FITC-labeled, heat-killed *E. faecium* by peritoneal macrophages (PM) was determined by FACS analysis as described in Materials and Methods. Results are expressed as the mean phagocytosis index, as defined by mean fluorescence x percent positive cells. (B) Killing capacity of PM is shown as percentage of killed *E. faecium* compared to t = 0. For (A) and (B), data are means ± SEM (n = 5). (C) Macrophages with phagocytosed *E. faecium*. Mice were injected with 10^8 CFU *E. faecium* intraperitoneally. One hour after the infection, mice were killed, cytospin preparations were made of peritoneal lavage fluid and cells were stained with Giemsa (magnification x 40).

Depletion of peritoneal macrophages by treatment with clodronate-liposomes

To elucidate the role of PM in *E. faecium* peritonitis in vivo, we selectively depleted PM by intraperitoneal injection of clodronate-liposomes. First, we confirmed that this treatment resulted in an effective and sustained depletion of PM in uninfected mice. As expected, uninfected mice remained > 90% (p<0.01) depleted of PM up to at least 5 days after the last clodronate-liposomes injection (Fig. 3).19 Hence, in the peritonitis experiments, >90% of PM was depleted at the time mice were infected with *E. faecium*, i.e. one day after the last injection with clodronate-liposomes. Remarkably, unlike uninfected mice, animals injected with *E. faecium* intraperitoneally at day 0 attracted new macrophages into the peritoneal cavity, a response that was evident 24 h post-infection (Fig. 4A). As a
consequence, 24 h after induction of *E. faecium* peritonitis, clodronate-liposome treated and control mice displayed comparable numbers of PM in their peritoneal cavity. Of note, in control mice, an initial decrease in PM was seen after *E. faecium* injection, which was followed by increased PM counts on day 1 and 2 after the infection. In all mice, PM numbers had returned to pre-infection levels on day 4. Blood monocyte counts did not change in either experimental group during the 4-day observation period (data not shown).

Intraperitoneal injection of *E. faecium* resulted in a strong increase in neutrophil counts in PLF in both groups of mice (Fig. 4B). Although clodronate-liposomes administered mice tended to have more neutrophils in their PLF after induction of peritonitis, the difference between groups was not statistically significant. Both experimental groups showed a modest rise in peripheral blood neutrophil counts after induction of *E. faecium* peritonitis which was not statistically significantly different between groups (data not shown).

Figure 3. Depletion of peritoneal macrophages by intraperitoneal clodronate-liposome injection. Mice were intraperitoneally injected with either clodronate containing liposomes (closed bars) or saline (open bars) at day -4 and day -1. Peritoneal cells were counted and differentiated 1 day and 5 days after the last injections. Clodronate treatment resulted in >90% reduction of peritoneal macrophages up to at least 5 days. Data are means ± SEM (n = 5 per group). **, p < 0.01 compared to control mice.
Delayed clearance of *E. faecium* in peritoneal macrophage-depleted mice

We previously demonstrated that normal wild-type mice rapidly clear *E. faecium* from their peritoneal cavity upon intraperitoneal infection. To obtain insight into the role of resident PM, we sequentially determined enterococcal loads in PLF and distant body sites in clodronate-liposome treated and control mice after induction of *E. faecium* peritonitis. Mice depleted of PM at the moment of *E. faecium* infection demonstrated an overall delay of *E. faecium* clearance (Fig. 5). Six h after the start of the infection, clodronate-liposome treated mice had about 1 log more *E. faecium* at the primary site of the infection (PLF) (Fig. 5A) as well as in the other cultured organs blood (Fig. 5B), liver (Fig. 5C) and lung (Fig. 5D). Two days after the start of the infection, clodronate-liposome treated mice had positive PLF and blood cultures, whereas control mice had cleared the enterococci from these compartments. In liver and lung, higher CFU were counted in clodronate-liposome treated mice at the earlier time points, whereas the *E. faecium* loads were comparable in both mouse groups from the second day after the infection onwards. Four days after infection, no bacteria were cultured from either PLF, blood or liver of any of the mice and comparable low amounts were cultured from lungs of both groups. These results indicate that resident PM play an important role in the clearance of *E. faecium* from the abdominal cavity and in reducing the dissemination of the infection.
Macrophages in *E. faecium* peritonitis

Figure 5. Mice depleted of peritoneal macrophages are impaired in peritoneal and systemic *E. faecium* clearance. Mice were intraperitoneally injected with either clodronate containing liposomes (closed symbols) or saline (open symbols) at day -4 and day -1. At time point 0, mice were injected intraperitoneally with $10^8$ CFU *E. faecium*. Mice were sacrificed at 6 h, 1, 2 or 4 days after the infection. An impairment in clearance was found in peritoneal fluid (A), blood (B), liver (C) and lung (D). Results are expressed as mean ± SEM (n = 8 per group per time point). *P*-values in the figures represent overall differences between groups. *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001 compared to control mice at the indicated time points.

Initial depletion of peritoneal macrophages causes higher cytokine responses, but no significant organ damage

*E. faecium* peritonitis in healthy mice causes a modest cytokine response, with detectable levels only early during the infection and low, or undetectable levels later than 6 h after induction of the infection. Correspondingly, in this study, 6 h after the infection only low levels of TNF-α, IL-6, MCP-1, IL-10, KC and LIX were measured in PLF of control mice (Fig. 6). Mice treated with clodronate-liposomes produced higher cytokine levels that were measurable up to 2 days (Fig. 6). These mice also had higher plasma TNF-α, IL-6 and MCP-1 levels 6 h after the infection, whereas the plasma levels of IL-10 did not differ between groups (Table 1). Consistent with our previous findings, plasma cytokine levels were undetectable at time points beyond 6 h post-infection. IL-12p70, IFN-γ and MIP-2 could not be detected at any time point.
In accordance with our previous report, *E. faecium* peritonitis did not result in histopathological changes in liver or lungs in control mice. Prolonged enterococcal loads and elevated cytokine levels in mice treated with clodronate-liposomes did not cause clear histopathological changes in these organs either (data not shown).

Figure 6: Enhanced peritoneal cytokine and chemokine response in peritoneal macrophage depleted mice. Mice were intraperitoneally injected with either clodronate containing liposomes (closed symbols) or saline (open symbols) at day -4 and day -1. At time point 0, mice were injected i.p. with $10^8$ CFU *E. faecium*. Cytokine levels were measured just before infection and 6 h, 1 day and 2 days after the infection. TNF-α (A), IL-6 (B), MCP-1 (C), IL-10 (D), KC (E) and LIX (F). Results are expressed as mean ± SEM (n = 8 per group per time point). *P*-values in the figures represent overall differences between groups, *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared to control mice at the indicated time points.
Table 1. Increased cytokine response in plasma of peritoneal macrophage-depleted mice, 6 h after infection with *E. faecium*.

<table>
<thead>
<tr>
<th>Cytokine (pg/ml)</th>
<th>Plasma Control mice</th>
<th>Macrophage-depleted mice</th>
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</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>38 ± 4</td>
<td>123 ± 15**</td>
</tr>
<tr>
<td>IL-6</td>
<td>140 ± 32</td>
<td>265 ± 75*</td>
</tr>
<tr>
<td>IL-10</td>
<td>53 ± 8</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>MCP-1</td>
<td>64 ± 16</td>
<td>209 ± 62*</td>
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Mice were inoculated intraperitoneally with 10⁸ CFU *E. faecium* with or without prior treatment with clodronate-encapsulated liposomes to deplete PM. Data are mean ± SEM, n = 8 mice/group. *, p<0.05; **, p<0.01 compared to control mice.

Discussion

Peritoneal macrophages are the most prominent leukocytes within the healthy peritoneal cavity. We here tested the hypothesis that resident PM are important for initial *E. faecium* clearance and initiation of an adequate innate immune response to *E. faecium* peritonitis. To test this hypothesis, we depleted mice of resident PM by injecting clodronate-liposomes intraperitoneally and subsequently infected these animals with *E. faecium*. The main finding was that PM-depleted mice displayed a strongly reduced capacity to clear *E. faecium* from the primary site of infection which was accompanied by higher enterococcal loads in blood. These data support the presence of a crucial role for PM in host defense against intraperitoneal infection with *E. faecium*.

Enterococci are increasingly reported as causing nosocomial infections worldwide, an observation which is mainly attributed to a rise in multi-drug resistant *E. faecium* strains specifically adapted to the hospital environment.5,20 In particular, severely ill, immunocompromised patients are vulnerable to infections with enterococci; in healthy humans, *E. faecium* only rarely causes infections. Knowledge of how the intact innate immune system deals with this bacterium is limited. We recently developed a model of *E. faecium* peritonitis in mice and examined the role of Toll-like receptor (TLR) signaling in host defense, demonstrating that TLR2 and its adaptor myeloid differentiation primary-response protein (MyD) 88 are important for the recognition of this bacterium by PM and that TLR2 dependent MyD88 signaling is important for effective enterococcal clearance after *in vivo* infection.10 Macrophages, which are present in all loose connective tissue in addition to lining blood vessels in some organs (liver, spleen, and bone marrow), have been implicated in host defense against infection and to contribute to instigation of
the initial inflammatory response. Macrophages participate in both innate and specific immunity and have numerous functions including phagocytosis, antigen processing/presentation and secretion of pro- and anti-inflammatory cytokines. Previous investigations have documented that, during the early phase of peritonitis residential macrophages and the lymphatic system (that drains to the thoracic lymphatic ducts and subsequently into the bloodstream) are important for containing the infection. Dunn et al. showed that the first line of host defense during peritonitis caused by Escherichia coli is determined by the capacity of PM and the diaphragmatic lymphatic system to inactivate and eliminate invading microorganisms. These authors instilled radiolabelled dead E. coli and found that one-half of the bacteria were mechanically cleared and one-third were engulfed by macrophages within minutes. Live E. coli were initially controlled by these mechanisms, but they then increased in number for up to 4 h before growth was curtailed by neutrophils. These data correspond with our results using E. faecium. We show that purified PM are capable of phagocytosing and killing E. faecium. The in vivo relevance of this finding was illustrated by the fact that PM had ingested E. faecium as early as 1 h after infection. Our own preliminary data suggest that neutrophils contribute to elimination of E. faecium from the peritoneal cavity in a later phase during the infection (data not shown). Together, these data suggest that neutrophil influx probably serves to eliminate those bacteria that have eluded the first line of defense that, in part, is provided by resident PM.

Previously, it has been shown that intraperitoneal deposition of clodronate-liposomes induces local and selective apoptosis of peritoneal and omental macrophages. Granulocytes and endothelial cells also incorporate liposomes, but are insensitive to the cytotoxic effect of clodronate. Clodronate-liposomes do not influence the viability of peritoneal mast cells. Repopulation from the bone marrow requires 7 days for PM and 7-28 days for macrophages of the omentum. Accordingly, in the present study intraperitoneal administration of clodronate-liposomes caused a sustained depletion of PM in uninfected mice for at least 5 d. However, after induction of E. faecium peritonitis, clodronate-liposome treated mice showed recruitment of PM resulting in PM counts in their peritoneal lavage fluid similar to those in infected control mice. These data are in accordance with earlier findings in a rat model of sterile peritonitis, in which clodronate-liposome treated rats displayed similar PM peritoneal fluid counts 1 day after intraperitoneal injection of Bacto-Tryptone. Together, these data suggest that peritoneal infection and inflammation stimulate the accumulation of PM and that prior treatment with clodronate-liposomes does not affect this response. Mononuclear cells might be recruited from tissues or the circulation by chemo-attractants secreted by cells other
than macrophages. Cells from the monocyte/macrophage lineage are mainly attracted by the CC chemokines, of which MCP-1 is an important member. This chemokine is produced by many cell types such as mast cells and eosinophils, and has been shown to cause monocyte accumulation in vivo.33-36 We found increased MCP-1 levels in the PLF of clodronate-liposome treated mice, 6 and 24 h after the induction of the peritonitis, which may have contributed to enhanced recruitment of macrophages into the peritoneal cavity. Of importance, in spite of the fact that new macrophages migrated into peritoneal fluid of clodronate-liposome administered mice, their antibacterial defense was strongly diminished, suggesting that the presence of resident PM is key for initiating an effective innate immune response upon initial invasion of the peritoneal cavity by E. faecium. Depletion of PM not only influenced the clearance of E. faecium but also the inflammatory response. Indeed, the local concentrations of cytokines and chemokines and the early release of cytokines into the circulation were enhanced in PM-depleted mice, whereas neutrophil numbers tended to be increased. Previous investigations have indicated that the effect of PM depletion on inflammatory responses depends on the inflammation inducing stimulus. Indeed, Ajuebor et al.33 showed that PM depletion inhibits neutrophil influx in LPS-induced inflammation, yet augments neutrophil influx in zymosan peritonitis, and has no effect in thioglycolate peritonitis. Furthermore, Knudsen et al.22 demonstrated that PM depletion inhibits neutrophil influx during Bacto-Tryptone induced peritonitis. Of interest, PM depletion was associated with enhanced release of the pro-inflammatory mediators TNF-α, MCP-1, KC and LIX, but not of the anti-inflammatory cytokine IL-10, at the primary site of infection, suggesting that PM have a predominant anti-inflammatory effect on the cytokine/chemokine network in the peritoneal cavity.

In contrast to our current data, Ajuebor et al.33 showed that reduced IL-10 production in PM-depleted mice might play a role in the amplified inflammatory response seen in zymosan peritonitis. The increased cytokine and chemokine production in the PM-depleted mice implies cells other than PM produce these mediators. Several studies have demonstrated the importance of mast cells in the inflammatory response and neutrophil migration to the inflamed peritoneal cavity, by virtue of their capacity to secrete pro-inflammatory mediators like TNF-α, leukotrienes, complement, opsonins and vasoactive substances.37-40 Ajuebor et al.33 demonstrated reduced KC and MCP-1 production in mast cell depleted (but not in macrophage depleted) mice during experimental inflammation. Moreover, other cell types, such as endothelial and mesothelial cells could, at least, in part be responsible for the production of these mediators.33,35,40-42 Knowledge about the pathogenesis of infections with multi-drug resistant E. faecium is limited. Using a mouse model of E. faecium peritonitis, we have demonstrated that
The depletion of PM hampers the initial clearance of *E. faecium* from the peritoneal cavity, whereas the inflammatory response is increased. These data indicate that PM play an important role in the eradication of bacteria and the regulation of the peritoneal inflammatory response during enterococcal peritonitis.

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**References**


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