Receptor for Advanced Glycation End Products Facilitates Host Defense during *Escherichia coli*–Induced Abdominal Sepsis in Mice

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**Background.** The receptor for advanced glycation end products (RAGE) mediates a variety of inflammatory responses.

**Methods.** To determine the role of RAGE in the innate immune response to abdominal sepsis caused by *Escherichia coli*, RAGE-deficient (RAGE−/−) and normal wild-type mice were intraperitoneally injected with *E. coli*. In a separate experiment, wild-type mice received either anti-RAGE immunoglobulin (Ig) G or control IgG.

**Results.** *E. coli* sepsis resulted in an up-regulation of RAGE in the liver but not in the lungs. RAGE-deficient mice demonstrated an enhanced bacterial outgrowth in their peritoneal cavity and increased dissemination of the infection, accompanied by increased hepatocellular injury and exaggerated systemic cytokine release and coagulation activation, 20 h after intraperitoneal administration of *E. coli*. Wild-type mice treated with anti-RAGE IgG also displayed a diminished defense against the growth and/or dissemination of *E. coli*. RAGE was important for the initiation of the host response, as reflected by a reduced immune and procoagulant response early after intraperitoneal injection of *E. coli* lipopolysaccharide.

**Conclusion.** These data are the first to suggest that intact RAGE signaling contributes to an effective antibacterial defense during *E. coli* sepsis, thereby limiting the accompanying inflammatory and procoagulant response.

Sepsis is the most common cause of death in noncoronary critical care units in the United States, with >750,000 cases per year [1]. Peritonitis is the second most common cause of sepsis [2], with *Escherichia coli* being 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.

The receptor for advanced glycation end products (RAGE) is a multiligand receptor of the immunoglobulin (Ig) superfamily that is expressed in all tissues on a wide range of cell types, including cells involved in the innate and adaptive immune system [4–6]. RAGE is one of the major signal transduction receptors for advanced glycation end products. Ongoing studies revealed, however, that RAGE is able to engage classes of unrelated (including non–advanced glycation end product) molecules, recognizing their tertiary structures rather than their amino acid sequences [4]. Its known ligands include the damage-associated molecular patterns (ie, endogenous molecules that signal tissue and cell damage [7]) high mobility group box 1 (HMGB1) [8, 9], S100A12 [10], S100B [11], and amyloid [12].

RAGE has been suggested to be involved in the inflammatory response in several ways. First, the cellular effects resulting from the activation of RAGE by above-mentioned endogenous proinflammatory ligands are mediated by multiple intracellular signaling pathways, including nuclear factor–κB, leading to the transcrip-
tion of proinflammatory factors [13, 14]. In addition to binding ligands that participate in inflammatory and immune responses, in vitro studies have shown that RAGE on endothelial and epithelial cells [15, 16] can function as an adhesive receptor that interacts with leukocyte β2-integrins, thereby being directly involved in inflammatory cell recruitment [15, 16].

RAGE deficiency improved survival in a model of abdominal polymicrobial sepsis induced by cecal ligation and puncture (CLP) [17, 18]. These studies suggested that inhibition of RAGE during sepsis attenuates the systemic inflammatory response and ensuing organ damage. However, the CLP model is less suitable to study the influence of an intervention on bacterial growth and dissemination, considering that the infection is polymicrobial, involving a large number of aerobic and anaerobic pathogens, and considering that the antibacterial response is dependent on the extent of necrosis of the cecum and the formation of a local abscess [19]. Therefore, we here investigated the role of RAGE during abdominal sepsis induced by one of its major involved pathogens, *E. coli*, focusing on the outgrowth of bacteria at the primary site of infection, the subsequent dissemination, and the accompanying systemic inflammatory response syndrome.

**METHODS**

**Mice.** Eight to 10–week-old female RAGE-deficient (RAGE−/−) mice on a C57Bl/6 background (backcrossed 10 times) were generated as described elsewhere [17]. Age- and sex-matched wild-type C57Bl/6 mice were obtained from Harlan Sprague Dawley. The Institutional Animal Care and Use Committee of the Academic Medical Center, University of Amsterdam, approved all experiments.

**Experimental groups.** *E. coli* peritonitis and lipopolysaccharide (LPS)–induced inflammation were induced as described elsewhere [20–22]. In some studies, wild-type mice were injected intraperitoneally with either rabbit anti-RAGE IgG polyclonal antibodies (200 μg given 20 h before and 4 h after infection; produced as described elsewhere [23]) or normal rabbit IgG (R & D Systems). Mice were sacrificed 20 h after *E. coli* injection. Assays were performed as described elsewhere [20, 21]. For measurements by enzyme-linked immunosorbent assay, liver and lung homogenates were lysed in lysis buffer and treated as described elsewhere [20, 21].

**Histological examination.** Lungs and livers were harvested 20 h after infection, were fixed in 4% formaldehyde, were embedded in paraffin, and were cut into 4-μm-thick sections for staining procedures. Immunostaining for RAGE was performed on paraffin slides after deparaffinization and rehydration by standard procedures. Primary antibodies were goat anti-mouse RAGE polyclonal antibodies (Neuromics), and secondary antibodies were biotinylated rabbit anti-goat antibodies (Dako-Cytomation). Hematoxylin-eosin stainings were performed as described elsewhere [24]. Liver and lung injury were semiquantitatively scored as described elsewhere [21]. Fibrin(ogen) stainings were performed as described elsewhere [20, 25].

**Statistical analysis.** All data are expressed as mean values ± standard errors. Differences between groups were analyzed by Mann-Whitney U test. *P* values <.05 were considered to represent a statistically significant difference.

**RESULTS**

**RAGE is up-regulated in the liver but not in lungs during *E. coli* peritonitis.** To determine whether RAGE expression changes during *E. coli* peritonitis, we performed immunohistochemical stainings of RAGE in liver and lung tissue from wild-type mice after intraperitoneal administration of *E. coli*. In accordance with other reports [26–28], we found that normal, healthy mice showed modest, if any, RAGE staining in liver tissues (figure 1A). Liver samples from mice injected with *E. coli* displayed clearly more-diffuse hepatic RAGE expression (figure 1B). Moreover, the surface of sinusoidal cells (capillaries between the hepatocytes; figure 1B), leukocytes in the vessels, and some smooth muscle cells were stained positively for RAGE (figure 1B). Remarkably, areas of necrosis showed decreased RAGE staining (figure 1B, arrows). In the lungs, RAGE was extensively present in both healthy mice and in mice infected with *E. coli* (figure 1D and 1E). Pulmonary RAGE expression was not increased in *E. coli*–injected mice, compared with healthy mice. Liver and lung tissues from RAGE−/− mice were used as negative controls for the RAGE staining and displayed only some background staining (figure 1C and 1F, respectively).

**RAGE−/− mice have enhanced bacterial outgrowth and dissemination to distant organs during *E. coli* peritonitis.** To examine whether RAGE deficiency influences bacterial outgrowth during peritonitis, we established the number of *E. coli* colony forming units at 20 h after infection in peritoneal lavage fluid (PLF), blood, liver, and lung samples from wild-type and RAGE−/− mice. RAGE−/− mice had significantly higher bacterial loads in their PLF samples than did wild-type mice (*P* <.01; figure 1G). In addition, blood, liver, and lung samples from RAGE−/− mice contained more bacteria than did those from wild-type mice (for blood and liver samples, *P* <.01; for lung samples *P* = .03; figure 1H–1J). Therefore, RAGE−/− mice demonstrated a clearly increased outgrowth of *E. coli* at the primary site of infection, which was associated with an enhanced dissemination of bacteria to distant organs.

**Inflammatory cell influx, chemokine, and cytokine levels.** At 20 h after infection, wild-type and RAGE−/− mice had similar numbers of total leukocytes, neutrophils, and macrophages in their PLF (table 1). The mouse CXC chemokines keratinocyte-derived chemokine (KC) and macrophage inflammatory protein (MIP)–2 are important mediators in the attraction of neutrophils during inflammation [29, 30]. Therefore, we measured...
Figure 1. Expression of receptor for advanced glycation end products (RAGE) during *Escherichia coli* peritonitis and increased local bacterial outgrowth and dissemination in RAGE-deficient (RAGE−/−) mice. Representative view of a liver (A) and lung (D) sample from a normal, uninfected wild-type (Wt) mouse, displaying modest if any staining in the liver and strong RAGE expression in the lung. B, Liver sample from a Wt mouse 20 h after intraperitoneal injection of 5 × 10^6 colony-forming units (CFUs) of *E. coli* showed more diffuse hepatic RAGE staining. The surface of sinusoidal cells (capillaries between the hepatocytes), leukocytes in the vessels, and some smooth muscle cells were stained RAGE positive. Arrows indicate areas of necrosis. C, Absence of RAGE positivity in the liver of a RAGE−/− mouse. Lungs from a Wt mouse 20 h after the inoculation of *E. coli* (E) and from a healthy Wt mouse (D). F, Absence of RAGE positivity in the lung of a RAGE−/− mouse. Original magnification, ×10. Bacterial loads in peritoneal lavage fluid (PLF) (G), blood (H), liver homogenate (I), and lung homogenate (J) specimens were determined for Wt (white bars) and RAGE−/− (black bars) mice 20 h after intraperitoneal injection of 5 × 10^6 CFU *E. coli* (6–16 mice per genotype). Data are mean values ± standard errors. **, compared with Wt mice; *** , compared with Wt mice. 

The concentrations of these chemokines in PLF specimens. RAGE−/− mice displayed higher MIP-2 levels than did wild-type mice (P < .05), whereas KC levels in PLF specimens were similar in both mouse strains (table 1). To determine whether RAGE influences the production of cytokines during septic peritonitis, local and systemic concentrations of pro- and anti-inflammatory cytokines were measured in wild-type and RAGE−/− mice (table 2). Tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10 levels were significantly higher in PLF specimens from RAGE−/− mice, compared with wild-type mice (P < .05). Moreover, levels of these 3 cytokines were higher in liver homogenates from RAGE−/− mice, whereas IL-6 and IL-10 levels were elevated in plasma specimens from these mice (table 2). In addition, RAGE−/− mice displayed higher concentrations of monocyte chemoattractant protein (MCP)-1 in PLF and liver, and MCP-1 levels tended to be higher in plasma samples from this mouse strain, compared with wild-type mice. The concentrations of cytokines and chemokines were similar in lung homogenates of wild-type and RAGE−/− mice (data not shown). Thus, RAGE deficiency was associated with an increased release of cytokines, in particular in the peritoneal cavity and liver.

**RAGE−/− mice display more-severe liver damage but unaltered lung inflammation.** Our model of *E. coli* peritonitis is associated with liver injury and focal hepatic necrosis [21]. Both wild-type and RAGE−/− mice showed signs of inflammation in liver tissue, as characterized by the influx of leukocytes into the hepatic parenchyma (figure 2A and 2B). RAGE−/− mice clearly showed more signs of liver necrosis, compared with wild-type mice (figure 2B, asterisks; figure 2C; P < .05). In addition, RAGE−/− mice demonstrated more thrombus formation, compared with wild-type mice (figure 2F,
**Table 1. Leukocyte Counts and Chemokine Levels in Peritoneal Lavage Fluid**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Wild-type mice</th>
<th>RAGE⁻⁻⁻ mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count, mean cells/mL × 10⁹ ± SE</td>
<td>227.1 ± 32.9</td>
<td>179.5 ± 17.3</td>
</tr>
<tr>
<td>Total</td>
<td>185.8 ± 28.5</td>
<td>150.7 ± 15.4</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>36.5 ± 6.6</td>
<td>28.3 ± 3.0</td>
</tr>
<tr>
<td>Macrophages</td>
<td>736 ± 101</td>
<td></td>
</tr>
<tr>
<td>Chemokines, mean pg/mL ± SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KC</td>
<td>3758 ± 1982</td>
<td>17859 ± 10400</td>
</tr>
<tr>
<td>MIP-2</td>
<td>422 ± 126</td>
<td>763 ± 101*a</td>
</tr>
</tbody>
</table>

**NOTE.** Cell counts and chemokine levels were measured in 14–16 mice per group at 20 h after intraperitoneal injection of 5 × 10⁹ colony forming units of *Escherichia coli*. KC, keratinocyte-derived chemokine; MIP-2, macrophage inflammatory protein-2; RAGE⁻⁻⁻, receptor for advanced glycation end products deficient; SE, standard error.

*a* *P* < .05, compared with wild-type mice.

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**Table 2. Cytokine and Chemokine Concentrations in Peritoneal Lavage Fluid (PLF), Plasma, and Liver 20 h after Intraperitoneal Injection of *Escherichia coli***

<table>
<thead>
<tr>
<th>Cytokine, location</th>
<th>Wild-type mice</th>
<th>RAGE⁻⁻⁻ mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>166 ± 51</td>
<td>266 ± 35*a</td>
</tr>
<tr>
<td>PLF</td>
<td>366 ± 206</td>
<td>759 ± 250</td>
</tr>
<tr>
<td>Plasma</td>
<td>26 ± 11</td>
<td>34 ± 6*a</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLF</td>
<td>3248 ± 1049</td>
<td>8269 ± 925*b</td>
</tr>
<tr>
<td>Plasma</td>
<td>3218 ± 1093</td>
<td>7146 ± 1455*a</td>
</tr>
<tr>
<td>Liver</td>
<td>221 ± 66</td>
<td>1251 ± 327*b</td>
</tr>
<tr>
<td>MCP-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLF</td>
<td>4674 ± 1128</td>
<td>10217 ± 793*b</td>
</tr>
<tr>
<td>Plasma</td>
<td>4355 ± 1260</td>
<td>5797 ± 1638</td>
</tr>
<tr>
<td>Liver</td>
<td>4429 ± 865</td>
<td>8838 ± 1019*b</td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLF</td>
<td>216 ± 144</td>
<td>454 ± 88*b</td>
</tr>
<tr>
<td>Plasma</td>
<td>78 ± 67</td>
<td>730 ± 233*a</td>
</tr>
<tr>
<td>Liver</td>
<td>501 ± 59</td>
<td>717 ± 88*a</td>
</tr>
</tbody>
</table>

**NOTE.** Cytokine and chemokine levels were measured in 14–16 mice per group at 20 h after intraperitoneal injection of 5 × 10⁹ colony forming units of *Escherichia coli*. IL, interleukin; MCP-1, monocyte chemotactic protein-1; TNF, tumor necrosis factor; RAGE⁻⁻⁻, receptor for advanced glycation end products deficient; SE, standard error.

*a* *P* < .05, compared with wild-type mice.

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arrow; figure 2C; *P* < .05). Clinical tests confirmed the existence of more profound hepatic cellular injury in RAGE⁻⁻⁻ mice; RAGE⁻⁻⁻ mice had significantly higher plasma aspartate aminotransferase and alanine aminotransferase levels, compared with wild-type mice (figure 2D; *P* < .05). Myeloperoxidase levels in liver homogenates were similar in wild-type and RAGE⁻⁻⁻ mice (data not shown). Pulmonary inflammation did not differ between the 2 mouse strains, as reflected by similar lung histology scores, relative lung weights, and myeloperoxidase levels in lung homogenates (data not shown). In conclusion, RAGE deficiency was associated with more-extensive hepatic cellular injury and necrosis and thrombus formation in the liver, whereas lung inflammation was not different.

**RAGE⁻⁻⁻ mice demonstrate enhanced coagulation activation during E. coli peritonitis.** This model of abdominal sepsis is associated with thrombin generation and activation of the coagulation system [20, 21]. To determine the role of RAGE herein, we performed fibrin(ogen) stainings on liver tissue slides. RAGE⁻⁻⁻ mice demonstrated increased fibrin(ogen) depositions compared with wild-type mice (compare figure 2F with figure 2E). Both wild-type and RAGE⁻⁻⁻ mice demonstrated strongly elevated thrombin-antithrombin complex (TATc) (figure 2G and 2H) and D-dimer (figure 2I and 2J) concentrations in plasma (figure 2G and 2I) and PLF specimens (figure 2H and 2J). Importantly, coagulation activation was more profound in RAGE⁻⁻⁻ mice, as reflected by higher TATc and D-dimer levels in plasma and PLF specimens, compared with wild-type mice (figure 2G–2J, *P* < .05). Together, these data indicate that RAGE deficiency enhances the activation of coagulation both locally and systemically during *E. coli* peritonitis.

**RAGE⁻⁻⁻ mice demonstrate a diminished inflammatory response to E. coli LPS.** We next investigated whether the exaggerated host response in RAGE⁻⁻⁻ mice during *E. coli* peritonitis was the consequence of the higher bacterial loads in these animals or an inhibitory effect of RAGE. For this, we injected wild-type and RAGE⁻⁻⁻ mice intraperitoneally with *E. coli* LPS and harvested plasma and PLF samples at 2 and 6 h after injection. RAGE⁻⁻⁻ mice had 2× lower neutrophil counts in their PLF specimens than did wild-type mice at 6 h after injection (table 3; *P* < .05); KC and MIP-2 levels did not differ in the PLF samples of RAGE⁻⁻⁻ and wild-type mice (table 3). Plasma TNF-α levels were clearly diminished in RAGE⁻⁻⁻ mice at 2 h after LPS injection (table 3; *P* < .01), whereas MCP-1, IL-6, and IL-10 levels were similar for both genotypes (data not shown). Finally, at 2 h after LPS injection, the plasma concentrations of TATc were diminished in RAGE⁻⁻⁻ mice, compared with wild-type mice (table 3; *P* < .05).

Anti-RAGE IgG enhances bacterial outgrowth and dissemination during *E. coli* peritonitis. To exclude the possibility that the results obtained with live *E. coli* bacteria in RAGE⁻⁻⁻ mice were attributable to compensatory changes unrelated to RAGE deficiency in these genetically modified animals, we treated wild-type mice with anti-RAGE IgG or control IgG antibodies and determined bacterial loads in various body compartments 20 h after induction of peritonitis. In strict accordance with the data generated in RAGE⁻⁻⁻ mice, wild-type mice with anti-RAGE IgG antibodies had more bacteria in their PLF and in distant body sites (data not shown). Furthermore, the
Figure 2. Receptor for advanced glycation end products (RAGE)–deficient (RAGE−/−) mice display more extensive liver necrosis and liver thrombi formation and enhanced systemic and local activation of coagulation. Wild-type and RAGE−/− mice were intraperitoneally injected with colony forming units of Escherichia coli. Representative hematoxylin-eosin stainings of liver tissue at 20 h after injection in wild-type (A) and RAGE−/− (B) mice. Original magnification, ×200. The arrow points out thrombi and asterisks indicate necrotic areas. C, Graphical representation of the degree of liver thrombi and necrosis, determined according to the scoring system described in the Methods section. D, Plasma concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Representative fibrin(ogen) stainings of liver tissue of wild-type (E) and RAGE−/− (F) mice. The arrow points out thrombi. Original magnification, ×20. Thrombin-antithrombin complex (TATc) (G and H) and D-dimer (I and J) concentrations were measured systemically (in plasma, G and I and locally (in peritoneal lavage fluid [PLF], H and J) in wild-type (white bars) and RAGE−/− (black bars) mice (6–16 mice per genotype). Dotted lines represent the mean values from normal (uninfected) mice. Data are mean values ± standard errors. *P < .05, compared with wild-type mice; **P < .01, compared with wild-type mice.

DISCUSSION

Gram-negative peritonitis is a life-threatening condition frequently associated with systemic dissemination of bacteria and septic shock. Host defense in peritonitis is an established domain of the innate immune system, as a rapid response to invading pathogens is essential for the host to survive. RAGE has the ability to activate signaling pathways, leading to proinflammatory gene expression on interaction with a range of distinct endogenous proinflammatory ligands. We here examined the in vivo role of RAGE during murine E. coli peritonitis with use of RAGE−/− mice and anti-RAGE IgG. Our key finding was that RAGE contributes to an effective antibacterial host response during E. coli infection. Indeed, RAGE deficiency caused 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 liver injury.

Knowledge of the role of RAGE in host defense against bacterial infection is highly limited. RAGE−/− mice displayed a reduced mortality after induction of polymicrobial sepsis produced by CLP [17, 18]. Moreover, anti-RAGE therapy conferred a survival advantage even when administered 24 h after CLP in mice receiving antibiotic treatment [18]. In the latter investigation, RAGE deficiency or anti-RAGE therapy was reported to not influence bacterial loads in PLF, liver, or spleen. It should be noted, however, that in this study, all mice were treated with broad spectrum antibiotics, and bacterial loads numbers of total leukocytes, neutrophils, and macrophages in PLF samples were similar in anti-RAGE– and control antibody–treated mice, whereas the local concentrations (in PLF) of the CXC chemokines KC and MIP-2 were higher in mice that had received anti-RAGE antibodies (data not shown); these results are in line with the data obtained in RAGE−/− mice.

Anti-RAGE IgG increased fibrin deposition during E. coli peritonitis. In accordance with the data obtained in RAGE−/− mice, livers of anti-RAGE IgG–treated mice showed more thrombi formation 20 h after infection than did livers of control mice (compare figure 3B, arrow, with figure 3A; figure 3C; P < .05). These data were supported by fibrin(ogen) stainings (compare figure 3F with figure 3E). However, in contrast to the RAGE−/− mice, anti-RAGE IgG treatment was not associated with enhanced liver necrosis, hepatocellular injury (figure 3C and 3D), or increased TATc levels (figure 3G and 3H).
were only determined in mice that survived (ie, not at predefined time points after CLP). Together with the fact that host defense against CLP, at least in part, relies on the extent of intestinal necrosis and the formation of a local abscess [19], the possible role of RAGE in antibacterial defense can not be easily determined from this earlier investigation [18]. Although our model does not resemble clinical abdominal sepsis as closely as does CLP, it is a relevant tool to study the role of endogenous receptors and/or mediators in limiting the growth and dissemination of bacteria after a primary intraabdominal infection and to determine the contribution of these host proteins to specific immune responses [20, 21]. As such, we here provide evidence, with use of RAGE−/− mice and anti-RAGE IgG, that RAGE signaling contributes to an effective antibacterial response. Most likely, RAGE exerts this effect indirectly and not via direct interaction with *E. coli*, considering that RAGE−/− leukocytes demonstrated an unaltered capacity to phagocytose and kill *E. coli* in vitro (data not shown). In addition, the observation that RAGE deficiency in general was associated with an exaggerated host response during *E. coli* sepsis and a reduced response to bolus *E. coli* LPS injection suggests that although RAGE is involved in the initiation of an immune reaction to *E. coli*, this function can be compensated for by other receptors in the presence of a growing bacterial load. Previously, in vitro data have shown that interaction of RAGE with its ligand HMGB1 can induce activation of intracellular signaling pathways [8, 9, 31] and thereby inflammation. Because HMGB1 has been reported to transduce cellular signals in vitro and in vivo by interacting with at least 2 other receptors (ie, Toll-like receptor (TLR)2 and TLR4 [32–35]), one possible explanation for the enhanced inflammation in the RAGE−/− mice could be that the absence of RAGE facilitates the interaction between HMGB1 and TLR2 and TLR4.

The current data do not necessarily contradict the previously described protective effect of RAGE deficiency with regard to CLP-induced mortality [17, 18]. The immune response to bacterial infection can act as a double-edged sword, on the one hand protecting the host against invading pathogens, on the other hand potentially damaging cells and tissues. It is conceivable that bacterial growth and dissemination do not impact significantly on the outcome of CLP-induced sepsis, in particular in the context of antibiotic therapy. Additional research is warranted to investigate RAGE-mediated antibacterial activity against other pathogens associated with abdominal sepsis, without the use of antibiotics. RAGE deficiency did not influence mortality in our *E. coli* model; 14 of 15 wild-type mice and all 13 RAGE−/− mice died, with the first lethals occurring 25 h after infection in both groups (data not shown). In this respect, it should be noted that the mortality curves after infection of previously healthy mice with this *E. coli* strain are very steep; whereas low doses do not cause lethality, doses that do cause lethality almost invariably do so. Therefore, we consider this model to be less suitable to determine the impact on mortality and rather make use of it to study early host defense mechanisms.

Plasma IL-10 levels were 10-fold higher in the RAGE−/− mice, compared with wild-type mice, whereas other plasma cytokine levels doubled at most. It had previously been established that the recovery of *E. coli* is diminished in IL-10−/− mice [36]. Similarly, treatment of mice with anti–IL-10 antibodies resulted in a reduced bacterial outgrowth in a model of peritonitis induced by the intraperitoneal injection of the gram-negative

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### Table 3. Impact of Receptor for Advanced Glycation End Products (RAGE) Deficiency on *Escherichia coli* Lipopolysaccharide–Induced Responses

<table>
<thead>
<tr>
<th>Location, measurement</th>
<th>Time after lipopolysaccharide administration</th>
<th>Time 0 h</th>
<th>Time 2 h</th>
<th>Time 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild-type mice</td>
<td>RAGE−/− mice</td>
<td>Wild-type mice</td>
<td>RAGE−/− mice</td>
</tr>
<tr>
<td>PLF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, cells/mL × 10⁶</td>
<td>54.4 ± 3.5</td>
<td>37.5 ± 9.5</td>
<td>14.6 ± 2.5</td>
<td>11.5 ± 3.8</td>
</tr>
<tr>
<td>Neutrophils, cells/mL × 10⁶</td>
<td>0.0 ± 0.0</td>
<td>1.0 ± 0.9</td>
<td>3.3 ± 0.7</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>Macrophages, cells/mL × 10⁶</td>
<td>50.9 ± 4.1</td>
<td>34.1 ± 3.5</td>
<td>10.0 ± 2.2</td>
<td>5.5 ± 2.7</td>
</tr>
<tr>
<td>KC, pg/mL</td>
<td>ND</td>
<td>ND</td>
<td>3881 ± 255</td>
<td>3175 ± 388</td>
</tr>
<tr>
<td>MIP-2, pg/mL</td>
<td>ND</td>
<td>ND</td>
<td>954 ± 129</td>
<td>763 ± 106</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>7.4 ± 2.1</td>
<td>7.2 ± 0.8</td>
<td>5184 ± 836.5</td>
<td>2543 ± 370.9</td>
</tr>
<tr>
<td>TATc, μg/L</td>
<td>5.8 ± 0.7</td>
<td>8.8 ± 1.6</td>
<td>29.5 ± 4.8</td>
<td>11.6 ± 1.4</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean values ± standard error for 4 (0 h) to 12 mice (2 and 6 h) per group at 0, 2, or 6 h after intraperitoneal injection of 250 μg of lipopolysaccharide from *Escherichia coli*. KC, keratinocyte-derived chemokine; MIP-2, macrophage inflammatory protein-2; ND, not determined; PLF, peritoneal lavage fluid, RAGE−/−, receptor for advanced glycation end products deficient; TNF, tumor necrosis factor; TATc, thrombin-anti-thrombin complexes.

* P < .05, compared with wild-type mice.

# P < .01, compared with wild-type mice.
Figure 3. Receptor for advanced glycation end products (RAGE) inhibition enhances hepatic fibrin(ogen) deposition during Escherichia coli–induced sepsis. Mice were intraperitoneally injected with 5 × 10⁴ colony forming units of E. coli and treated with either anti-RAGE IgG antibodies (anti-RAGE) or control IgG antibodies (control). Representative hematoxylin-eosin stainings of liver tissue at 20 h after injection in IgG control– (A) and anti-RAGE IgG–treated (B) mice. Original magnification, ×200. The arrow points out thrombi. C, Graphical representation of the degree of the liver thrombi and necrosis, determined according to the scoring system described in the Methods section. D, Plasma concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (9–10 mice per group). Representative fibrin(ogen) staining of liver tissue of control IgG– (E) and anti-RAGE IgG–treated (F) mice. Original magnification, ×20. Thrombin-antithrombin complex (TATc) (G and H) concentrations were measured systemically (plasma, G) and locally peritoneal lavage fluid (PLF, H). Data are mean values ± standard errors. *P < .05, compared with control antibodies.

bacterium Klebsiella pneumoniae [37]. In the latter study, all mice received gentamicin. Together, these and our data suggest a (direct or indirect) association of high IL-10 levels with enhanced bacterial outgrowth during gram-negative peritonitis. Of note, IL-10⁻/⁻ mice demonstrated more organ damage during E. coli peritonitis, despite an accelerated bacterial clearance [36]. In that report, anti–TNF-α partially attenuated neutrophil recruitment and multiple organ damage in the IL-10⁻/⁻ mice. These results imply that although endogenous IL-10 facilitates bacterial outgrowth during E. coli peritonitis, it protects mice from organ damage by a mechanism that involves inhibition of TNF-α release. In contrast to these IL-10⁻/⁻ mice, our RAGE⁻/⁻ mice show more-severe organ (liver) damage and elevated TNF-α concentrations. Therefore, the role of elevated IL-10 in RAGE⁻/⁻ mice during E. coli peritonitis remains to be elucidated.

RAGE deficiency resulted in enhanced organ injury with more necrosis of the liver in our model of E. coli–induced sepsis. Sepsis is also associated with organ failure of the heart, which contributes to hypotension, impaired perfusion, and mortality [38]. The precise mechanism of this sepsis-related myocardial dysfunction is unknown. Similar to some innate immune cells, cardiomyocytes are able to respond to “danger” signals with an innate immune inflammatory response [39, 40]. In addition, cardiomyocytes express multiple TLRs that signal predominantly through nuclear factor–κB when stimulated by pathogen-associated molecular patterns, leading to decreased cardiocyte contractility [41]. Boyd et al [42] demonstrated that RAGE coimmunoprecipitated with both S100A8 and S100A9 in hearts of mice injected with LPS. Furthermore, it has been demonstrated that myocardial RAGE expression is up-regulated in a model of ischemia/reperfusion in rats and that RAGE-deficient mice are protected from ischemia/reperfusion injury of the heart [43, 44]. It remains to be established whether myocardial RAGE plays a role during E. coli sepsis. Results of immunochemistry of RAGE in mice have not been published before, and unfortunately, we obtained unreliable results with multiple commercial antibodies.

In line with findings published elsewhere [20], E. coli peritonitis was associated with activation of the coagulation system. RAGE⁻/⁻ mice displayed more activation of coagulation, as reflected by increased TATc and D-dimer concentrations in PLF and plasma and more fibrin deposition in the liver. Knowledge of the involvement of RAGE in activation of coagulation is limited. In a model of chronic vascular inflammation in diabetic apolipoprotein E–deficient mice, administration of soluble RAGE for 6 weeks suppressed aortic levels of tissue factor [45], the main initiator of coagulation in sepsis in general [46] and in our model of abdominal sepsis in particular [20]. In addition, anti-RAGE IgG has been reported to inhibit tissue factor expression by monocytes stimulated with serum amyloid A in
vitro [47]. Clearly, these data cannot be readily extrapolated to our model of severe acute bacterial infection. Our finding that RAGE−/− mice demonstrated lower plasma TATc levels upon bolus injection of LPS suggest that RAGE may play a role in acute activation of coagulation. The enhanced coagulation activation in mice with attenuated RAGE function during E. coli sepsis at least in part may have been caused by the higher bacterial loads in these animals. As such, our results provide evidence that although RAGE signaling may contribute to coagulation activation during acute infection, it is not essential for the procoagulant response during sepsis.

Although overall the effects of RAGE deficiency and anti-RAGE IgG on the host response to E. coli peritonitis were largely similar, some differences were observed. It should be noted that RAGE deficiency results in a complete absence of signaling via RAGE, whereas anti-RAGE IgG treatment most likely only partially prevents RAGE signaling. Furthermore, these differences in effects could also be attributed to an additional deficiency of soluble RAGE in the RAGE-deficient mice (in contrast to the anti-RAGE IgG-treated mice that still can express soluble RAGE). It is important to note that most RAGE ligands (which can be bound by soluble RAGE) are promiscuous and are able to activate other cell-associated receptors besides RAGE [8–12, 48].

The present study is the first, to our knowledge, to document that intact RAGE signaling contributes to an effective antibacterial defense during abdominal sepsis caused by E. coli, thereby limiting the ensuing host systemic inflammatory and procoagulant response to infection. These data further illustrate the existence of a delicate balance between inflammation and anti-inflammation during severe bacterial infection, where a certain degree of inflammation is required to combat invading pathogens and exaggerated inflammation can result in tissue injury.

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References