Urokinase-Type Plasminogen Activator Receptor Plays a Role in Neutrophil Migration during Lipopolysaccharide-Induced Peritoneal Inflammation but Not during Escherichia coli–Induced Peritonitis

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Background. Urokinase-type plasminogen activator receptor (uPAR) is expressed on many different cells, including leukocytes. uPAR has been implicated to play a role in neutrophil migration to sites of inflammation.

Methods. To determine the role that uPAR plays in neutrophil recruitment in response to bacterial products or intact bacteria, uPAR gene–deficient (uPAR⁻/⁻) and wild-type (wt) mice were injected intraperitoneally with either Escherichia coli or lipopolysaccharide (LPS) derived from this bacterium.

Results. uPAR⁻/⁻ mice demonstrated a decreased LPS-induced neutrophil migration into peritoneal lavage fluid, whereas the chemokine and cytokine response was unaltered. In contrast, during E. coli–induced peritonitis, uPAR⁻/⁻ mice had a normal neutrophil migration into the primary site of infection. The unaltered neutrophil trafficking in uPAR⁻/⁻ mice during bacterial infection was corroborated by histological assessment of liver and lung tissue and myeloperoxidase levels in tissue homogenates. uPAR⁻/⁻ mice displayed slightly but significantly lower bacterial loads in the peritoneal cavity, together with a decreased dissemination to the circulation early during the infection.

Conclusion. These data suggest that uPAR, in part, mediates neutrophil migration into the peritoneal cavity on local instillation of LPS but that this function of uPAR can be compensated for during peritonitis caused by intact E. coli.

Effective recruitment of inflammatory cells to the site of an infection is of crucial importance for an adequate host defense against invading microorganisms. The urokinase-type plasminogen activator (uPA) receptor (uPAR; CD87) has been implicated to play an important role in this process [1, 2]. uPAR is a high-affinity receptor for uPA and is expressed by many different cell types, including leukocytes, endothelial cells, and tumor cells.

The expression of uPAR on monocytes and neutrophils is up-regulated on exposure to bacteria or lipopolysaccharide (LPS) [3–5]. uPAR can influence cellular movement in different ways. The binding of uPA by uPAR results in the formation of plasmin at the leading edge of cells, and this facilitates cell migration by pericellular proteolysis of extracellular matrix proteins [2, 6]. Besides functioning as a proteinase receptor, uPAR also affects cellular migration, adhesion, differentiation, and proliferation through intracellular signaling, which occurs, in part, independently of the proteolytic activity of uPA. Because uPAR is glycosylphosphatidylinositol linked and lacks an intracellular domain, it needs to form functional transmembrane units with other molecules before it can transduce signals into the cellular interior. uPAR has been found to form such functional units with several components, including members of the integrin adhesion receptor superfamily [7, 8].
Recent studies have demonstrated that uPAR plays a role in the immune response to bacterial infection. uPAR gene-deficient (uPAR−/−) mice displayed strongly decreased neutrophil recruitment to the pulmonary compartment after induction of *Pseudomonas* or pneumococcal pneumonia, and this decreased recruitment was associated with an impaired antibacterial defense [9, 10]. Moreover, in uPAR−/− mice, cerebrospinal fluid pleocytosis was significantly attenuated during experimental pneumococcal meningitis [11]. To date, the contribution of uPAR to leukocyte trafficking toward the peritoneal cavity has been studied only in the model of sterile peritonitis induced by intraperitoneal (ip) administration of thioglycollate, and uPAR−/− mice were found to have a profoundly decreased neutrophil migration [12]. In the present study, we sought to determine the contribution of uPAR to neutrophil migration into the peritoneal cavity during peritonitis induced by bacterial products or intact bacteria. Because *Escherichia coli* is the most common pathogen involved in intra-abdominal infections in humans [13], we investigated the role that uPAR plays in the inflammatory response to ip injection of *E. coli* LPS and in the host defense against peritonitis induced by intact *E. coli*. We compared the levels of inflammatory cell migration, chemokine and cytokine responses, bacterial outgrowth, and organ damage in uPAR−/− and wild-type (wt) mice.

### MATERIALS AND METHODS

**Mice.** uPAR−/− mice on a C57BL/6J background (backcrossed at least 6 times) were purchased from Jackson Laboratory and bred in the animal facilities at the University of Amsterdam. In addition, wt C57BL/6J mice were purchased from Harlan and housed in the local animal facilities for at least 2 weeks before experiments were begun. All experiments were performed with 8–10-week-old female mice. The Committee on Use and Care of Animals of the Academic Medical Center at the University of Amsterdam approved all experiments. Animal experimentation guidelines were followed in all experiments.

**LPS-induced inflammation.** uPAR−/− and wt mice received an ip injection of 200 μg of LPS (*E. coli* serotype 0111:B14; Sigma), and groups of mice were killed before and at 3, 6, and 20 h after LPS injection. In a separate experiment, mice received an ip injection of 500 ng of LPS and were killed at 20 h after LPS injection.

**E. coli–induced peritonitis.** Peritonitis was induced as described elsewhere [14–16]. In brief, *E. coli* serotype O18:K1 was cultured in Luria-Bertani medium (Difco) at 37°C, harvested at midlog phase, and washed twice with sterile saline before injection. Mice received an ip injection of 1 × 10^7 cfu of *E. coli* in 200 μL of sterile isotonic saline.

**Sample harvesting.** Mice were anesthetized by inhalation

### Table 1. Leukocyte counts in peritoneal lavage fluid (PLF) after injection of *Escherichia coli* lipopolysaccharide (LPS).

<table>
<thead>
<tr>
<th>Cell type</th>
<th>500 ng of LPS</th>
<th>200 μg of LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt mice</td>
<td>uPAR−/− mice</td>
</tr>
<tr>
<td>All leukocytes</td>
<td>58.0 ± 11.2</td>
<td>41.7 ± 2.6</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>20.5 ± 2.6</td>
<td>10.9 ± 3.1</td>
</tr>
<tr>
<td>Macrophages</td>
<td>31.7 ± 7.3</td>
<td>26.8 ± 3.7</td>
</tr>
<tr>
<td>Other</td>
<td>5.7 ± 2.1</td>
<td>4.1 ± 1.6</td>
</tr>
</tbody>
</table>

**NOTE.** Data are expressed as means ± SEs (n = 8 mice/group/time point) after intraperitoneal injection of 500 ng or 200 μg of *E. coli* LPS. uPAR−/−, urokinase-type plasminogen activator receptor gene deficient; wt, wild type.

* P < .05, vs. wt mice at the same dose (Mann-Whitney U test).

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Unaltered lipopolysaccharide (LPS)–induced chemokine release in urokinase-type plasminogen activator receptor gene–deficient (uPAR−/−) mice. Macrophage inflammatory protein (MIP)-2 and keratinocyte-derived chemokine (KC) levels were measured in peritoneal lavage fluid (PLF) from uPAR−/− (black squares) and wild-type (wt; white squares) mice at 0, 3, 6, and 20 h after intraperitoneal injection of 200 μg of LPS (n = 8 mice/group/time point). Data are expressed as means ± SEs.
of 2% isoflurane (Abbott Laboratories) in 2 liters of O₂. A peritoneal lavage was then performed with 5 mL of sterile isotonic saline and an 18-gauge needle, and peritoneal lavage fluid (PLF) was collected in sterile tubes (Plastipack; Becton Dickinson). After collection of PLF, deeper anesthesia was induced by ip injection of 0.07 mL of FFM mixture (fentanyl [0.315 mg/mL]-fluanisone [10 mg/mL; Janssen] and midazolam [5 mg/mL]-fluanisone [10 mg/mL; Janssen] and midazolam [5 mg/mL; Roche]) per gram of body weight. The abdomen was opened, and blood was drawn from the vena cava inferior into a sterile syringe, transferred to tubes containing heparin, and immediately placed on ice. Thereafter, liver and lungs were harvested and processed for measurements of colony-forming units, chemokines, and cytokines and for histological assessment, as described below.

**Cell counts and differentials.** Cell counts in PLF were determined by use of a hemacytometer (Beckman Coulter). Subsequently, the pellet was diluted in PBS to a final concentration of 1 × 10⁵ cells/mL, and differential cell counts were performed on cytospin preparations stained with a modified Giemsa stain (Diff-Quick; Dade Behring).

**Assays.** Tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10 levels were measured in peritoneal lavage fluid (PLF) and plasma from uPAR−/− (black squares) and wild-type (wt; white squares) mice at 0, 3, 6, and 20 h after intraperitoneal injection of 200 μg of LPS (n = 8 mice/group/time point). Data are expressed as means ± SEs.

**Figure 2.** Unaltered lipopolysaccharide (LPS)-induced cytokine responses in urokinase-type plasminogen activator receptor gene-deficient (uPAR−/−) mice. Tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10 levels were measured in peritoneal lavage fluid (PLF) and plasma from uPAR−/− (black squares) and wild-type (wt; white squares) mice at 0, 3, 6, and 20 h after intraperitoneal injection of 200 μg of LPS (n = 8 mice/group/time point). Data are expressed as means ± SEs.

**Table 2.** Leukocyte counts in peritoneal lavage fluid (PLF) during *Escherichia coli*-induced peritonitis.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>6 h after injection</th>
<th>20 h after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt mice</td>
<td>uPAR−/− mice</td>
</tr>
<tr>
<td>All leukocytes</td>
<td>110 ± 11.3</td>
<td>73.7 ± 5.25⁰</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>73.3 ± 8.63</td>
<td>53.6 ± 5.46</td>
</tr>
<tr>
<td>Macrophages</td>
<td>32.8 ± 5.38</td>
<td>19.3 ± 5.87</td>
</tr>
<tr>
<td>Other</td>
<td>3.60 ± 0.67</td>
<td>1.53 ± 0.54</td>
</tr>
</tbody>
</table>

**NOTE.** Data are expressed as means ± SEs ×10⁶ per milliliter of PLF (n = 8 mice/group/time point) at 6 or 20 h after intraperitoneal injection of 1 × 10⁹ cfu of *E. coli*; uPAR−/−, urokinase-type plasminogen activator receptor gene deficient; wt, wild type.

⁰ P < .05, vs. wt mice at the same time point (Mann-Whitney U test).
which included the migration of leukocytes and the presence of endothelitis; and (3) the presence and degree of necrosis (liver only). Inflammation and hepatic necrosis were rated separately in 5 random microscopic fields (magnification, X 100 [liver] or X200 [lungs]); (2) the presence and degree of inflammation, thrombi were homogenized at 4°C in 5 volumes of sterile isotonic saline by use of a tissue homogenizer (Biospect Products), which was carefully cleaned and disinfected with 70% ethanol after each homogenization. Serial 10-fold dilutions in sterile saline were made from these homogenates, blood, and PLF, and 50-μL volumes were plated onto sheep blood agar plates and incubated in 5% CO₂ at 37°C. Colony-forming units were determined after 20 h of incubation. Colony-forming units were counted after 5% CO₂ at 37°C. Colony-forming units were counted after 20 h of incubation.

Histological assessment. Immediately after mice were killed, liver and lung samples were fixed with 4% formalin and embedded in paraffin. Paraffin sections were cut 4 μm thick and stained with hematoxylin-eosin. All slides were coded and scored by a pathologist who did not have knowledge of the strain of mice. Liver and lung sections were scored in accordance with the following parameters: (1) the number of thrombi counted in 5 random microscopic fields (magnification, ×100 [liver] or ×200 [lungs]); (2) the presence and degree of inflammation, which included the migration of leukocytes and the presence of endothelitis; and (3) the presence and degree of necrosis (liver only). Inflammation and hepatic necrosis were rated separately on a scale from 0 to 3, in which 0 meant that the condition was absent, 1 meant that it was occasionally present, 2 meant that it was regularly present, and 3 meant that it was present in massive amounts. Granulocyte staining was performed as described elsewhere [10, 16]. Granulocytes were counted in 5 random microscopic fields (magnification, ×200 [liver] or ×400 [lungs]).

Statistical analysis. Data are expressed as means ± SEs, unless indicated otherwise. Comparisons between groups were conducted using the Mann-Whitney U test. P < .05 was considered to be statistically significant.

RESULTS

Diminished LPS-induced neutrophil migration into the peritoneal cavity in uPAR−/− mice. Previous work has implicated uPAR as a regulator of cell recruitment to sites of inflammation [1, 3, 9-12]. Therefore, we investigated whether uPAR deficiency affects cell migration during E. coli LPS–induced peritoneal inflammation. Leukocyte counts and differentials in PLF were determined after ip injection of a low (500 ng) or a high (200 μg)
dose of LPS. Both doses induced a migration of immune cells, particularly neutrophils, into the peritoneal cavity at 20 h after LPS injection. After the low dose of LPS, the number of neutrophils in uPAR<sup>−/−</sup> mice decreased to 47% of that in wt mice, and after the high dose of LPS, the number decreased to 39% of that in wt mice (for both, \( P < .05 \) ) (table 1).

**Normal chemokine and cytokine responses to LPS in uPAR<sup>−/−</sup> mice.** Because glutamic acid–leucine–arginine–positive (ELR<sup>+</sup>) CXC chemokines have been implicated in the attraction of neutrophils to the site of an infection [17, 18], we measured the primary mouse ELR<sup>+</sup> CXC chemokines MIP-2 and KC in PLF. LPS injection increased MIP-2 and KC levels in PLF from both wt and uPAR<sup>−/−</sup> mice. The levels of both chemokines were similar in the 2 groups of mice (figure 1), ruling out the possibility that decreased levels of these neutrophil attractants were responsible for the decreased neutrophil migration in uPAR<sup>−/−</sup> mice.

Furthermore, to investigate whether uPAR deficiency might influence the local or systemic cytokine response to *E. coli* LPS, we measured the levels of TNF-\( \alpha \), IL-6, and IL-10 in PLF and plasma. In PLF, TNF-\( \alpha \) and IL-6 levels were increased at 2, 6, and 20 h after LPS injection, whereas IL-10 remained undetectable; there were no differences in cytokine levels between uPAR<sup>−/−</sup> and wt mice (figure 2). In plasma, LPS induced a strong increase in all cytokine levels; however, none of the cytokine levels differed between uPAR<sup>−/−</sup> and wt mice (figure 2). These data indicate that uPAR<sup>−/−</sup> mice are able to mount a normal local and systemic cytokine response to *E. coli* LPS-induced peritoneal inflammation.

**uPAR and mediation of neutrophil trafficking during *E. coli*–induced peritonitis.** Having established that uPAR<sup>−/−</sup> mice showed a decreased neutrophil response to LPS injection, we next investigated whether uPAR also mediates neutrophil recruitment to the peritoneal cavity during peritonitis induced by intact *E. coli*. To this end, wt and uPAR<sup>−/−</sup> mice received ip injection of \( 1 \times 10^4 \) cfu of *E. coli*. *E. coli* injection resulted in
stainings of liver tissue and measured MPO levels in liver homogenates (figure 3). On histopathological examination, both groups of mice showed liver tissue necrosis and intralobular thrombi. Histological scores of the liver sections (quantified in accordance with the scoring system described in Materials and Methods) did not differ between wt and uPAR-/- mice. Granulocyte stainings revealed that the number of granulocytes in 5 random microscopic fields, determined in all liver sections, did not differ between wt and uPAR-/- mice. These findings were confirmed by MPO levels in liver homogenates, which were not different between wt and uPAR-/- mice at 6 or 20 h after E. coli injection. Finally, to obtain insight into the role that uPAR plays in neutrophil migration to a more distant organ during E. coli-induced peritonitis, lungs were harvested at 20 h after E. coli injection. Lungs of both wt and uPAR-/- mice showed clear signs of inflammation, as was reflected by an accumulation of leukocytes in the interstitium (figure 4). In addition, lungs of both wt and uPAR-/- mice contained multiple thrombi. Although the mean total histological score tended to be lower in uPAR-/- mice than in wt mice, the difference did not reach statistical significance (P = .19). When granulocyte stainings were performed and the number of granulocytes in the lung sections (per 5 random microscopic fields) was counted, uPAR-/- mice were found to have a slightly lower number of neutrophils than wt mice (the difference was not significant). In agreement with these data, MPO levels in lung homogenates, measured at 6

Figure 5. Bacterial outgrowth. Nos. of Escherichia coli colony-forming units were measured in peritoneal lavage fluid (PLF), blood, and liver from urokinase-type plasminogen activator receptor gene-deficient (uPAR-/-; black bars) and wild-type (wt; white bars) mice at 6 and 20 h after intraperitoneal injection of 1 × 10⁶ cfu of E. coli (n = 8 mice/group/time point). Data are expressed as means ± SEs. *P < .05, vs. wt mice (Mann-Whitney U test).

a strong migration of leukocytes into the peritoneal cavity and, as occurred during LPS-induced peritoneal inflammation, neutrophils were primarily the cells that were recruited (table 2). Remarkably, at both 6 and 20 h after E. coli injection, neutrophil counts in PLF from uPAR-/- and wt mice were similar (table 2). To obtain further proof for the finding that uPAR does not contribute to neutrophil trafficking during E. coli–induced peritonitis, we performed histological analyses with granulocyte stainings of liver tissue and measured MPO levels in liver homogenates (figure 3). On histopathological examination, both uPAR-/- and wt mice showed inflammation of the hepatic parenchyma, as characterized by leukocyte migration into the interstitium. In addition, both groups of mice showed liver tissue necrosis and intralobular thrombi. Histological scores of the liver sections (quantified in accordance with the scoring system described in Materials and Methods) did not differ between wt and uPAR-/- mice. Granulocyte stainings revealed that the number of granulocytes in 5 random microscopic fields, determined in all liver sections, did not differ between wt and uPAR-/- mice. These findings were confirmed by MPO levels in liver homogenates, which were not different between wt and uPAR-/- mice at 6 or 20 h after E. coli injection. Finally, to obtain insight into the role that uPAR plays in neutrophil migration to a more distant organ during E. coli–induced peritonitis, lungs were harvested at 20 h after E. coli injection. Lungs of both wt and uPAR-/- mice showed clear signs of inflammation, as was reflected by an accumulation of leukocytes in the interstitium (figure 4). In addition, lungs of both wt and uPAR-/- mice contained multiple thrombi. Although the mean total histological score tended to be lower in uPAR-/- mice than in wt mice, the difference did not reach statistical significance (P = .19). When granulocyte stainings were performed and the number of granulocytes in the lung sections (per 5 random microscopic fields) was counted, uPAR-/- mice were found to have a slightly lower number of neutrophils than wt mice (the difference was not significant). In agreement with these data, MPO levels in lung homogenates, measured at 6

Figure 6. Unaltered chemokine levels in peritoneal lavage fluid (PLF) from urokinase-type plasminogen activator receptor gene-deficient (uPAR-/-) mice during Escherichia coli–induced peritonitis. Macrophage inflammatory protein (MIP)-2 and keratinocyte-derived chemokine (KC) levels were measured in PLF from uPAR-/- (black bars) and wild-type (wt; white bars) mice at 6 and 20 h after intraperitoneal injection of 1 × 10⁶ cfu of E. coli (n = 8 mice/group/time point). Data are expressed as means ± SEs.
and 20 h after *E. coli* injection, also tended to be lower in uPAR−/− mice than in *wt* mice (the difference was not significant). Altogether, these data indicate that uPAR does not make an important contribution to neutrophil trafficking during *E. coli*-induced peritonitis.

**Bacterial outgrowth and chemokine/cytokine response.** In theory, uPAR deficiency can impair the host defense against bacterial infection in at least 2 ways. First, impaired neutrophil recruitment to the site of infection is expected to facilitate the outgrowth of bacteria. Second, uPAR has been implicated to play a role in bacterial phagocytosis [19]. If uPAR−/− mice had higher bacterial loads in the peritoneal cavity, this could have masked a relatively impaired neutrophil migration—that is, the higher bacterial load would have provided a more potent proinflammatory stimulus, and the response to it would have overcome the deficient recruitment of uPAR−/− neutrophils. Therefore, we compared the number of *E. coli* colony-forming units in PLF at 6 and 20 h after injection; in addition, blood and liver were cultured at the same time points (figure 5). Remarkably, at 6 h after *E. coli* injection, uPAR−/− mice had slightly but significantly fewer bacteria in the peritoneal cavity and blood than did *wt* mice (for both, *P* < .05). At 20 h after *E. coli* injection, uPAR−/− mice still had lower mean bacterial loads in both compartments, but the differences were no longer statistically significant. The bacterial loads in liver did not differ between the 2 groups of mice at either time point. To examine whether uPAR deficiency influences chemokine or cytokine responses, we measured chemokine levels in PLF and cytokine levels in both PLF and plasma. uPAR−/− and *wt* mice displayed similar MIP-2 and KC levels at the site of infection at both 6 and 20 h after *E. coli* injection (figure 6). In PLF, uPAR−/− mice had lower cytokine levels at 6 h after *E. coli* injection than did *wt* mice, and the difference in IL-10 levels between the 2 groups of mice was significant (figure 7). In plasma, uPAR−/− mice tended to have lower TNF, IL-6, and IL-10 levels at both time points than did *wt* mice, but the differences did not reach statistical significance (figure 7).

**DISCUSSION**

Peritonitis is the second most common cause of sepsis [20], and *E. coli* is a major pathogen involved in the disease [13]. uPAR has been implicated as an important receptor for the regulation of leukocyte trafficking to sites of inflammation and infection [1, 2, 6]. The requirement for uPAR before cell invasion into the peritoneal cavity can occur has been previously demonstrated in a noninfectious inflammation model [12]. To our knowledge, the present study is the first to investigate the role that uPAR plays in neutrophil migration into the peritoneal cavity in response to locally instilled LPS or intact *E. coli*. We demonstrated that uPAR participates in neutrophil migration to the peritoneal cavity after ip injection of LPS but that this function of uPAR is compensated for during peritonitis caused by intact *E. coli*.

uPAR is a multifunctional protein involved in different inflammatory responses, including cell-associated proteolysis, cell
adhesion, chemotaxis, cell migration, and proliferation [1, 2, 6]. uPAR, which lacks an intracellular domain and therefore is not able to directly activate intracellular pathways, can induce cellular responses by interacting with other molecules, such as vitronectin, caveolin, and integrins. A great deal of research on the functional and physical interaction between uPAR and β2 integrins, including leukocyte function associated antigen (LFA)-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), has been conducted. In a previous study, uPAR−/− mice displayed a 50% decrease in leukocyte counts in PLF after ip administration of thioglycollate; treatment with a blocking anti–LFA-1 antibody strongly decreased the leukocyte migration in wt mice, whereas it only marginally affected the leukocyte counts in PLF from uPAR−/− mice, suggesting that the deficiency in the leukocyte migration in uPAR−/− mice was due to a perturbed LFA-1 function [12]. During Pseudomonas pneumonia, anti-CD11b treatment profoundly inhibited the neutrophil recruitment into the lungs of wt mice but did not further diminish the already strongly decreased the neutrophil migration in uPAR−/− mice [9]. Although these data clearly establish a biologically relevant interaction between uPAR and β2 integrins, the functional consequence of the association between uPAR and individual members of the integrin family likely depends on the site of inflammation and the nature of the inflammatory stimulus. Indeed, in contrast to CD11a, CD11b does not play a significant role in neutrophil recruitment to the peritoneal cavity during thioglycollate-induced peritonitis [21, 22]; however, during polymicrobial peritonitis induced by cecal ligation and puncture, CD11b gene–deficient mice had a decreased neutrophil migration into the peritoneal cavity, compared with that in wt mice [23]. Moreover, neutrophil recruitment during pneumococcal pneumonia, which occurs via a CD11b/CD18-independent mechanism [24, 25], is largely dependent on the presence of uPAR [10]. Our finding that uPAR−/− mice have a decreased capacity to recruit neutrophils to the site of inflammation after LPS injection is in agreement with earlier findings for neutrophil recruitment during thioglycollate-induced peritonitis. The mechanism by which uPAR mediates this response remains to be investigated, although an interaction with 1 member or more of the integrin family seems likely. It is quite conceivable that locally produced ELR+ CXC chemokines, such as MIP-2 and KC, contribute to this characteristic innate immune response [17, 18]. In the present study, uPAR−/− and wt mice had similar MIP-2 and KC levels in PLF after LPS injection, indicating that these chemokines could not have contributed to the differences in neutrophil recruitment between these groups of mice.

Neutrophils constitute an important component of the early host defense against bacterial infection. A decreased capacity of neutrophils to migrate to the site of an infection is likely to be associated with an impaired local antibacterial defense, indicating a protective role for migrating neutrophils [23, 26, 27].

Because we found impaired LPS-induced neutrophil migration to the peritoneal cavity in uPAR−/− mice, we hypothesized that uPAR−/− mice would also show decreased neutrophil recruitment after E. coli injection and, as a consequence, an impaired antibacterial host defense. Surprisingly, we did not find a significant difference in the neutrophil migration into PLF at 6 and 20 h after E. coli injection between uPAR−/− and wt mice. Of note, in PLF obtained 6 h after E. coli injection, total leukocyte counts were slightly but significantly lower in uPAR−/− mice, compared with those in wt mice, and this decrease was caused by small, statistically insignificant decreases in the numbers of all leukocyte subsets. We consider the biological relevance of these small differences between uPAR−/− and wt mice to be limited, and the notion that uPAR does not participate in neutrophil trafficking to a significant extent during E. coli–induced peritonitis was further supported by the finding that the numbers of neutrophils in liver and lungs, as determined by neutrophil staining and measurement of MPO levels, did not differ between uPAR−/− and wt mice. A possible explanation for the discrepant roles that uPAR plays during LPS- and E. coli–induced neutrophil migration may lie in the much stronger and more long-lived proinflammatory stimulus provided by intact E. coli, as is shown by the higher number of total neutrophils and the higher levels of MIP-2, TNF-α, and IL-6 in PLF, compared with those after LPS injection. Thus, during E. coli–induced peritonitis, the effect that the uPAR deficiency has on neutrophil recruitment to the site of infection possibly was compensated for by other potent chemotactic mediators, such as complement factors and CXC chemokines [18, 28].

Gyetko et al. showed that neutrophils from uPAR−/− mice had a decreased bacterial phagocytosis capacity in vitro [19]. In contrast, we found that the bacterial outgrowth was attenuated in uPAR−/− mice soon after injection. Furthermore, uPAR−/− mice showed lower cytokine levels in PLF at 6 h after injection. This latter finding was most likely the result of a lower inflammatory stimulus due to the lower bacterial load in the peritoneal cavity. We have no clear explanation for the differences in bacterial loads between uPAR−/− and wt mice at 6 h after injection. Because all differences disappeared during a more advanced stage of infection, they likely did not have a significant impact on the course of the infection.

uPAR is believed to play an important role in neutrophil migration to sites of inflammation and infection. To our knowledge, the role that uPAR plays during bacterial infection has, to date, been demonstrated only in experimental models of pneumonia and meningitis, which clearly revealed the contribution of this receptor to the neutrophil migration into the lungs and the cerebrospinal fluid [9–11]. In the present study, we showed that, although uPAR is involved in the neutrophil migration into the peritoneal cavity in response to the local administration of LPS, it is not indispensable for adequate neu-
neutrophil recruitment during peritonitis induced by intact *E. coli*. Remarkably, in preliminary studies, we found that uPAR deficiency resulted in an enhanced rather than a decreased neutrophil migration into the peritoneal cavity during peritonitis induced by *Streptococcus pneumoniae* (authors' unpublished data). Together, these data suggest that the role that uPAR plays in neutrophil trafficking may depend on the site and the type of infection.

Acknowledgments

We thank Joost Daalhuisen and Anita de Boer, for their excellent technical assistance.

References