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Ligands of the receptor for advanced glycation end products (RAGE), including high mobility group box (HMGB) 1, limit bacterial dissemination during Escherichia coli peritonitis

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Under revision

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Abstract

Objective
The receptor for advanced glycation end products (RAGE) mediates a variety of inflammatory responses. Soluble RAGE (sRAGE) has been suggested to function as a decoy abrogating cellular activation. High mobility group box (HMGB) 1 is a high-affinity binding ligand for RAGE with cytokine activities and plays a role in sepsis.

Design
Controlled, in vivo laboratory study.

Setting
Research laboratory of a health sciences university.

Subjects
C57BL/6 mice.

Interventions
Peritonitis was induced by intraperitoneal injection of Escherichia coli (E. coli). Mice received soluble RAGE or anti-HMGB1 IgG, or the appropriate control treatment.

Measurements and Main Results
sRAGE treated mice demonstrated an enhanced bacterial dissemination to liver and lungs accompanied by increased hepatocellular injury and exaggerated systemic cytokine release, 20 hrs after intraperitoneal administration of E. coli. Remarkably, lung inflammation was unaffected. Furthermore, HMGB1 release was enhanced during peritonitis and anti-HMGB1 treatment was associated with higher bacterial loads in liver and lungs.

Conclusions
These data are the first to suggest that RAGE ligands, including HMGB1, limit bacterial dissemination during gram-negative sepsis.
**Introduction**

*Escherichia (E.) coli* is the most common pathogen involved in intraabdominal infection in humans (1). Peritonitis is a life-threatening infection characterized by the presence of bacteria in the normally germ-free peritoneal cavity. Mortality rates of peritonitis range between 30 and 50% despite advances in surgery and antimicrobial therapy. A serious complication originating from peritonitis is systemic inflammation and sepsis with mortality rates of up to 80% (2).

The multiligand receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily, being expressed as a cell surface molecule by a range of cell types and playing a key role in diverse inflammatory processes (3). Known RAGE ligands include amyloid (4), β-sheet fibrils (5) and some members of the S100 family, such as S100A12 (6), S100B (7) and S100P (8). Another high-affinity binding ligand for RAGE is high mobility group box (HMGB)1 (9, 10). HMGB1 is a potent pro-inflammatory cytokine that plays an important role in a variety of inflammatory conditions, including sepsis and arthritis (11, 12). Engagement of these and other ligands to RAGE can induce inflammatory responses via activation of several intracellular signaling cascades, including the NF-κB pathway (13, 14).

Soluble RAGE (sRAGE), a truncated form of the full length cell surface receptor, is composed of the extracellular ligand-binding domain (V-C-C’) only, lacking the cytosolic and transmembrane domains (i.e. the parts that transfer a signal into the cell). sRAGE has been suggested to be involved in inflammatory processes in several ways. First, the circulating levels of soluble RAGE are associated with various inflammatory diseases in patients (15-17) and in mouse models of experimental acute lung injury (17). Secondly, it is thought that sRAGE can compete with full length cell-surface RAGE for ligand binding, preventing these ligands to bind to their receptors (including RAGE), and/or to exert effects otherwise. Indeed, the exogenous administration of sRAGE reduced inflammatory responses in several animal models, including models of hepatic injury (18-20), diabetic atherosclerosis (21, 22), delayed type hypersensitivity (23, 24), type II collagen-induced arthritis (24) and experimental auto-immune encephalomyelitis (25). HMGB1 is one possible candidate to be targeted by sRAGE in these diseases and inhibition of HMGB1 might be partially responsible for the observed effects of sRAGE. Many RAGE ligands are promiscuous with regard to receptor use. The assumption that sRAGE not only prevents HMGB1 and other RAGE ligands to bind to RAGE but also to other receptors is supported by the finding that administration of sRAGE also decreased the delayed type hypersensitivity response in RAGE deficient mice (23). In addition, sRAGE might be involved in inflammation because it has pro-inflammatory and chemotactic properties itself via interaction with the β2-integrin Mac-1 (26).
RAGE and HMGB1 have been implicated as mediators of lethality in abdominal sepsis caused by cecal ligation and puncture (CLP) (23, 27-29). The CLP model is less suitable to study the impact of an intervention on bacterial growth and dissemination due to the polymicrobial nature of the infection and the fact that the antibacterial response is dependent on the extent of necrosis of the cecum and the formation of a local abscess (30). Our laboratory has used a model of abdominal sepsis induced by intraperitoneal injection of \textit{E. coli} to investigate host defense mechanisms against this most common pathogen in peritonitis (31-34). This model is suitable to study factors contributing to local growth within the peritoneal cavity and subsequent dissemination of bacteria, as well as the occurrence of systemic inflammation and organ injury. We here used this model to determine the effect of sRAGE and anti-HMGB1 on host defense against septic peritonitis.

**MATERIALS AND METHODS**

**Mice**
8-10-week-old female C57Bl/6 mice were purchased from Harlan Sprague Dawley Inc. (Horst, The Netherlands). The Institutional Animal Care and Use Committee of the Academic Medical Center, University of Amsterdam, approved all experiments.

**Soluble RAGE**
Murine sRAGE was prepared and purified from a baculovirus expression system as previously described (21). Any detectable lipopolysaccharide was removed by Detoxigel columns (Pierce Chemical Co., Rockford, IL) (35). sRAGE was administered intraperitoneally at a dose of 100 μg in 200 μl 0.5 hrs after infection. Control mice received equal volumes of vehicle (phosphate-buffered saline [PBS]).

**Anti-HMGB1 antibodies**
Polyclonal antibodies against HMGB1 B box were raised in rabbits as described previously (27) and titers were determined by immunoblotting. Anti-HMGB1 B Box antibodies were affinity-purified by using cyanogens bromide-activated Sepharose beads following standard procedures. Neutralizing activity of anti-HMGB1 was confirmed in HMGB1-stimulated macrophage cultures by assay of tumor necrosis factor (TNF)-α release. In the presence of the antibody, neutralizing antibody was defined as inhibition (>80%) of HMGB1-induced TNF-α release. Anti-HMGB1 antibodies were administered intraperitoneally at a dose of 500 μg 0.5 hrs before infection. Control mice received equal volumes of non-immune rabbit IgG (item I5006, Sigma-Aldrich, St. Louis, MO).
Induction of peritonitis
Peritonitis was induced as described previously (31-34). In brief, *E. coli* O18:K1 was cultured in Luria-Bertani medium (LB, Difco, Detroit, MI) at 37 ºC, harvested at mid-logarithmic phase and washed in pyrogen-free 0.9% NaCl (2x) before injection. Mice were injected intraperitoneally with 5 x 10^4 colony forming units (CFUs) of *E. coli* in 200 µl of sterile 0.9% NaCl. The inoculum was plated on blood agar plates to determine viable counts. Mice were sacrificed 20 hrs after *E. coli* injection. Quantitative cultures were made of peritoneal lavage fluid (PLF), blood, liver and lung as described previously (32, 34, 36).

Sample harvesting
At the time of sacrifice, mice were first anesthetized by inhalation of isoflurane (Abbott Laboratories LTD., Kent, UK). A peritoneal lavage was then performed with 5 ml of sterile 0.9% NaCl using an 18-gauge needle and PLF was collected in sterile tubes and put on ice. After collection of PLF, deeper anesthesia was induced by intraperitoneal injection of ketamine (Eurovet Animal Health BV, Bladel, The Netherlands) and medetomidine (Pfizer Animal Health BV, Capelle aan de IJssel, The Netherlands). 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, livers and lungs were harvested and processed for histology and measurements of CFUs, cytokines and myeloperoxidase (MPO) as described (32, 34, 36). The supernatants were frozen at -20 ºC until assayed. Cell counts and differentials were performed as described earlier (31-34).

Assays
Keratinocyte-derived chemokine (KC) and macrophage inflammatory protein 2 (MIP-2) were measured by ELISA according to instruction of the manufacturer (R&D systems, Abingdon, United Kingdom). TNF-α, interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1 and IL-10 were measured by cytometric bead array multiplex assay (BD Biosciences, San Jose, CA) in accordance with the manufacturer’s recommendations. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined with commercially available kits (Sigma-Aldrich, St. Louis, MO), using a Hittachi analyzer (Boehringer Mannheim, Mannheim, Germany). MPO was measured by ELISA (Hycult Biotechnology BV, Uden, The Netherlands). Thrombin-antithrombin complexes (TATc) were measured by ELISA (Enzygnost TAT Micro, Dade Behring, Marburg, Germany). HMGB1 was measured by Western immunoblotting (27, 37).

Histological examination
Lungs and livers were harvested after 20 hrs, fixed in 4% formaldehyde, embedded in paraffin and cut in 4-µm thick sections for staining procedures. Hematoxylin-eosin stainings were performed as described (38) and analyzed by a pathologist who had no
knowledge of the genotype of the mice. Liver and lung injury were semi-quantitatively scored as described (32). Neutrophil and fibrin(ogen) stainings were performed as described (36, 39-41).

**Statistical analysis**
All data are expressed as mean ± SEM. Differences between groups were analyzed by Mann-Whitney U test. Values of $p < .05$ were considered to represent a statistically significant difference.

**RESULTS**

**sRAGE facilitates bacterial dissemination to distant organs during *E. coli* peritonitis**
To obtain insight in the effect of sRAGE on bacterial outgrowth and dissemination, mice were inoculated intraperitoneally with *E. coli* together with sRAGE or vehicle. We established the number of *E. coli* CFUs at 20 hrs after infection in PLF (the primary site of infection), blood, liver and lungs (to evaluate to which extent the infection became systemic). Mice treated with sRAGE had similar CFU counts in PLF and blood (Fig. 1A and B). However, livers from sRAGE treated mice clearly contained more bacteria compared with mice treated with vehicle ($p < .005$, Fig. 1C). In addition, there was more bacterial outgrowth in the lungs from mice administered with sRAGE ($p < .05$, Fig. 1D). Therefore, administration of sRAGE was associated with an increased bacterial dissemination during *E. coli* peritonitis, predominantly to the liver.

![Figure 1](image-url) **Figure 1.** sRAGE treated mice demonstrate an enhanced dissemination. Number of *Escherichia coli* (*E. coli*) CFUs in peritoneal lavage fluid (PLF, A), blood (B), liver (C) and lungs (D) at 20 hrs after intraperitoneal injection of $5 \times 10^4$ CFUs of *E. coli* in mice treated with either vehicle (white bars) or sRAGE (black bars) ($n = 8-10$ mice/group). Data are mean ± SEM; *$p < .05$ and ***$p < .005$ vs. vehicle treated mice.
Inflammatory cell influx and chemokine levels in PLF

The recruitment of leukocytes to the site of an infection is an essential part of the host defense to invading bacteria. sRAGE has been shown to interact with Mac-1, thereby acting as an important chemotactic stimulus for neutrophils (26). Hence, we determined leukocyte counts and chemokine levels in PLF at 20 hrs after E. coli injection in sRAGE and vehicle treated mice. E. coli injection resulted in a profound increase in total leukocyte numbers in PLF compared to 0 hrs (data not shown), which was mainly due to neutrophil influx (Table 1).

Table 1. Leukocyte counts and chemokine levels in peritoneal fluid

<table>
<thead>
<tr>
<th></th>
<th>vehicle</th>
<th>sRAGE</th>
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<tbody>
<tr>
<td><strong>Cell count (x 10^4/ml PLF)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>215.4 ± 23.2</td>
<td>151.8 ± 11.2</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>189.4 ± 19.2</td>
<td>139.6 ± 13.3</td>
</tr>
<tr>
<td>Macrophages</td>
<td>33.6 ± 6.1</td>
<td>17.5 ± 3.2</td>
</tr>
<tr>
<td><strong>Chemokines (pg/ml PLF)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KC</td>
<td>24180 ± 3172</td>
<td>29939 ± 901</td>
</tr>
<tr>
<td>MIP-2</td>
<td>3587 ± 957</td>
<td>4495 ± 876</td>
</tr>
</tbody>
</table>

sRAGE, soluble receptor for advanced glycation end products; PLF, peritoneal lavage fluid; KC, keratinocyte derived chemokine; MIP-2, macrophage inflammatory protein.

Mice received 5 x 10^4 CFUs Escherichia coli intraperitoneally. All mice were killed 20 hrs later.

Administration of sRAGE did not result in a change in peritoneal leukocyte counts or differentials. The mouse CXC chemokines KC and MIP-2 have been implicated to play an important role in the attraction of neutrophils during inflammation (42, 43). Therefore, we measured the levels of these chemokines in PLF. In line with leukocyte counts and differentials in PLF, concentrations of both KC and MIP-2 were similar in the sRAGE and vehicle treated mice (Table 1).

Cytokine response

To determine whether sRAGE influences the local or systemic release of cytokines during septic peritonitis, TNF-α, IL-6, MCP-1 and IL-10 concentrations were measured in PLF and plasma of sRAGE and vehicle treated mice (Table 2). Levels of MCP-1 in PLF and IL-10 in plasma were significantly higher in sRAGE treated mice, while IL-6 concentrations tended to be higher in PLF and plasma, although these differences did not reach statistical significance.
Table 2. Cytokine levels

<table>
<thead>
<tr>
<th>Cytokines (pg/ml)</th>
<th>vehicle</th>
<th>sRAGE</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PLF</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>63 ± 7</td>
<td>66 ± 5</td>
</tr>
<tr>
<td>plasma</td>
<td>354.5 ± 80.6</td>
<td>337.2 ± 86.8</td>
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<tr>
<td>IL-6</td>
<td>707.5 ± 180</td>
<td>1570 ± 345.8, p = 0.05</td>
</tr>
<tr>
<td>plasma</td>
<td>14538 ± 3542</td>
<td>25667 ± 4308, p = 0.08</td>
</tr>
<tr>
<td>MCP-1</td>
<td>2614 ± 375.4</td>
<td>7209 ± 540.8b</td>
</tr>
<tr>
<td>plasma</td>
<td>5054 ± 852.5</td>
<td>5024 ± 701.5</td>
</tr>
<tr>
<td>IL-10</td>
<td>154.7 ± 45.7</td>
<td>237.2 ± 42.2</td>
</tr>
<tr>
<td>plasma</td>
<td>466.1 ± 98.0</td>
<td>1093 ± 161.3a</td>
</tr>
</tbody>
</table>

sRAGE, soluble receptor for advanced glycation end products; TNF, tumor necrosis factor; PLF, peritoneal lavage fluid; IL, interleukin; MCP, monocyte chemoattractant protein.
Mice received 5 x 10⁴ CFUs Escherichia coli intraperitoneally. All mice were killed 20 hrs later.

*p < .005 vs. vehicle treated mice; **p <.0001 vs. vehicle treated mice.

sRAGE treated mice have more severe liver damage
This experimental model of abdominal sepsis is associated with profound liver injury (32-34). Considering the strongly increased bacterial outgrowth in the livers from sRAGE treated mice, we examined the influence of sRAGE administration on liver damage after E. coli injection. Upon histopathological examination, both sRAGE and vehicle treated mice showed mild inflammation of liver tissue as characterized by the influx of leukocytes into the hepatic parenchyma (Fig. 2A and B). Although total histology scores did not differ between sRAGE and vehicle treated mice (Fig. 2C), clinical chemistry showed more profound hepatocellular injury in sRAGE treated mice: the mice which received sRAGE had higher plasma levels of AST, although ALT concentrations were similar compared to vehicle treated mice (Fig. 2D). In line, the mice administered with sRAGE demonstrated more fibrin/thrombus formation (Fig. 2B, arrow and Fig. 2G, p < .05 vs. vehicle) and showed markedly more fibrin(ogen) depositions (Fig. 2F vs. 2E).

sRAGE administration increases neutrophil recruitment in the liver interstitium
Having shown that sRAGE facilitates bacterial dissemination to the liver, we then asked ourselves which factors might be involved in the early spread of E. coli. Given that leukocyte recruitment to the site of infection is an important part of host defense during bacterial infection, we next investigated influx of neutrophils in the livers. Therefore, we performed granulocyte stainings of liver tissue. Compared to vehicle treated mice, sRAGE
Figure 2. sRAGE worsens liver damage. Mice were treated with either vehicle or sRAGE intraperitoneally at 0.5 hrs after 5 x 10^4 CFUs *Escherichia coli* (*E. coli*) injection. Representative hematoxylin-eosin stainings of liver tissue at 20 hrs after *E. coli* infection in vehicle (A) and sRAGE treated (B) mice. Original magnification x10. Arrow points out thrombus. Graphical representation of the degree of liver inflammation (C) and of fibrin and thrombi (G) determined according to the scoring system described in the Materials and Methods section. Plasma AST and ALT levels (D). Representative fibrin(ogen) immunostaining of liver tissue of mice administered with either vehicle (E) or sRAGE (F) after injection with *E. coli*. Original magnification x10. White bars represent vehicle treated and black bars indicate sRAGE treated mice (n = 8-10 mice/group). Data are mean ± SEM; *p < .05 vs. vehicle treated mice.

Figure 3. sRAGE increases hepatic neutrophil influx. Mice were treated with either vehicle or sRAGE intraperitoneally at 0.5 hrs after 5 x 10^4 CFUs *Escherichia coli* (*E. coli*) injection. Representative Ly-6 stainings of liver tissue at 20 hrs after *E. coli* infection in vehicle (A) and sRAGE treated (B) mice. Original magnification x10. Myeloperoxidase (MPO; C), KC (D) and MIP-2 (E) levels in liver homogenate in mice treated with control (white bars) or sRAGE (black bars) (n = 8-10 mice/group). Data are mean ± SEM; *p < .05 vs. vehicle treated mice, **p < .01 vs. vehicle treated mice.
administered mice showed more hepatic neutrophil influx (Fig. 3B vs. 3A). In line, sRAGE treated mice had higher MPO levels (reflecting the neutrophil content of an organ) in liver homogenates (Fig. 3C, $p < .01$ vs. vehicle). CXC chemokines have been implicated in the attraction of neutrophils to the site of an infection (42, 43). To investigate whether a difference in local chemokine levels could have influenced the neutrophil influx in the interstitial liver tissue, we determined KC and MIP-2 levels in liver homogenates. Mice that had received sRAGE showed higher levels of MIP-2 in their liver homogenates than mice that received vehicle (Fig. 3E, $p < .005$ vs. vehicle). KC levels were similar between the two mouse groups (Fig. 3D).

**Increased cytokine responses in sRAGE treated mice**

Finally, we measured the pro-inflammatory cytokines TNF-α, IL-6 and MCP-1 and the anti-inflammatory cytokine IL-10 in liver homogenates. TNF-α, IL-6 and MCP-1 liver concentrations were clearly elevated in the mice that received sRAGE (all $p < .001$, Fig. 4A-C). IL-10 levels were higher in the sRAGE treated mice as well ($p < .05$, Fig. 4D).

![Figure 4](image_url)

**Influence on pulmonary inflammation**

To determine the role of sRAGE in the development of inflammation in another organ even more susceptible to inflammation-induced injury, lungs were harvested at 20 hrs after *E. coli* infection. Pulmonary inflammation was present as reflected by the accumulation of leukocytes in the interstitium (Fig. 5A-B). The total histological scores did not differ between the two groups (Fig. 5C). MPO concentrations in lung homogenates, however, were slightly higher in the mice treated with sRAGE ($p < .05$, Fig. 5D). Neutrophil stainings of lung tissue were similar in both groups (Fig. 5E-F).
Having established that sRAGE treatment adversely affects the host response to E. coli peritonitis, we next questioned whether HMGB1, one of the high-affinity binding ligands for RAGE (9, 10), plays a role therein. To investigate whether HMGB1 is released in our model of abdominal sepsis, we measured HMGB1 concentrations in PLF from healthy mice and from mice 20 hrs after E. coli injection. HMGB1 was not or barely measurable in PLF from uninfected mice and was clearly increased after induction of E. coli peritonitis (Fig. 6A, \( p < .0001 \)).

We next asked ourselves what the contribution of endogenous HMGB1 is in host defense during peritonitis. Therefore, we treated mice with either anti-HMGB1 IgG or control IgG antibodies and quantified the number of bacteria in various body compartments 20 hrs after intraperitoneal injection of E. coli. Anti-HMGB1 IgG treatment resulted in an enhanced dissemination of E. coli from the primary site of infection, as reflected by higher bacterial loads in liver, lungs and blood, albeit in blood the difference with vehicle treated mice did not reach statistical significance (Fig. 6C-E). In PLF, bacterial loads were virtually identical in anti-HMGB1 IgG and control IgG treated mice (Fig. 6B). Anti-HMGB1 IgG did not influence inflammatory responses (cell influx or CXC chemokine levels in PLF, cytokine release in PLF and plasma, plasma AST and ALT, liver and lung pathology; data not shown).

**Figure 5.** Influence of sRAGE on pulmonary inflammation. Mice were treated with either vehicle or sRAGE intraperitoneally at 0.5 hrs after 5 x 10^4 CFUs Escherichia coli (E. coli) injection. Representative hematoxylin-eosin stainings of lung tissue at 20 hrs after E. coli infection in vehicle (A) and sRAGE treated (B) mice. Original magnification x20. Graphical representation of the degree of lung inflammation (C) determined according to the scoring system described in the Materials and Methods section and myeloperoxidase (MPO) levels in lung homogenate (D) in mice treated with vehicle (white bars) or sRAGE (black bars) (n = 8-10 mice/group). Representative Ly-6 stainings of lung tissue at 20 hrs after E. coli infection in vehicle (E) and sRAGE treated (F) mice. Original magnification x10. Data are mean ± SEM. * \( p < .05 \) vs. vehicle treated mice.
Discussion

The clinical syndrome of sepsis is the result of a systemic response of the host to a severe infection, characterized by the concurrent activation of various mediator systems. In this study we show that administration of sRAGE resulted in an enhanced bacterial outgrowth in the distant organs liver and lungs together with increased inflammation and damage in the liver. Additionally, we here report that the administration of an antibody against one of the high-affinity targets of sRAGE, HMGB1, led to a similar pattern in bacterial dissemination, but without enhancement of hepatocellular injury. Together these data suggest that during severe peritonitis RAGE ligands, and in particular HMGB1, play a
role in limiting the dissemination of *E. coli* from the primary site of infection.

Peritonitis is characterized by recruitment of leukocytes to the site of infection (31, 36, 44). Theoretically, sRAGE can influence cell influx by binding HMGB1, which we show here to be released into the peritoneal cavity during *E. coli* peritonitis, thereby abrogating the chemotactic properties of HMGB1 (45). In accordance with this possibility is our recent observation in RAGE deficient mice in which we found a reduced neutrophil influx into PLF upon intraperitoneal administration of recombinant HMGB1 (46). However, we did not find a decreased neutrophil influx to the peritoneal cavity in mice treated with sRAGE. One possible explanation for this is that sRAGE itself can exert chemotactic effects which could counteract the abrogation of the chemotactic features of HMGB1: an *in vivo* study showed that the intraperitoneal administration of sRAGE alone caused an increase in leukocyte (and neutrophil) counts in PLF (26) and in an *in vitro* chemotaxis assay neutrophils were found to migrate toward sRAGE (26). These potential opposite effects of sRAGE could have resulted in a net equal amount of neutrophils in sRAGE and vehicle treated mice. Notably, the number of neutrophils in liver and lungs as determined by measurement of MPO concentrations, was higher in the sRAGE treated mice. This was most likely the consequence of the increased bacterial load, providing a more potent pro-inflammatory stimulus.

sRAGE treated mice displayed more fibrin/thrombi formation in their livers, in combination with higher cytokine and increased plasma AST concentrations. This is remarkable since administration of sRAGE has been shown to diminish hepatic damage in mice subjected to ischemia and reperfusion, massive hepatectomy and acetaminophen-induced hepatotoxicity (18-20). In addition, anti-HMGB1 IgG did not impact on liver damage, whereas anti-HMGB1 IgG decreased inflammation and liver damage after CLP (28) and attenuated hepatic ischemia/reperfusion injury (47). Considering that anti-HMGB1 IgG had less of a negative impact on the hepatic bacterial load than sRAGE, these data suggest that the detrimental effect of sRAGE on the growth of *E. coli* in the liver is only partially mediated by inhibition of HMGB1. Moreover, it remains to be established whether sRAGE and anti-HMGB1 IgG can exert liver protective effects during abdominal sepsis: such possible liver protective effects might have been overruled in our peritonitis model by the increased number of bacteria in the livers of sRAGE and anti-HMGB1 IgG treated mice, whereby sRAGE treatment even was associated with enhanced hepatocellular injury due to the 100-fold higher bacterial load.

In contrast to the increased liver damage observed after sRAGE administration, no differences in lung pathology were detected between sRAGE and vehicle treated mice, as reflected by similar histology lung scores after induction of *E. coli* peritonitis. MPO levels were only slightly elevated in the sRAGE treated mice. Endogenous sRAGE has been reported to be increased in acute lung injury, *i.e.* sRAGE was elevated in BALF from rats intratracheally instilled with either lipopolysaccharide or hydrochloric acid (17).
and in the pulmonary edema fluid of patients with acute lung injury/acute respiratory
distress syndrome (17). A clear function of sRAGE in the lungs has yet to be elucidated,
although it likely serves to limit potential damage caused by the release of RAGE ligands.
Considering that anti-HMGB1 did not influence pulmonary inflammation, our current
data suggest that if RAGE ligands other than HMGB1 play a role in pulmonary pathology
during E. coli sepsis, this effect is compensated by concurrent reduction in the bacterial
load in this compartment.
HMGB1 is a nuclear protein that is released from necrotic cells, as well as from
macrophages, dendritic cells and natural killer cells upon activation by infectious agents
(48). HMGB1 binds to RAGE with high affinity (9, 10). Elevated HMGB1 plasma
levels are readily detected in clinical and experimental sepsis (37, 49, 50). The function
of endogenous HMGB1 in bacterial sepsis has thus far only been studied in the model
of CLP induced abdominal sepsis, wherein anti-HMGB1 treatment improved survival
and attenuated organ damage (27, 28, 37). In the current study, we first documented the
release of HMGB1 in PLF after intraperitoneal infection with E. coli. The finding that
HMGB1 is secreted locally at the site of infection extends our report demonstrating high
HMGB1 concentrations in abdominal fluid of patients with peritonitis (49). Remarkably,
anti-HMGB1 IgG treatment was associated with an enhanced bacterial dissemination
from the abdominal cavity: whereas the bacterial load in PLF was not affected by anti-
HMGB1 IgG, more E. coli was cultured from especially the liver and lungs in mice that
had received this antibody. Of interest, adenoid-derived and recombinant HMGB1
have been reported to exert antibacterial barrier defense system in the upper respiratory
tract (51). Our data suggest that endogenous HMGB1 may also serve as an antibacterial
factor in sepsis. The fact that in contrast to sRAGE anti-HMGB1 IgG did not impact
on inflammatory responses during E. coli sepsis suggests that RAGE ligands other than
HMGB1 play a role in the host responses that were exaggerated by sRAGE.

Conclusions

Peritonitis is a common cause of sepsis in humans. Intraperitoneal administration of live
E. coli results in a clinical condition commonly associated with septic peritonitis, with
diaphragmatic lymphatic drainage and systemic bacteremia and endotoxemia. We here
used this model to investigate the function of endogenous RAGE ligands and more
specifically HMGB1 in host defense against septic peritonitis. Both inhibition of multiple
RAGE ligands, by the administration of sRAGE, and inhibition of HMGB1 resulted in
an enhanced dissemination of E. coli from the primary site of infection. These results
identify a novel beneficial role for endogenous RAGE ligands, including HMGB1, in the
innate antibacterial response to abdominal sepsis.
ACKNOWLEDGEMENTS

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Chapter 7


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