CRITICAL CARE

The role of receptor for advanced glycation endproducts (RAGE) in infection

Marieke AD van Zoelen*, Ahmed Achouiti, Tom van der Poll

Introduction

During evolution, multicellular organisms have developed an impressive arsenal of defense and repair mechanisms to counteract threats such as infection and trauma. Such an inflammatory response begins with the detection of the potential life-threatening event by recognizing so-called danger signals. These signal molecules have been classically divided into: i) Exogenous, pathogen-associated molecular patterns (PAMPs) [1], which are conserved motifs on pathogens that are not found in higher eukaryocytes; and ii) endogenous innate danger molecules, also named damage-associated molecular patterns (DAMPs) or alarmins, which are structurally diverse proteins rapidly released by the host itself during infection or (sterile) tissue damage [2].

Known PAMPs include lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria, peptidoglycan (present in most bacteria), lipoteichoic acid (in many Gram-positive bacteria), bacterial DNA, viral DNA/RNA and mannans in the yeast cell wall. PAMPs are recognized by pattern recognition receptors (PRRs), in particular Toll-like receptors (TLRs) and Nod-like receptors (NLRs), leading to an inflammatory response via several signaling pathways, including nuclear factor-kappa B (NF-κB) activation and subsequent tumor necrosis factor (TNF)-α production.

Examples of putative DAMPs, the endogenous equivalents of PAMPs, are high-mobility group box 1 (HMGB1), some S100 proteins (S100A8/A9, S100A12), interleukins such as IL-1α, heat-shock proteins (HSPs), and nucleosomes [3]. DAMPs can be secreted either actively or passively following necrosis but are not released by apoptotic cells [4] and have activating effects on receptor-expressing cells engaged in host defense. DAMPs can also be detected by TLRs and NLRs and their engagement induces NF-κB activation as well, suggesting that DAMPs and PAMPs use, at least partially, the same receptors and signaling pathways. Liu et al. [5] however, propose that the immune system treats DAMPs and PAMPs differently; they suggest that DAMPs – but not PAMPs – bring CD24-Siglec G/10 into the proximity of TLRs/NLRs, resulting in repressed DAMP-induced TLR/NLR signaling.

When invaded by pathogens, host defense systems encounter PAMPs from microorganisms and DAMPs that are released from tissues, which are recognized by TLRs and NLRs to warn the host of imminent danger. In addition, the multiligand receptor for advanced glycation endproducts (RAGE) is regarded as a prototypic DAMP receptor that can bind several DAMPs, including HMGB1 and S100A12 [6]. Other known RAGE ligands include amyloid, β-sheet fibrils, S100B and S100P [7]; furthermore, β2 integrins can interact with RAGE [8]. RAGE is expressed at high levels in the lungs and at low levels in normal adult tissues, including on cells involved in the innate immune system, e.g., neutrophils, T and B lymphocytes, monocytes, macrophages, dendritic cells, and endothelial cells [7]. Engagement of RAGE by its ligands leads to receptor-dependent signaling and activation of NF-κB and mitogen-activated protein kinase (MAPK) pathways [7]. Activation of RAGE plays a role in diverse experimentally-induced sterile inflammatory and infectious diseases, including cecal ligation and puncture (CLP)-induced abdominal sepsis [9], diabetic nephropathy, delayed type hypersensitivity, type II collagen induced arthritis, hepatic injury, and diabetic atherosclerosis [7,10–12]. This review focuses on new insights into the pathogenesis of infectious diseases, including sepsis, peritonitis and pneumonia, offered by studies conducted in the RAGE research field.

*Correspondence: M.A.D.vanZoelen@umcutrecht.nl
Center for Experimental and Molecular Medicine and Center for Infection and Immunity, Academic Medical Center, Meibergdreef 9, Room G2-130, 1105 AZ Amsterdam, the Netherlands

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RAGE: a multiligand receptor

RAGE consists of three immunoglobulin-like regions, a transmembrane domain, and a highly charged short cytosolic tail that is essential for intracellular signaling [13]. The V domain in the extracellular part of RAGE is essential for binding of its ligands. Because of its ability to recognize three-dimensional structures rather than specific amino acid sequences, RAGE can interact with a wide range of ligands. RAGE was first identified as a receptor for advanced glycation endproducts (AGEs), explaining its name. AGEs are products of the non-enzymatic glycation and oxidation of lipids, proteins and other macromolecules that appear, in particular, under conditions of increased availability of reducing sugars and/or enhanced oxidative stress, especially when molecules turn over slowly and aldose levels are elevated. Further investigations showed that RAGE can recognize a diverse array of endogenous molecules that warn the immune system and induce a defensive immune response; the alarmins or DAMPs.

Putative RAGE ligands in infectious diseases

HMGB1

HMGB1 is a non-histone DNA-binding protein that serves as a structural component to facilitate the assembly of nucleoprotein complexes in the nucleus [14]. Extracellularly, HMGB1 functions as a cytokine. In response to inflammatory stimuli, including PAMPs, HMGB1 can be actively released into the extracellular environment from a variety of cells including monocytes, macrophages, endothelial cells, enterocytes, pituicytes, dendritic cells, and natural killer cells [14]. HMGB1 can also be passively secreted into the extracellular milieu when cells die in a non-programmed way (necrosis), whereas apoptotic cells modify their chromatin so that HMGB1 binds irreversibly and consequently is not released [4]. During infectious diseases, increased HMGB1 concentrations may be due to active as well as passive release. Detection methods of HMGB1 that are currently used (and published) do not distinguish between these (and possible other) different forms of HMGB1. More studies are necessary to: 1) Report the biological activity of (different forms of) HMGB1; and 2) develop HMGB1 ELISA assays that can distinguish between these (possibly also functionally) different forms of HMGB1. Most investigations on HMGB1 and infection involve sepsis, the second leading cause of death in non-coronary intensive care units (ICUs) and the 10th leading cause of death overall. Patients with severe sepsis display elevated circulating HMGB1 levels [15–17] and HMGB1 is predominantly released at the site of infection; patients with pneumonia and those with peritonitis showed increased concentrations in fluid obtained from the bronchoalveolar space and abdomen, respectively [17]. In an animal model of CLP-induced sepsis, the kinetics of HMGB1 secretion *in vivo* was delayed and more sustained when compared with the release of pro-inflammatory cytokines, like TNF-α, IL-1β and IL-6 [18,19]. Similarly, various interventions that inhibit HMGB1 activity or production, such as anti-HMGB1 antibodies, the A-box segment of HMGB1, ethyl pyruvate, and nicotine, reduced CLP-induced sepsis and/or LPS lethality even if treatment was delayed for many hours, up to one day after the challenge [20,21]. An implicated crucial event in sepsis patho-physiology is apoptosis of immune cells, playing a major role in immunosuppression and lethality [22]. HMGB1 seems to be a downstream factor of apoptosis in the final common pathway to organ damage in severe sepsis as indicated by observations that prevention of lymphocyte apoptosis improved survival after CLP [23], whereas anti-HMGB1 treatment reduced lethality in the same model without influencing apoptosis [19]. This indicates that HMGB1 secretion is a relatively late event in sepsis that contributes significantly to a worsened outcome. In addition, it has been reported that very pure HMGB1 does not have cytokine-inducing capacity itself, but activates cells indirectly by first acquiring immune stimulating CpG DNA [24], which is released in the bloodstream during bacterial sepsis. However, a recent study reported that HMGB1-mediated induction of macrophage cytokine production requires binding to TLR4, and that binding and signaling are dependent on a molecular mechanism that requires cysteine in position 106 within the B box [25]. Together these data indicate that HMGB1 may exert pro-inflammatory effects in a direct TLR4-dependent way and an indirect way via binding of DAMPs and other agonistic molecules.

S100A12

S100A12 is a calcium binding protein expressed in the cytoplasm of neutrophils, where it comprises 5% of the total protein content. Furthermore, S100A12 – also known as EN-RAGE (extracellular newly identified ligand of RAGE) or myeloid-related protein (MRP)-6 – is found in monocytes and lymphocytes and provokes pro-inflammatory responses in endothelial cells [26]. Although many RAGE ligands are promiscuous with regard to receptor use, S100A12 has only been shown to bind to RAGE. S100A12 expression is high in inflammatory diseases such as atherosclerosis, rheumatoid arthritis, Crohn's disease, Kawaski disease, and cystic fibrosis. Within the lungs, S100A12 and RAGE are increased during acute lung injury (ALI) [26]. S100A12 expression may reflect activation of neutrophils during pulmonary inflammation and may contribute to endothelial activation via binding to RAGE [26].
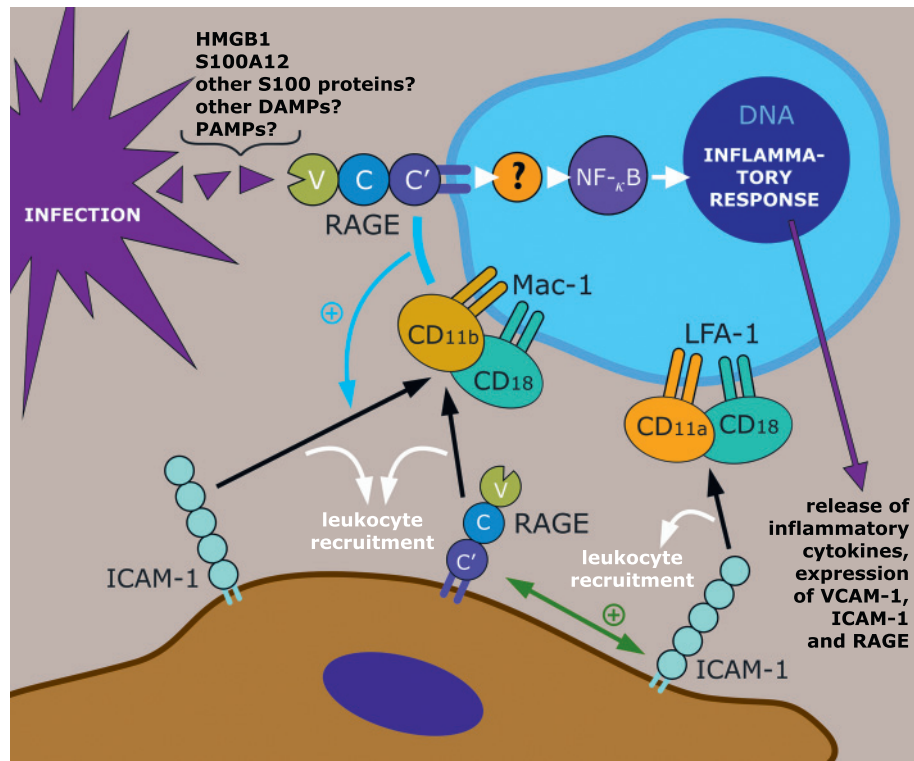
Figure 1. Putative involvement of the receptor for advanced glycation endproducts (RAGE) during infection. The damage-associated molecular patterns (DAMPs), high-mobility group box 1 (HMGB1) and S100A12, are released during infection ([15, 17], and unpublished data) and bind to and activate RAGE. It has to be determined whether other S100 proteins and other DAMPs are RAGE ligands (indicated as purple shapes) released during infection. It would be interesting to investigate whether RAGE can directly bind to, become activated and mount a first immune reaction after ligation with specific PAMPs as well. Engagement of RAGE by its ligands results in receptor-dependent signaling and activation of NF-κB leading to a pro-inflammatory response; the signaling pathway is largely unknown. In addition, RAGE interacts as an endothelial (and epithelial) adhesion receptor with the leukocyte integrin, CD11b/CD18 (Mac-1) (lower section) [8]. Furthermore, lateral (*in cis*) RAGE-Mac-1 interaction on the leukocyte surface is mediated by HMGB1 and activates Mac-1-intercellular adhesion molecule (ICAM)-1 dependent adhesion and migration and augments leukocyte recruitment [27] (indicated by the blue line and blue „+"). Moreover, a recent report shows that endothelially expressed RAGE acts in concert with ICAM-1 in mediating β_2 integrin–dependent leukocyte adhesion during acute trauma–induced inflammation [28] (indicated by the green line and green „+").

β2 integrins

Recruitment of leukocytes to the site of infection is an essential step in host defense during infectious diseases against invading pathogens. RAGE plays a role in the regulation of cell migration in several ways. First of all, RAGE is a counter-receptor for integrins on leukocytes; in particular, RAGE has been identified as a binding partner for the β2 integrins, Mac-1 and p150, 95 [8]. Second, by the interaction of RAGE with β2 integrin-mediated leukocyte recruitment *in vivo*: RAGE^{-/-} mice displayed a diminished number of adherent inflammatory cells on the peritoneum after CLP [9] and a reduction in neutrophil influx in the peritoneal cavity after thioglycollate peritonitis [8]. Interestingly, HMGB1 can activate lateral (*in cis*) RAGE-Mac-1 interactions on the leukocyte cell surface, enhancing Mac-1-intercellular adhesion molecule (ICAM)-1-dependent adhesion and migration [27] (Fig. 1, indicated by the blue line and blue

"+"). Furthermore, a recent report shows that endothelial expressed RAGE acts in concert with ICAM-1 in mediating β_2 integrin-dependent leukocyte adhesion during acute trauma-induced inflammation [28] (Fig. 1, indicated by the green line and green "+").

RAGE: a signal transducing receptor

The signaling cascade(s) of RAGE – induced by engagement of its various ligands – that ultimately activates NF-κB is largely unknown. The predicted cytosolic portion of RAGE, consisting of 43 amino acids, is short compared to other PRRs, the TLRs and IL-1 receptors, and does not include a known signaling domain or motif. A RAGE mutant lacking this intracellular tail does not activate NF-κB and behaves like a dominant negative, preventing pro-inflammatory cytokine release from macrophages. These data indicate a critical role of this cytosolic portion in transducing the signal from the cell surface to the nucleus.

One possibility is that RAGE uses as yet unknown adaptors framing a whole `new' signaling cascade to NF-κB. Another possibility is that the RAGE tail interacts with a Toll/IL-1 receptor (TIR)-containing protein which then recruits the downstream TIR-containing proteins in a way analogous to TLR-mediated signaling pathways. Finally, RAGE could transduce signals from the cell surface to the nucleus by bypassing the TIR-containing adaptor, directly interacting with member(s) of the signaling cascade.

In addition to triggering NF-κB activation, RAGE engagement by its myriad ligands is linked to an array of signaling pathways, including MAPK family members, such as Jun-N-terminal kinase (JNK), p38 and extra-cellular signal-regulated kinase (ERK), PI3K/Akt, Rho GTPases, Jak/STAT and Src family kinases [7].

This rather extraordinary variety of observed signals may be due to the broad expression of RAGE, its diversity of ligands, and possible contaminating elements in the preparations used in experiments.

Soluble RAGE (sRAGE)

The truncated form of full-length RAGE, soluble RAGE (sRAGE), consists of only the extracellular ligand-binding domain (V-C-C') lacking the cytosolic and transmem-brane domains (i.e., the parts that transfer a signal into the cell) and circulates in the bloodstream. sRAGE has been indicated to be involved in inflammatory processes in several ways. First, sRAGE blood concentrations are associated with various inflammatory diseases in patients and in rats with experimentally induced ALI [29]. Furthermore, it is suggested that sRAGE can compete with full length cell-surface RAGE for ligand engagement, preventing these ligands from binding to RAGE or other receptors and/or exerting effects otherwise. Exogenous sRAGE treatment indeed attenuated inflammatory res-ponses in several animal models, including models of type II collagen-induced arthritis, hepatic injury, diabetic atherosclerosis, delayed type hypersensitivity, and experi-mental auto-immune encephalomyelitis [7]. The involve-ment of sRAGE during infection is not known. Based on experimental studies in rats and in patients with ALI, sRAGE has been described as a marker of lung injury [29].

RAGE during infection

An increasing amount of research suggests a role for RAGE in the pathogenesis of pneumonia, peritonitis and sepsis, indicating that RAGE (ligand)-directed therapies might offer new treatment opportunities for human disease in the future.

RAGE during pneumonia
Localization and role of RAGE in the lungs
Recent studies point to an important role of RAGE in the pulmonary compartment in physiological as well as in pathological circumstances. Physiologically, RAGE is expressed at high basal levels in the lungs relative to other tissues [26,29–34], suggesting that RAGE may have lung-specific functions distinct from the role of RAGE in other adult tissues. In particular, although the kidney is dramatically affected by microangiopathy and fibrosis – which is substantially attributed to RAGE – in patients with diabetes, the lungs, with a significantly higher baseline RAGE expression than the kidney, remain unaffected. In addition, RAGE has been found to be specifically localized near the basal cell membrane within alveolar pneumocytes [32,35,36]. These two observations raise the question as to whether RAGE has a function in normal healthy lungs. Indeed, Englert et al. documented that aged RAGE^{-/-} mice develop pulmonary fibrosis-like alterations spontaneously; lungs from 19 to 24 month-old RAGE^{-/-} mice showed increased collagen staining and displayed increased levels of hydroxyproline relative to wild type mice [31]. RAGE knockdown in pulmonary fibroblasts increased their proliferation and migration *in vitro*, suggesting an important protective function of RAGE in the lungs and that loss of RAGE may be related to functional changes of pulmonary cell types resulting in fibrotic disease [34]. Another study demonstrated that RAGE on epithelial cells promoted their adherence to human collagen (a major component of the alveolar basal lamina) and a spreading morphology, which may facilitate gas exchange and alveolar stability *in vivo* [34,35]. Together, these data suggest that RAGE plays a role in maintaining lung homeostasis in normal, healthy lungs. Further studies are needed to unravel the function(s) of pulmonary RAGE in physiology in more detail.

This putative functional role of RAGE in healthy lungs may be the explanation for the finding that the inhibition of RAGE signaling attenuates pathological sterile inflam-matory responses in diverse non-pulmonary experimen-tal studies [9–12], whereas in pulmonary non-infectious pathological inflammatory conditions, somewhat con-flicting results emerge. Lung injury induced by either bleomycin or hyperoxia is diminished in RAGE^{-/-} mice [37,38], suggesting a deteriorating attribution of RAGE. In contrast, Englert et al. showed that RAGE^{-/-} mice developed more severe lung fibrosis after asbestos administration as measured by histological scoring and total lung hydroxyproline quantification [31]. Of note, in all these studies, the mice were much younger at the time of sacrifice than the aged (19–24 month-old) RAGE^{-/-} mice that developed pulmonary fibrosis spontaneously in the experiment by Englert et al. [31]. Interestingly, lung homogenates and bronchoalveolar lavage (BAL) fluid from patients suffering from idiopathic pulmonary fibrosis reveal reduced membrane bound (and soluble) RAGE protein levels compared to healthy donor samples [31,34].

RAGE expression during pneumonia

Community-acquired pneumonia (CAP) is distinguished from hospital-acquired pneumonia (HAP) according to the time of acquisition of pneumonia and the pathogens involved. The Gram-positive bacterium, *Streptococcus pneumoniae*, is the single most frequent pathogen causing CAP, responsible for up to 60% of cases; *Klebsiella pneumoniae, Haemophilus influenzae, Staphylococcus aureus*, and viruses are isolated in about 10% of cases each and *Mycobacterium tuberculosis* is more prevalent in developing countries. Knowledge of the expression and role of RAGE in host defense during pneumonia is limited. Morbini et al. observed increased RAGE expression in patients with interstitial and post-obstructive pneumonia [32]; this report left unanswered whether patients with bacterial pneumonia were included in the analysis. Notably, two other studies showed that constitutively present RAGE was not upregulated during pulmonary inflammation associated with ALI or acute respiratory distress syndrome (ARDS): First, rats with ALI induced by intratracheally administered LPS displayed no change in the distribution of RAGE-expressing cells [29]; and second, patients with ARDS did not have increased pulmonary expression of RAGE [26].

sRAGE has been suggested to be a lung injury marker based on studies in patients with ALI and on experimental studies in rats [29]. sRAGE was increased in pulmonary edema fluid and serum from patients with either ALI or ARDS and with hydrostatic pulmonary edema, and in BAL fluid from rats with either LPS- or hydrochloric acid-induced ALI [29].

Considering the ubiquitous expression of RAGE in the lungs, its putative involvement in the regulation of lung inflammation and the somewhat inconsistent findings which currently exist in the literature, we investigated its role during pneumonia, a major cause of morbidity and mortality world-wide. Recently, we reported that murine pneumonia induced by *S. pneumoniae* and by influenza A virus was associated with an upregulation of intra-alveolar (membrane bound) RAGE expression [39,40]. Furthermore, lung tissue of mice intranasally infected with the *K. pneumoniae* or with *M. tuberculosis* also showed increased RAGE expression (unpublished data). These clinically very different types of pulmonary infection and the involvement of RAGE therein will be discussed below.

Role of RAGE in pneumonia caused by different pathogens

Levels of the high-affinity RAGE ligand, HMGB1, were higher in BAL fluid from patients with pneumonia compared to BAL fluid from healthy controls [17]. In experimentally induced pneumococcal pneumonia, the presence of RAGE was detrimental: Mice lacking RAGE had a better survival rate together with a lower pulmonary bacterial load and decreased dissemination of *S. pneumoniae* to blood and spleen compared to wild-type mice [39]. The difference was possibly partially due to an increased killing capacity of RAGE$^{-/-}$ alveolar macrophages. Additionally, lung injury and neutrophil recruitment were reduced in the RAGE$^{-/-}$ mice, which parallels findings on RAGE as an endothelial counter receptor for the β2 integrin, Mac-1, [8] and the interplay between RAGE and Mac-1 on leukocytes, required for HMGB1-mediated inflammatory cell recruitment [27]. In addition, blockade of the RAGE-HMGB1 interaction and prevention of the subsequent pro-inflammatory stimulus might be an explanation for the less severe pulmonary damage in the RAGE$^{-/-}$ mice during *S. pneumoniae* pneumonia.

Interestingly, in contrast to Gram-positive pneumonia, preliminary data from our laboratory reveal that RAGE plays a beneficial role in mice during the host response to Gram-negative pneumonia (unpublished data). Indeed, RAGE deficiency was associated with increased mortality and increased bacterial outgrowth and dissemination after *K. pneumoniae* inoculation (unpublished data). Relative to wild type mice, lung inflammation was similar and cytokine and chemokine levels were slightly – if at all – elevated. Moreover, RAGE$^{-/-}$ mice showed an unaltered response to intranasally instilled *Klebsiella* LPS with respect to pulmonary cell recruitment and local release of cytokines and chemokines. Together, these findings indicate that RAGE contributes to an effective anti-bacterial host response during *K. pneumoniae* pneumonia, whereas RAGE plays an insignificant part in the lung inflammatory response to either intact *Klebsiella* or *Klebsiella* LPS.

It is unclear whether RAGE can also interact with ligands from pathogens. If so, this could be part of the explanation for our observation that RAGE involvement during Gram-positive and –negative pneumonia had such opposite effects on mortality. In addition, RAGE-mediated effects on other first-line defense mechanisms, such as chemotaxis, phagocytosis, killing (including respiratory burst), may depend on the pathogen and may contribute to the observed effects in Gram-positive and -negative pneumonia models. However, this remains speculative until investigations have been performed to analyze this interesting issue.

In addition to its potential to cause pandemics, seasonal influenza A virus infection causes over 200,000 hospitalizations and approximately 41,000 deaths in the United States annually, being the 7th leading cause of mortality. We demonstrated that RAGE deficiency resulted in a better outcome from pulmonary influenza A virus infection as indicated by a relative protection from influenza A virus-induced lethality in mice [40]. This was accompanied by improved viral clearance and enhanced

cellular T cell response and activation of neutrophils, suggesting that endogenous RAGE impairs the cellular immunity against respiratory tract infection with influenza A virus. RAGE ligand, HMGB1, as well as sRAGE were upregulated in BAL fluid during influenza A virus pneumonia. Hence, similar to pneumonia induced by the Gram-positive bacterium *S. pneumoniae*, RAGE is detrimental during pneumonia caused by influenza A virus. This is of particular interest, since it has been suggested that the greatest proportion of the mortality associated with influenza A virus infection is due to secondary bacterial pneumonia, with *S. pneumoniae* as the most frequent pathogen of the superinfection. Therefore, RAGE is a potential treatment target in postinfluenza pneumococcal pneumonia and further research is warranted to investigate this.

RAGE during abdominal sepsis

The role of RAGE in abdominal sepsis has been investigated in a limited number of studies so far. RAGE-deficient mice showed decreased mortality after induction of polymicrobial sepsis induced by CLP in two reports [9,41]. Moreover, anti-RAGE antibody yielded a better survival even when the anti-RAGE therapy was delayed up to 24 hours after CLP in mice receiving antibiotics [41]. The protective effect provided by the absence of RAGE was related to a firm inhibition of NF-κB activation, suggesting that the lack of excessive NF-κB activation in RAGE$^{-/-}$ mice might have contributed to their reduced mortality [9]. In addition, RAGE deficiency resulted in fewer inflammatory cells in the peritoneum [9], which parallels the results of an earlier investigation by the same group of authors identifying RAGE as a counter-receptor for the β2 integrin, Mac-1 (CD11b/CD18), and thereby as a mediator of leukocyte recruitment and adhesion [8]. Furthermore, the protective effect of RAGE inhibition in this CLP model could at least in part be the consequence of the inhibition of one of its ligands, HMGB1. Indeed, HMGB1 is secreted into the circulation after CLP and anti-HMGB1 antibody led to increased survival after CLP-induced peritonitis [18].

In the same surgically (CLP)-induced model of sepsis, RAGE deficiency and anti-RAGE therapy were reported not to affect bacterial outgrowth in the peritoneum, liver, or spleen [41]. Notwithstanding, a possible role of RAGE in antibacterial defense cannot be easily evaluated from this study because host defense against CLP depends, at least in part, on the extent of intestinal necrosis and the formation of a local abscess. Also, all mice in this experiment received broad spectrum antibiotics and bacterial outgrowth was only determined in mice that survived (i.e., not at predefined time points after CLP). For this reason, we used our model of abdominal sepsis

induced by injection of the Gram-negative bacterium *Escherichia coli* into the peritoneum [42,43] to study whether RAGE affects antibacterial defense. This model is a relevant tool to investigate the role of receptors/mediators in limiting the growth and dissemination of bacteria after a primary intra-abdominal infection and to assess the contribution of these proteins to specific immune responses. RAGE expression was upregulated during *E. coli* induced sepsis [42]. RAGE deficiency (either pharmacologically using anti-RAGE IgG antibodies or genetically using RAGE knock out mice) was related to a higher bacterial load and dissemination [42]. These data indicate that RAGE signaling contributes to an effective antibacterial response during abdominal sepsis. RAGE exerted this effect probably indirectly and not via direct interaction with *E. coli*, considering the observation that leukocytes from RAGE$^{-/-}$ mice had an unaltered capacity to phagocytose and kill *E. coli in vitro*. Furthermore, the finding that deficiency of RAGE in general was associated with an exaggerated host response during *E. coli* sepsis [42] on the one hand, and with an attenuated inflammatory response and better survival in (other) sterile models of intraperitoneal injection of LPS derived from *E. coli* [42,44] on the other hand, suggests that although RAGE is involved in the immune reaction to *E. coli*, this function can be compensated for by other receptors in the presence of a growing bacterial load. The high-affinity RAGE ligand, HMGB1, is secreted into the circulation systemically during clinical sepsis [16–18] as well as in our experimental sepsis model of *E. coli* [43]. Importantly, HMGB1 has been shown to transduce cellular signals *in vitro* and *in vivo* by interacting with at least three other receptors, i.e., TLR2, TLR4 and TLR9 when HMGB1 is complexed with CPG DNA [24,44,45]. One possible explanation for the increased response in the RAGE lacking mice during *E. coli* sepsis is, therefore, that the absence of RAGE could facilitate the interaction between HMGB1 and TLR2, TLR4 and/or TLR9.

Evidence of involvement of ligands of RAGE and HMGB1 in host defense in *E. coli* abdominal sepsis was recently published by our laboratory [43]. Inhibition of multiple RAGE ligands (by the administration of sRAGE) and inhibition of HMGB1 (by the administration of anti-HMGB1 antibodies) led to an enhanced bacterial dissemination of *E. coli*, denoting an advantageous role of RAGE ligands, including HMGB1, in the antibacterial response during Gram-negative sepsis.

Interestingly, we recently found that S100A12, another high-affinity ligand of RAGE, is released systemically in patients during (abdominal) sepsis and also locally during peritonitis (unpublished data). Additionally, intravenous injection of LPS in healthy humans raised circulating S100A12 levels, implying that LPS might partially contribute to this upregulation during Gram-negative infection.

Payen et al. reported that, in patients with septic shock, mRNA S100A12 expression by circulating leukocytes was decreased during the recovery phase [46]. One possible function of S100A12 in host defense during infection and sepsis is its role as a DAMP. NF-κB mediated expression of pro-inflammatory cytokines and upregulation of ICAM-1 and vascular cell adhesion molecule (VCAM)-1 on endothelium has been documented *in vitro* after S100A12 stimulation [47]. Furthermore, S100A12 could be of benefit for the host during infection and sepsis due to its (more direct) antibacterial activity. Cole et al. determined that S100A12 has activity primarily against Gram-negative bacteria, including *E. coli* [48]. Because of the absence of S100A12 in rodents, a potential functional role of S100A12 during sepsis cannot be easily investigated by inhibiting/deleting S100A12 in animals. Altogether, the role of S100A12 during sepsis has yet to be evaluated using non-rodent models.

Bopp et al. documented that septic patients have elevated circulating sRAGE levels and that non-survivors show higher plasma sRAGE concentrations than survivors, suggesting that sRAGE is related to severity and clinical outcome in sepsis [49]. Knowledge on the role of endogenous sRAGE in sepsis is scarce. Hudson et al. demonstrated that sRAGE levels might represent an early marker of microvascular dysfunction, a phenomenon also present in sepsis [50]. Furthermore, increased sRAGE concentrations in sepsis might represent the acute inflammatory status as splice variants of RAGE or as split off variants of the cell surface RAGE, the latter analogous to ICAM-1, another member of the immunoglobulin superfamily, which is a marker of cellular damage during sepsis. Another possibility is that systemic sRAGE levels might be elevated in parallel with HMGB1/S100A12 levels as a counter-system against HMGB1/S100A12 elicited tissue effects. More research is needed to clarify the functional role of sRAGE in sepsis and its putative role as a new sepsis marker.

Conclusion

The innate immune response is the first line of defense against pathogens. The experimental studies described herein provide further insight into the role of RAGE and its ligands in host defense during clinically important infections, which eventually may contribute to better therapies against specific pathogens. While interpreting the results from preclinical investigations, one has to keep in mind that a careful balance between the inflammatory and anti-inflammatory response is vital in order to survive or recover from a severe infection.

The observation that lack of RAGE is of benefit in one pneumonia model and detrimental in another, clearly adds to the notion that the way in which RAGE mediates host defense against different pathogens relies on distinct mechanisms. It would be highly interesting to evaluate whether RAGE can directly bind to, become activated, and mount a first immune reaction after ligation with specific PAMPs. Furthermore, RAGE-mediated effects on other first-line defense mechanisms, such as chemotaxis, killing, phagocytosis and respiratory burst could depend on the pathogen. As such, targeting RAGE may be ineffective or even harmful in some infectious conditions. Therefore, more studies are necessary to justify clinical trials targeting RAGE in patients with severe infections. In this respect one could think of research on RAGE inhibition in pneumococcal and influenza A viral pneumonia. Additionally, experiments in which RAGE targeting is delayed until after bacterial/viral infection and combined with antibiotic/antiviral therapy should be considered. Moreover, more studies need to be conducted on the role of RAGE in critical organ derangements involved in the pathogenesis of severe infection, including activation of the coagulation system and the complement system. RAGE remains a potential yet promising therapeutic target that awaits further research.

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