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Chapter 12

S100A12 and soluble RAGE levels during severe infection

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ABSTRACT

S100A12 is highly expressed, and serum levels correlate with individual disease activity in patients with inflammatory diseases. We here sought to determine the extent of S100A12 release and its soluble high-affinity receptor for advanced glycation end products (sRAGE) in patients with severe sepsis stratified to the three most common infectious sources (lungs, abdomen, and urinary tract) and to determine S100A12 and sRAGE concentrations at the site of infection during peritonitis. Two patient populations were studied: (a) 51 patients with sepsis due to (i) peritonitis (n = 12), (ii) pneumonia (n = 29), or (iii) urinary tract infection (n = 10); and (b) 17 patients with peritonitis. In addition, eight healthy humans were studied after intravenous injection of lipopolysaccharide (4 ng/kg). Compared with healthy volunteers, patients with severe sepsis displayed increased circulating S100A12 concentrations at day 0 (591.2 ± 101.0 vs. 106.2 ± 15.6 ng/mL [control subjects], P < 0.0001) and at day 3 (637.2 ± 111.2 vs. 106.2 ± 15.6 ng/mL [control subjects], P < 0.0001). All three severe sepsis subgroups had elevated serum S100A12 concentrations at both time points (sepsis due to [i] peritonitis [393.5 ± 89.9 at day 0 and 337.9 ± 97.2 at day 3 vs. 106.2 ± 15.6 ng/mL, control subjects, P < 0.005 and P < 0.05, respectively]; [ii] pneumonia [716.9 ± 167.0 at day 0 and 787.5 ± 164.7 at day 3 vs. 106.2 ± 15.6 ng/mL, control subjects, P < 0.0001 and P < 0.05, respectively]); and [iii] urinary tract infection [464.2 ± 115.6 at day 0 and 545.6 ± 254.9 at day 3 vs. 106.2 ± 15.6 ng/mL, control subjects, P < 0.0001 and P < 0.05, respectively]). Remarkably, patients with sepsis due to pneumonia had the highest S100A12 levels (716.9 ± 167.0 and 787.5 ± 164.7 ng/mL at days 0 and 3, respectively). S100A12 levels were not correlated to either Acute Physiology and Chronic Health Evaluation II scores (r = -0.185, P = 0.19) or Sepsis-Related Organ Failure Assessment scores (r = -0.194, P = 0.17). Intravenous lipopolysaccharide injection in healthy humans elevated systemic S100A12 levels (peak levels at 3 h of 59.6 ± 22.0 vs. 12.4 ± 3.6 ng/mL; t = 0 h, P < 0.005). In contrast to S100A12, sRAGE concentrations did not change during severe sepsis or human endotoxemia. During peritonitis, S100A12 concentrations in abdominal fluid (12945.8 ± 4142.1 ng/mL) were more than 100-fold higher than in concurrently obtained plasma (121.2 ± 80.4 ng/mL, P < 0.0005), whereas sRAGE levels in abdominal fluid (148.8 ± 36.0 pg/mL) were lower than those in plasma (648.7 ± 145.6 pg/mL, P < 0.005) and did not increase. In conclusion, in severe sepsis, S100A12 is released systemically irrespective of the primary source of infection. During abdominal sepsis, S100A12 release likely predominantly occurs at the site of infection. Concentrations of its high-affinity sRAGE do not change during infection or human endotoxemia.
INTRODUCTION

Sepsis remains a common and serious problem in clinical medicine today. Attempts to improve the outcome of sepsis by immune modulatory strategies have been largely unsuccessful, at least in part because of an incomplete understanding of the septic process. In sepsis, the lung is the primary source of infection (35%–54%), followed by the abdomen (20%–28%) and the urinary tract (8%–13%) [1,2].

S100A12 (or myeloid-related protein (MRP)-6 or calcitermin) is a calcium binding protein expressed in the cytoplasm of neutrophils [3]. It was identified and isolated from resting neutrophils, wherein it comprises 5% of the cytosolic protein [4]. Besides in neutrophils, S100A12 is also found in monocytes and lymphocytes [4]. S100A12 was the first S100 protein for which a receptor had been described. A specific binding with the C1 domain of RAGE has been reported [5]. Of note, S100A12 has been suggested as a biomarker of neutrophil activation in inflammatory diseases, including sepsis and pulmonary infections [6–8]. Previously, a multivariate regression model predicting for S100A12 revealed that clinical history of cardiovascular disease and inflammation were independently associated with S100A12 levels in hemodialysis patients [9].

RAGE is a multiligand receptor of the immunoglobulin superfamily that is expressed at a high level in the lungs and at a lower level in normal adult tissues [10]. The interaction of S100A12 as well as other ligands with RAGE leads to activation of proinflammatory signaling cascades. RAGE deficiency improved survival in murine models of abdominal polymicrobial sepsis induced by cecal ligation and puncture (CLP) [11,12] and pneumonia induced either by Streptococcus pneumoniae or by influenza A virus [13,14]. Since a functional s100a12 gene is absent in the murine genome [15], and other RAGE ligands have been described to play a role in pneumonia and sepsis [16–18], non-S100A12 mediated RAGE effects play an important role in the pathogenesis of pneumonia and sepsis as well.

Soluble RAGE (sRAGE) is a truncated form of the full length cell surface receptor and 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 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; sRAGE administration decreased inflammatory responses in several animal models. In humans, S100A12 is one possible candidate to be targeted by sRAGE in inflammatory diseases, and S100A12 concentrations are high in serum of patients with inflammatory bowel disease, cystic fibrosis, rheumatoid arthritis and severe infection [6–8]. Although many RAGE ligands are promiscuous with regard to receptor use, S100A12 has only been shown to bind to RAGE.
In this study, we sought to determine S100A12 and sRAGE levels in patients with severe sepsis stratified to the three most common primary sources of infection (lungs, abdomen and urinary tract) and in human endotoxemia. In addition, we measured local S100A12 and sRAGE concentrations in abdominal fluid obtained from patients with peritonitis in a separate population.

MATERIALS AND METHODS

Patients and design
All studies were approved by the scientific and ethics committees of the Academic Medical Center (Amsterdam, the Netherlands), the St. Luc University Hospital (Brussels, Belgium), and Cliniques St. Pierre's (Ottignies, Belgium). Written informed consent was obtained from all subjects or their relatives. The study included two separate patient populations described in detail previously [19,20]. The first population comprised 51 patients with severe sepsis (68 ± 2 years, 31 males), defined as a known or suspected infection plus a systemic inflammatory response syndrome and failure of at least one organ [21]; these patients were admitted to the intensive care unit (ICU) of either St. Luc University Hospital or Cliniques St. Pierre's on the day the diagnosis of severe sepsis was made (day 0), and serum was obtained at days 0 and 3. A total of 31 healthy subjects served as control subjects. The second population consisted of 17 patients with peritonitis (61 ± 4 years, nine males) admitted to the surgical ward of the Academic Medical Center; these patients were part of a previous study examining the effect of peritonitis on coagulation and fibrinolysis [22]. In this study, patients were included with secondary peritonitis, requiring emergency laparotomy, caused by perforation of a visceral organ (n = 8), anastomotic leakage (n = 7), or other causes (n = 2). Exclusion criteria were Acute Physiology and Chronic Health Evaluation (APACHE) II score of 10 points or less (to ensure comparable disease severity), age younger than 18 or older than 80 years, abdominal infection related to continuous ambulant peritoneal dialysis or endoscopy (within 24 h), and acute pancreatitis. Abdominal fluid was aspirated with a syringe from an abdominal drain in cavum Douglasi. EDTA-anticoagulated blood and abdominal fluid samples were taken at index laparotomy for peritonitis (t = 0) and after 1, 2, and 3 days.

In addition to these two patient populations, eight healthy males (22.6 ± 0.6 years) were studied after intravenous injection of *Escherichia (E.) coli* lipopolysaccharide (LPS) (lot G, United States Pharmacopeial Convention, Rockville, MD) at a dose of 4 ng/kg. EDTA anticoagulated blood was obtained before and 0, 3, 6, 8 and 24 h after challenge. Parts of the results of this study have been published before [19,20].
Measurements and assays
Data were collected prospectively from patient records, patient data management system (at the intensive care unit) and hospital information system. The following variables were collected when appropriate: APACHE-II and sepsis-related organ failure assessment (SOFA) scores (at admission [day 0]), date of birth, sex, presence of septic shock, of organ dysfunction (both defined according to the consensus published in 2009 [21], length of intensive care stay and of hospital stay, blood culture results and date of death. Data from the two distinct patient studies (and from the LPS volunteer study) were analyzed separately. S100A12 concentrations were analyzed by an enzyme-linked immunosorbent assay as described before [7,23]. sRAGE levels were determined using a commercially available enzyme-linked immunosorbent assay kit (Quantikine, R&D systems, Minneapolis, Minn.) according to the manufacturer’s protocol.

Statistical analysis
Data are presented as mean ± SEM or median with 25th and 75th percentiles, where appropriate. Differences between sepsis groups (and control subjects) were assessed with nonparametric repeated-measures analysis of variance of Kruskal-Wallis test. Differences in time after intravenous LPS in healthy volunteers were compared using nonparametric repeated measures of variances. Differences between time points within groups were compared using the Wilcoxon signed rank test. Correlations were calculated using Spearman’s rho test. A Mann-Whitney U test was used to compare S100A12 levels between abdominal fluid and blood samples within the group of patients with peritonitis. P < 0.05 was considered to represent a statistically significant difference.

RESULTS

Severe sepsis results in elevated systemic S100A12 levels irrespective of the source of infection, whereas sRAGE concentrations do not change
The demographic and clinical characteristics of patients with severe sepsis are presented in Table 1. In total, 51 patients were included, with an overall in-hospital mortality of 45%. The primary source of infection was the lungs in 29 patients (52%), the abdomen in 12 patients (24%), and the urinary tract in 10 patients (20%). Compared with healthy volunteers (aged 64 ± 6.1 years, mean ± SEM, 65% male), the overall group of patients with sepsis (aged 68 ± 1.6 years, mean ± SEM, 61% male) showed increased circulating S100A12 levels both at day 0 (591.2 ± 101.0 vs. 106.2 ± 15.6 ng/mL [control subjects], P < 0.0001) and at day 3 (637.2 ± 111.2 vs. 106.2 ± 15.6 ng/mL [control subjects], P < 0.0001; Fig. 1A). All three severe sepsis subgroups with (i) peritonitis, (ii) pneumonia, or (iii) urinary tract infection as primary infection displayed elevated serum S100A12
concentrations at days 0 and 3 relatively to the control subjects (sepsis due to [i] peritonitis [393.5 ± 89.9 at day 0 and 337.9 ± 97.2 at day 3 vs. 106.2 ± 15.6 ng/mL, healthy volunteers; P < 0.005 and P < 0.05, respectively; Fig. 1B], [ii] pneumonia [716.9 ± 167.0 at day 0 and 787.5 ± 164.7 at day 3 vs. 106.2 ± 15.6 ng/mL, healthy volunteers; both P < 0.0001; Fig. 1C); and [iii] urinary tract infection [464.2 ± 115.6 at day 0 and 545.6 ± 254.9 at day 3 vs. 106.2 ± 15.6 ng/mL, healthy volunteers; P < 0.0001 and P < 0.05, respectively; Fig. 1D]). Remarkably, patients with sepsis due to pneumonia had the highest S100A12 serum levels (716.9 and 787.5 ng/mL at days 0 and 3, respectively; Fig. 1C). There was no apparent correlation between serum S100A12 concentrations and the severity of disease at admission (day 0): serum S100A12 did not correlate with either APACHE II scores (r = −0.185, P = 0.19) or SOFA scores (r = −0.194, P = 0.17). Median serum S100A12 levels in survivors and nonsurvivors were 365 and 265 ng/mL, respectively (with 25%–75% IQR of 173 and 91–835, respectively).

S100A12 is a high-affinity ligand for (s)RAGE [5,24]. We next measured sRAGE in these patients with severe sepsis. Relative to healthy controls, neither the total sepsis population nor any of the subgroups with different infectious sources demonstrated changes in sRAGE concentrations at day 0 or day 3 (Fig. 2A-D). Together, these data suggest that sepsis results in a sustained elevation of serum S100A12 concentrations irrespective of

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients with severe sepsis, n=51(100)</th>
<th>Patients with sepsis due to peritonitis, n=12 (24)</th>
<th>Patients with sepsis due to pneumonia, n=29 (57)</th>
<th>Patients with sepsis due to UTI, n=10 (20)</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>68 ± 1.6</td>
<td>63 ± 4.0</td>
<td>69 ± 1.9</td>
<td>69 ± 3.6</td>
</tr>
<tr>
<td>Male sex</td>
<td>31 (61)</td>
<td>9 (75)</td>
<td>16 (55)</td>
<td>6 (60)</td>
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<td>APACHE II score</td>
<td>27 ± 1.1</td>
<td>27 ± 1.9</td>
<td>26 ± 1.5</td>
<td>29 ± 2.6</td>
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<tr>
<td>SOFA score</td>
<td>10 ± 0.6</td>
<td>10 ± 1.1</td>
<td>9 ± 0.9</td>
<td>11 ± 0.7</td>
</tr>
<tr>
<td>Shock*</td>
<td>35 (69)</td>
<td>8 (67)</td>
<td>21 (72)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Respiratory failure*</td>
<td>32 (63)</td>
<td>2 (17)</td>
<td>25 (86)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Renal failure*</td>
<td>25 (49)</td>
<td>4 (33)</td>
<td>14 (48)</td>
<td>7 (70)</td>
</tr>
<tr>
<td>Metabolic acidosis*</td>
<td>20 (39)</td>
<td>6 (50)</td>
<td>8 (28)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Thrombocytopenia*</td>
<td>17 (33)</td>
<td>6 (50)</td>
<td>5 (17)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>INR</td>
<td>1.6 ± 0.1</td>
<td>2.4 ± 0.5</td>
<td>1.3 ± 0.1</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Use of steroids</td>
<td>25 (56)</td>
<td>8 (67)</td>
<td>13 (45)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>ICU stay, d</td>
<td>10 (4-25)</td>
<td>15 (7-29)</td>
<td>10 (4-22)</td>
<td>8 (2-14)</td>
</tr>
<tr>
<td>Hospital stay, d</td>
<td>23 (12-41)</td>
<td>30 (13-49)</td>
<td>20 (12-36)</td>
<td>31 (14-52)</td>
</tr>
<tr>
<td>Positive blood culture</td>
<td>23 (45)</td>
<td>6 (50)</td>
<td>11 (38)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Mortality</td>
<td>23 (45)</td>
<td>8 (67)</td>
<td>12 (41)</td>
<td>3 (30)</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of patients with severe sepsis, stratified to the source of infection Data are mean ± SEM, median (25%-75% interquartile range), or no. (%). *Defined according to the 1992/2009 American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference guidelines [21]. UTI indicates urinary tract infection; INR, international normalized ratio.
Figure 1. Systemic S100A12 levels in patients with severe sepsis. Serum S100A12 concentrations from patients with severe sepsis (all patients, n = 51, A) and subgroups of patients with sepsis with different infectious sources: abdomen (peritonitis, n = 12, B), lungs (pneumonia, n = 29, C), and urinary tract (UTI, n = 10, D) and from control subjects (n = 31). Data are mean ± SEM. *P < 0.05, **P < 0.005, ***P < 0.0005 vs. control subjects.
Figure 2. Systemic sRAGE levels in patients with severe sepsis. Serum sRAGE concentrations from patients with severe sepsis (all patients, n = 51, A) and subgroups of patients with sepsis with different infectious sources: abdomen (peritonitis, n = 12, B), lungs (pneumonia, n = 29, C), and urinary tract (UTI, n = 10, D) and from control subjects (n = 31). Data are mean ± SEM.
the source of the infection and that this response is not related to the severity of disease. Concurrently, the levels of its high-affinity decoy-receptor sRAGE are unaffected during sepsis.

**Human endotoxemia is associated with systemic S100A12 release but does not influence circulating sRAGE levels.**

To investigate whether intravenous LPS induces S100A12 release in humans *in vivo*, peripheral blood was obtained from healthy volunteers intravenously injected with endotoxin and S100A12 was measured before and 3, 6, 8 and 24 h thereafter. Infusion of LPS was associated with a rapid increase in S100A12 concentrations, reaching peak levels after 3 h of 59.6 ± 22.0 vs. 12.4 ± 3.6 ng/mL; t = 0 h, P < 0.005 (Fig. 3A). At 24 hrs, S100A12 concentrations had returned to baselines levels (Fig. 3A). To determine whether human endotoxemia affects systemic sRAGE concentrations, we measuredsRAGE levels in concurrently obtained samples. Unlike S100A12, sRAGE levels were not influenced by intravenous LPS injection (Fig. 3B).

**Figure 3.** Lipopolysaccharide-induced S100A12 and sRAGE release in healthy humans. Plasma S100A12 (A) and sRAGE (B) concentrations in healthy volunteers (n = 8) intravenously (i.v.) challenged with LPS (4 ng/kg, t = 0 h). Data are mean ± SEM. **P < 0.005 vs. t = 0 h. These data are part of a manuscript in preparation.

**Peritonitis is associated with local S100A12 but not sRAGE release.**

To evaluate whether S100A12 and sRAGE release occurs at the site of infection, we measured these molecules in the abdominal fluid of patients with peritonitis (n = 17). The demographic and clinical characteristics are presented in Table 2. At t = 0 hrs, S100A12 concentrations in abdominal fluid (12,945.8 ± 4,142.1 ng/mL) were more than 100-fold higher than in concurrently obtained plasma (121.2 ± 80.4 ng/mL, P < 0.0005), and these
local levels remained elevated throughout the 3-day sampling period, but declined to about relatively 50- and 25-fold higher than in plasma ($p < .0005$ at all time points) (Fig. 4A). Of note, the S100A12 plasma concentrations of the patients with peritonitis during the whole sampling-period were higher than the pre-LPS values of the healthy volunteers (with a mean of 12 ng/ml at $t = 0$ h; Fig. 3A). In contrast to the S100A12 values in the patients with peritonitis, concentrations of sRAGE in abdominal fluid were lower than in plasma ($648.7 \pm 145.6$ pg/mL, $P < 0.005$) and did not change in both compartments during peritonitis (Fig. 4B).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients with peritonitis (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61 ± 4.0</td>
</tr>
<tr>
<td>Male sex</td>
<td>9 (53)</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>14 ± 0.8</td>
</tr>
<tr>
<td>SOFA score</td>
<td>7 ± 1.4</td>
</tr>
<tr>
<td>ICU stay, d</td>
<td>5 (1-20)</td>
</tr>
<tr>
<td>Hospital stay, d</td>
<td>30 (17-53)</td>
</tr>
<tr>
<td>28 day mortality</td>
<td>0</td>
</tr>
</tbody>
</table>

**Etiology of peritonitis**
- Perforation 8 (47)
- Anastomotic leakage 7 (41)
- Abscess 1 (6)
- Other 1 (6)

Table 2. Characteristics of patients with severe sepsis, stratified to the source of infection. Data are mean ± SEM, median (25%-75% interquartile range), or no. (%).

**Figure 4.** Local S100A12 and sRAGE concentrations during peritonitis. S100A12 (A) and sRAGE (B) concentrations in abdominal fluid and plasma from patients with peritonitis (n = 17). Data are mean ± SEM. **P < 0.005, ***P < 0.0005 for abdominal fluid vs. plasma.
DISCUSSION

Sepsis remains a life-threatening condition associated with bacterial systemic dissemination and shock. S100A12 is highly expressed at sites of local inflammation, and serum concentrations correlate with individual disease activity in patients with inflammatory diseases [7,25]. We here sought to determine the extent of S100A12 release and its (soluble) receptor sRAGE in patients with severe sepsis. We demonstrate that the levels of S100A12 are increased in the circulation of patients with sepsis of various origins, whereas sRAGE levels do not change. Importantly, patients with peritonitis showed strongly elevated S100A12 levels in abdominal fluid compared with plasma values. These data show for the first time that, in patients with severe infection, S100A12 release occurs at the primary site of infection.

Our findings of elevated serum concentrations of S100A12 in patients with severe infection confirm and extend results from a previous study. In this article describing S100A12 as a neutrophil product during chronic active inflammatory bowel disease, Foell et al. [7] reported increased S100A12 serum levels in patients either with Crohn disease, colitis ulcerosa, or severe bacterial infection compared with healthy volunteers. From these patient groups, the patients with severe bacterial infection (n = 15) demonstrated the highest values with a mean of 630 ng/mL (233 ng/mL), which is comparable with our observed S100A12 serum levels in patients with severe sepsis. Kikkawa et al. [26] investigated postoperative S100A12 serum concentrations in patients who underwent emergency surgery because of sepsis secondary to perforation of the lower gastrointestinal tract. They found higher S100A12 levels in patients who developed acute lung injury (ALI) relative to patients who did not develop ALI. With the aim of that study to determine whether S100A12 (or sRAGE) is a useful marker for the development of ALI in patients with postoperative sepsis, serum levels of healthy humans were not reported. Furthermore, the detection method of S100A12 differed from the present and former [7] study, making the results difficult to interpret or to compare with our studies. In addition, Payen et al. [27] demonstrated that mRNA S100A12 expression by circulating leukocytes in patients with septic shock is diminished during the recovery phase. Other studies found that S100A12 protein is strongly expressed in inflammatory diseases such as cystic fibrosis, atherosclerosis, and rheumatoid arthritis [6,28–31].

Previously, Wittkowski et al. [32] excluded the possibility that complexes of S100A12 with sRAGE interfere with the measurements of S100A12 or sRAGE, for example, by masking of epitopes important for the detection in ELISAs. In that report, quantitative analyses with and without coincubation of these two proteins were compared. S100A12 binding to sRAGE did not affect the detection of both proteins in any of the ELISA systems used [32].
We here show that patients with sepsis resulting from either pneumonia, peritonitis, or a urinary tract infection have elevated serum S100A12 concentrations at the time severe sepsis is diagnosed and 3 days thereafter. Additional research is warranted to further explore the potential differences in the kinetics of S100A12 release in patients with sepsis depending on the primary source of the infection.

S100A12 concentrations in the present study did not correlate either with the severity of sepsis as reflected by APACHE II and SOFA scores. The observation that systemic administration of LPS to healthy volunteers induces a rise in circulating S100A12 levels implicates that LPS might at least in part be responsible for this upregulation during gram-negative infection.

The remarkable difference in S100A12 levels between the abdominal fluid and blood samples of the patients with peritonitis could be explained by a local release of S100A12 at the site of infection. In an animal model of abdominal sepsis, Zhu et al. [33] reported a similar difference in macrophage inflammatory protein 1[alpha] quantities between peritoneal fluid and blood samples of mice that underwent CLP.

Moreover, the observation that the striking difference in S100A12 concentrations in these patients with peritonitis between their systemic (blood) and local (abdominal) values was maintained for several days is not specific for S100A12, because earlier clinical studies have shown this same phenomenon for interleukin 8, macrophage chemoattractive protein 1, high-mobility group box 1, and MRP8/MRP14 (or calprotectin or S100A8/A9) [19,20,34].

Because S100A12 is not present in rodents [15], the role of S100A12 during sepsis cannot be investigated by inhibiting/deleting S100A12 in mice. One possible function of S100A12 in host defense during infection and sepsis might be to warn the host for eminent danger by exerting proinflammatory effects. Such endogenous proteins that are released by activated or damaged cells under conditions of cell stress have been called damage-associated molecular patterns (or "endokines" or "alarmins"). Indeed, nuclear factor [kappa]B–mediated expression of proinflammatory cytokines, such as tumor necrosis factor [alpha], has been documented after S100A12 stimulation. Also, other in vitro experiments indicate that S100A12 enhances the expression of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 on endothelium [35]. These proinflammatory effects of S100A12 could help to kill or contain pathogens during infection. Furthermore, S100A12 could be of benefit for the host during infection and sepsis because of its (more direct) antibacterial activity. Cole et al. [36] documented that S100A12 has activity primarily against gram-negative bacteria, including E. coli. On the other hand, S100A12-induced proinflammatory effects may underlie the tissue injury/damage associated with the infection and/or inflammation and therefore be detrimental for the host. The proinflammatory effects of S100A12 might in part be mediated by interaction with RAGE. Taken together, the role of S100A12 during sepsis...
S100A12 and sRAGE in sepsis

(or infection)—either beneficial or detrimental for the host—has still to be investigated using nonmurine models.

Knowledge about (s)RAGE involvement in host defense during sepsis or infection is limited. Bopp et al. [37] showed that sRAGE plasma levels are increased in patients with sepsis and that nonsurvivors had higher circulating sRAGE concentrations than did survivors, suggesting that sRAGE is related to severity and outcome of sepsis. We here did not find an upregulation of sRAGE blood samples either during severe sepsis or during human endotoxemia. Inflammatory stimuli such as LPS can increase transcription of RAGE via nuclear factor [kappa]B, resulting in increased expression of full-length RAGE. Therefore, full-length RAGE can be upregulated during sepsis. Indeed, we found that RAGE expression is enhanced in lungs and livers in mice during abdominal sepsis [38]. Possibly, there is a regulated equilibrium between membrane-bound and sRAGE in which sRAGE might function as a decoy receptor for proinflammatory ligands as S100A12 in patients with sepsis. Our finding that S100A12 levels increase in the absence of a rise in sRAGE concentrations suggests that relatively more S100A12 is available for binding to the transmembrane (signaling) form of RAGE.

RAGE knockout mice (deficient in full length and sRAGE) displayed a reduced mortality after induction of polymicrobial sepsis produced by CLP [11,12]. Moreover, anti-RAGE therapy conferred a survival advantage even when administered 24 hrs after CLP in mice receiving antibiotic treatment [12]. Similarly, RAGE deficient mice and wild-type mice treated with an anti-RAGE antibody displayed a reduced mortality during pneumococcal pneumonia [13,39]. As mentioned before, mice do not express S100A12 and because other RAGE ligands have been described to play a role in sepsis [16,17], non-S100A12 mediated RAGE effects play an important role in sepsis pathogenesis as well. Notably, we previously showed that another high-affinity ligand of RAGE, high-mobility group box 1 (HMGB1), is also released systemically during sepsis and locally during peritonitis (measured in the same patient population as in the present study) [19].

A limitation of our study is that we measured all sRAGE isoforms. These include isoforms produced by truncation of full-length RAGE as well as by alternative splicing from the single rage gene. The rage gene has been demonstrated in humans to generate ~20 alternative splice variants [40]. They have in common the ligand-binding domain of the full-length RAGE protein, but not its transmembrane and signaling domains. Splice variants of RAGE may affect the S100A12-binding domain by insertion of removal of parts of the V or C1 domain [40]. The sRAGE ELISA we here used does not distinguish between the different isoforms. Further experiments are needed to (i) investigate separate activities of different isoforms of sRAGE and (ii) develop sRAGE ELISA assays, which distinguish between these (possibly also functionally) different forms of sRAGE.
CONCLUSIONS

We here extend a previous finding on S100A12 levels in patients with severe bacterial infection [7], showing that S100A12 release occurs in sepsis irrespective of the primary source of infection. In patients with severe infection, S100A12 release may predominantly occur at the site of infection. The levels of sRAGE (likely functioning as a decoy receptor for S100A12) are not altered during sepsis. The role of S100A12 in sepsis still has to be examined in nonmurine models.
REFERENCES


