

## Review

**Bench-to-bedside review: The inflammation-perpetuating pattern-recognition receptor RAGE as a therapeutic target in sepsis**Christian Bopp<sup>1</sup>, Angelika Bierhaus<sup>2</sup>, Stefan Hofer<sup>1</sup>, Axel Bouchon<sup>3</sup>, Peter P Nawroth<sup>2</sup>, Eike Martin<sup>1</sup> and Markus A Weigand<sup>1</sup><sup>1</sup>Department of Anesthesiology, University of Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany<sup>2</sup>Department of Medicine I, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany<sup>3</sup>Bayer CropScience, Alfred-Nobel-Str. 50, 40789 Monheim, GermanyCorresponding author: Markus A Weigand, [markus.weigand@med.uni-heidelberg.de](mailto:markus.weigand@med.uni-heidelberg.de)

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*Critical Care* 2008, **12**:201 (doi:10.1186/cc6164)**Abstract**

Sepsis still represents an important clinical and economic challenge for intensive care units. Severe complications like multi-organ failure with high mortality and the lack of specific diagnostic tools continue to hamper the development of improved therapies for sepsis. Fundamental questions regarding the cellular pathogenesis of experimental and clinical sepsis remain unresolved. According to experimental data, inhibiting macrophage migration inhibitory factor, high-mobility group box protein 1 (HMGB1), and complement factor C5a and inhibiting the TREM-1 (triggering receptor expressed on myeloid cells 1) signaling pathway and apoptosis represent promising new therapeutic options. In addition, we have demonstrated that blocking the signal transduction pathway of receptor of advanced glycation endproducts (RAGE), a new inflammation-perpetuating receptor and a member of the immunoglobulin superfamily, increases survival in experimental sepsis. The activation of RAGE by advanced glycation endproducts, S100, and HMGB1 initiates nuclear factor kappa B and mitogen-activated protein kinase pathways. Importantly, the survival rate of RAGE knockout mice was more than fourfold that of wild-type mice in a septic shock model of cecal ligation and puncture (CLP). Additionally, the application of soluble RAGE, an extracellular decoy for RAGE ligands, improves survival in mice after CLP, suggesting that RAGE is a central player in perpetuating the innate immune response. Understanding the basic signal transduction events triggered by this multi-ligand receptor may offer new diagnostic and therapeutic options in patients with sepsis.

**Introduction**

In the United States, sepsis is the main cause of death in non-cardiac intensive care units and is linked with increasing costs for patient care. Sepsis represents a range of disorders

involving bacterial, fungal, or viral infections that can be disseminated by the bloodstream [1]. Epidemiological data from North America show an incidence of 3.0 cases per 1,000 persons. The overall mortality is approximately 50% in patients with severe septic shock [2]. Even high-priority engagement in sepsis research has led to only slight improvements in existing treatment strategies for sepsis. Currently, the detailed mechanisms linking the foreign bacterial agent (for example, in the bloodstream or in the abdomen) with the sophisticated ongoing transcription work of the cell nucleus are not completely understood.

The combined use of the pre-existing innate and inducible adaptive immune systems ensures that the host will be able to mount an appropriate immune response against different types of pathogenic agents [1]. The first line of defense is the innate immune system, which is characterized by non-clonally distributed leukocytes that react rapidly to microbial products without antigenic specificity. Host innate responses to bacterial or fungal infections are primarily mediated by neutrophils and monocytes/macrophages. These cells express germline-encoded pattern-recognition receptors (PRRs), which recognize certain invariable pathogen-associated molecular patterns, or PAMPs, shared by groups of microorganisms. PRRs trigger signaling pathways that initiate an inflammatory response to infection [3]. Activating isoforms are truncated in their cytoplasmic tails and deliver stimulatory signals by associating with transmembrane adapter proteins, such as CD3 $\gamma$ , the  $\gamma$ -chain of Fc receptors, and DAP12 (also

AGE = advanced glycation endproduct; ALI = acute lung injury; CLP = cecal ligation and puncture; DTH = delayed-type hypersensitivity; EAE = experimental allergic (or autoimmune) encephalomyelitis; EN-RAGE = extracellular newly identified receptor of advanced glycation endproducts-binding protein; ERK-1/2 = extracellular signal-regulated kinase 1/2; HMGB1 = high-mobility group box protein 1; ICAM-1 = intercellular adhesion molecule 1; IL = interleukin; JNK = c-jun N-terminal kinase; KO = knockout; LPS = lipopolysaccharide; MAPK = mitogen-activated protein kinase; NF- $\kappa$ B = nuclear factor kappa B; PRR = pattern-recognition receptor; RAGE = receptor of advanced glycation endproducts; SAPK = stress-activated protein kinase; sRAGE = soluble receptor of advanced glycation endproducts; TLR = Toll-like receptor; TNF = tumor necrosis factor; VCAM-1 = vascular cell adhesion molecule 1.

known as KARAP) [4]. Innate immune cells, however, also receive continuous off signals via inhibitory receptors that recognize ubiquitously expressed endogenous molecules. These receptors transmit their inhibitory signals through a cytoplasmatic immunoreceptor tyrosine-based inhibitory motif, or ITIM [5].

The balance between activating and inhibitory signals generated by the engagement of these receptors ultimately controls neutrophil- and macrophage-mediated phagocytosis, respiratory burst, and the release of proinflammatory cytokines. Under certain circumstances, an excessive inflammatory response to infectious agents can lead to septic shock. The disastrous endpoint of an overstimulated immune system is multiple organ failure as a result of endorgan damage. This process is characterized by the massive release of proinflammatory mediators such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1, macrophage migration inhibitory factor, and high-mobility group box protein 1 (HMGB1) [6,7].

The receptor of advanced glycation endproducts (RAGE), a member of the immunoglobulin superfamily, is involved in the signal transduction from pathogen substrates to cell activation during the onset and perpetuation of inflammation. Recent data suggest (a) that RAGE perpetuates and amplifies inflammation and (b) that targeting this receptor might attenuate hyperinflammation. Therefore, this multi-ligand receptor should be viewed as a PRR. Gaining further knowledge about the ligands and basic mechanisms of this receptor may offer new diagnostic and therapeutic options in patients with sepsis.

### Receptor of advanced glycation endproducts

RAGE was first identified in lung tissue [8-10]. It is located on the basolateral membranes of alveolar epithelial type I cells [11], but RAGE mRNA has also been found in alveolar epithelial type II cells [12]. Originally, RAGE was identified as a receptor for advanced glycation endproducts (AGEs), explaining the choice of this name. AGEs are products of non-enzymatic glycation and oxidation of proteins, lipids, 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 [13,14].

RAGE expression occurs both constitutively and inducibly, depending upon cell type and developmental stage. Whereas RAGE is constitutively expressed during embryonal development, RAGE expression is downregulated in adult life. Known exceptions are skin and lung, which constitutively express RAGE throughout life [15]. However, downregulated cells can be induced to express RAGE in situations in which inflammatory mediators and ligands accumulate [16,17]. The activation of RAGE initiates nuclear factor kappa B (NF- $\kappa$ B) [18,19] and mitogen-activated protein kinase (MAPK) pathways [20]. Additionally, RAGE-mediated cellular stimulation promotes increased expression of the receptor itself. This

positive feedback loop, characterized by ligand-receptor interaction followed by increased expression of the receptor, suggests that RAGE functions as a propagation and perpetuation factor: the two-hit model of RAGE engagement is based on this finding [21]. The transcription factors regulating RAGE in this setting include specificity protein-1, activator protein-2, NF- $\kappa$ B, and NF-IL-6 [22]. Takada and colleagues [23] reported that matrix metalloproteinase-9 (gelatinase B) plays a critical role in concordant expression, at least in human pancreatic cancer cells.

### Localization and structure of RAGE

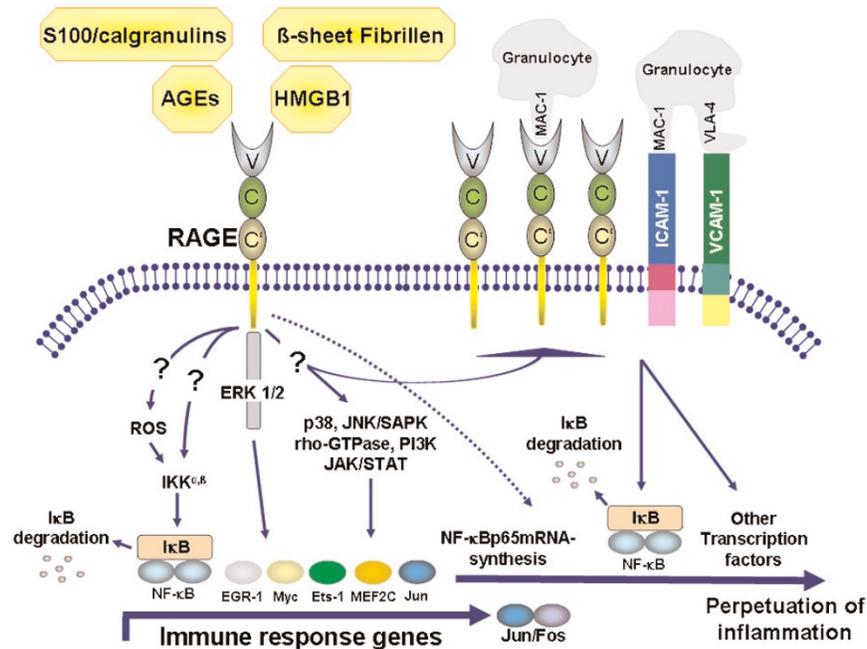
The gene for RAGE is located on chromosome 6 near the major histocompatibility complex III in humans and mice, in the proximity of genes encoding TNF, lymphotoxin, and the homebox gene *HOX12* [24,25]. The extracellular domain of RAGE consists of one V-type immunoglobulin domain followed by two C-type immunoglobulin domains. The V-type domain, in particular, interacts with the potential extracellular ligands [9,10,19,20]. The rest of the molecule is a single transmembrane-spanning domain completed by a 43-amino acid, highly charged cytosolic tail. This cytosolic tail lacks known signaling motifs such as phosphorylation sites or kinase domains. Hofmann and colleagues [26] showed that the cytosolic tail is essential for signal transduction of RAGE because a truncated form of RAGE, in which the cytosolic tail is deleted, can bind both ligands and the wild-type receptor but does not mediate any cellular activation. In the rat lung, extracellular signal-regulated kinase 1/2 (ERK-1/2) was shown to bind intracellularly to the cytoplasmic tail of RAGE, suggesting that ERK may play a role in RAGE signaling through interaction with RAGE [27]. The existence of truncated and partly secreted RAGE isoforms from the same gene implies that the pre-mRNA of RAGE in humans can be subjected to alternative splicing [13]. In contrast, the truncated isoforms in mice seem to be produced by carboxyl-terminal truncation [28].

Although only little is known about the physiologic role of RAGE, it may fit with the concept of pleiotropic antagonism [29]. This concept of an evolutionary basis for the development of age-related diseases postulates that genes that are beneficial during the reproductive phase of life may become deleterious to development later on. Formerly, this interest was mainly focused on the role of RAGE in chronic diseases. Particularly under pathologic conditions, RAGE is up-regulated in blood vessels, neurons, and transformed epithelia and is involved in several chronic diseases, such as rheumatoid arthritis, diabetes, inflammatory kidney disease, arteriosclerosis, inflammatory bowel disease, neurodegenerative disorders (especially Alzheimer disease), and wound-healing disorders [14].

### RAGE interactions with its ligands in acute inflammation and sepsis

RAGE is a multi-ligand receptor and interacts with different structures to transmit a signal into the cell and recognizes

Figure 1



Receptor of advanced glycation endproducts (RAGE)-mediated signal transduction. AGE, advanced glycation endproduct; C, C-type immunoglobulin domain; ERK-1/2, extracellular signal-regulated kinase 1/2; HMGB1, high-mobility group box protein 1; ICAM-1, intercellular adhesion molecule 1; I $\kappa$ B, inhibitor of kappa B; IKK $\alpha/\beta$ , inhibitor of kappa B kinase; JAK, Janus kinase; JNK, c-jun N-terminal kinase; MAC-1, macrophage-1 antigen; NF- $\kappa$ B, nuclear factor kappa B; P13K, phosphoinositide 3-kinase; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; STAT, signal transducer and activator of transcription; V, V-type immunoglobulin domain; VCAM-1, vascular cell adhesion molecule 1; VLA-4, very late antigen 4.

three-dimensional structures rather than specific amino acid sequences. Therefore, RAGE seems to fulfill the requirements of a PRR. As a member of the immunoglobulin superfamily, it interacts with a diverse class of ligands, including AGEs [9,10], S100/calgranulins [26], HMGB1 [30], amyloid  $\beta$ -peptide [31], amyloid A [32], leukocyte adhesion receptors [33], prions [34], *Escherichia coli* curli operons [35], and  $\beta$ -sheet fibrils [14].

### The AGE-RAGE interaction

AGEs are a heterogeneous group of compounds produced by non-enzymatic glycation and oxidation of proteins and lipids that exhibit characteristic absorbance and fluorescence properties, *N*<sup>ε</sup>-(carboxymethyl)lysine being a highly reactive AGE [36,37]. They are protease-resistant and can cause irreversible tissue damage. AGEs can bind to various cellular surface receptors and thereby induce post-receptor signaling, activation of transcription factors, and gene expression *in vitro* and *in vivo*. Several receptors that bind AGEs, including AGE-R1, AGE-R2, AGE-R3, the scavenger receptor II, and RAGE, have been identified [38]. Binding of AGEs (and other ligands) to RAGE generates intracellular reactive oxygen species and depletes antioxidant defense mechanisms at the same time [39,40]. As a result, AGEs binding to RAGE, reduced glutathione, and ascorbic acid are diminished.

Depletion of glutathione leads to reduced glyoxalase-1 recycling and decreased *in situ* activity. Glyoxalase-1, however, has an important role in reducing the cellular AGE load [38,41]. Furthermore, the myeloperoxidase in human phagocytes generates *N*<sup>ε</sup>-(carboxymethyl)lysine at sites of inflammation and thus sustains cellular activation via RAGE [36]. AGE-RAGE interaction activates intracellular signal transduction pathways, such as the ERK-1/2 kinases [27], the p38 MAPK, the stress-activated protein kinase/c-jun N-terminal kinase (SAPK/JNK) kinases [30,42], rho-GTPases, phosphoinositide 3-kinases, JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway [17,43,44], and the NF- $\kappa$ B pathway [14] (Figure 1). In addition to activating the NF- $\kappa$ B pathway, triggering RAGE induces *de novo* p65 mRNA synthesis; this results in a growing pool of transcriptionally active NF- $\kappa$ Bp65, which appears to overwhelm endogenous autoregulatory feedback inhibitory loops [18]. However, no proximal signaling events directly downstream of the receptor have been discovered yet. Only direct binding of ERK to the intracellular domain of RAGE has been demonstrated thus far [27].

NF- $\kappa$ B is frequently present in sepsis, hyperglycemia, and oxidative stress. As already described, these conditions favor formation of 'advanced glycation endproducts', which can

trigger RAGE and subsequently lead to sustained inflammation. Intensive insulin therapy interferes with this pathway and therefore may explain, in part, why this treatment modality is effective.

### Relevance of RAGE-S100/calgranulin interaction

In addition to binding AGEs, RAGE binds proteins of the S100/calgranulin family, including S100-A12, also known as extracellular newly identified RAGE-binding protein (EN-RAGE), and S100B [18,26,45]. Most of the S100/calgranulins are encoded on human chromosome 1q21 and represent a family of multiple members that have important intracellular properties that are linked to homeostatic properties, such as calcium binding [46,47].

S100/calgranulin members such as S100A12 and S100B activate endothelial cells, macrophages, smooth muscle cells, and peripheral blood mononuclear cells (including T cells) via RAGE, thus triggering activation of signaling cascades and generation of cytokines and proinflammatory adhesion molecules [26,48]. In addition, S100P stimulates cell proliferation and survival via RAGE [49]. However, whereas nanomolar concentrations of S100B induce trophic effects in RAGE-expressing cells, micromolar concentrations promote apoptosis, likely through oxidant stress [50]. RAGE-S100 interactions have been implicated in inflammation, too, since binding of S100A12 from the S100/calgranulin family to RAGE in murine macrophages resulted in the elaboration of IL-1 $\beta$ , TNF- $\alpha$ , and IL-2 [26]. Furthermore, EN-RAGE induced intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression on endothelial cells. EN-RAGE also decreased NF- $\kappa$ B activation and proinflammatory cytokine expression by blocking RAGE engagement. Intravenous infusion of EN-RAGE into mice enhanced VCAM-1 expression in the lungs, which was abrogated by soluble RAGE (sRAGE), neutralizing anti-EN-RAGE or anti-RAGE monoclonal antibody and lending support to the *in vitro* findings. In addition, treatment with sRAGE in murine models *in vivo* strongly diminished delayed-type hypersensitivity (DTH) and inflammatory colitis [26].

The precise mechanism by which transcription and translation of S100/calgranulins are regulated is still poorly understood. However, there is evidence that these molecules are released by activated monocytes, promoting the presence of S100/calgranulin at sites of inflammation [26,47]. Interaction of these polypeptides and RAGE, therefore, might represent a proximal step in the cascade of events perpetuating inflammation. We [51] demonstrated that S100 species are increased in septic patients. However, we did not observe any significant difference between survivors and non-survivors.

### Amphoterin/HMGB1 as RAGE ligand

Amphoterin, one of the HMGB DNA-binding proteins (amphoterin is another name for HMGB1), also acts as a

signal-transducing ligand of RAGE. HMGB1, encoded on human chromosome 13q12-13, is a nuclear protein present in almost all eukaryotic cells. It stabilizes nucleosome function and acts as a transcription factor-like protein that regulates the expression of several genes [52]. The non-histone chromosomal protein HMGB1 not only has intracellular functions, but also may exist extracellularly and on the surface of cells, especially on migrating cells in neuronal development and tumors [43,53,54]. It is secreted as a cytokine by activated macrophages, mature dendritic cells, and natural killer cells in response to cell stimulation [55]. Active release is observed after acetylation in the nucleus, blocking re-entry into the nucleus by interacting with the nuclear-importer protein complex [56]. Thereafter, cytosolic HMGB1 migrates to cytoplasmic secretory vesicles, where it is released into the immunological synapse or into the extracellular space. Together with S100 [26], heat shock proteins [57], ATP [58], and uric acid [59], HMGB1 [60] is one of the main prototypes of the group of so-called damage-associated molecular pattern molecules: all of these molecules are released in response to infection or other inflammatory stimuli, especially during tissue damage (for example, by necrotic cells). Whereas HMGB1 is released from the nucleus, the other molecules are localized in the cytosol.

Cellular migration, invasion, and proliferation are enhanced when RAGE is engaged in tumor cells via HMGB1 [43]. A COOH-terminal motif in HMGB1 (amino acids 150 to 183) seems to be responsible for RAGE binding [61]. HMGB1 has a propagating role in inflammatory responses [6,62] and seems to be an important RAGE ligand in sepsis and acute inflammation [52,63,64]. Recent studies have shown that the monocyte-derived HMGB1 is a late-acting cytokine mediator of endotoxin lethality. In animal experiments, the time-dependent induction of HMGB1 release by macrophage cultures could be detected 8 hours after lipopolysaccharide (LPS) stimulation. Furthermore, endotoxemia leads to a systemic increase in HMGB1 levels in mice [6,64]. Systemic HMGB1 levels were also measured during endotoxemia in the serum of mice after injection of LPS. HMGB1 was first detected in serum after 8 hours and increased to a plateau from 16 to 32 hours after LPS stimulation [6]. Interestingly, this delay is one of the typical observations in patients with sepsis, when clinical signs appear several hours after the first infection-associated cytokines are detected in the bloodstream, and opens a therapeutic window. Examinations in healthy volunteers and septic patients showed (a) no HMGB1 in the serum of healthy humans, (b) dramatically increased HMGB1 levels in septic patients, and (c) markedly higher HMGB1 levels in non-survivors of septic shock than in patients who survived [6].

HMGB1 amplifies the cytokine cascade during systemic inflammation [62,64]. In addition, HMGB1 seems to be an autocrine/paracrine regulator of monocyte invasion, involving RAGE through the endothelium [65]. The proinflammatory

activity of HMGB1 is exerted by the B-box of the protein. When this HMGB1 B-box was added to enterocytic monolayers, intestinal permeability increased [66]. These effects were strongly diminished in the presence of an anti-RAGE antibody, suggesting a significant role of RAGE in HMGB1-initiated pathogenic events. Using bone marrow macrophages from RAGE knockout (KO) mice, Kokkola and colleagues [67] recently provided formal proof that a major component of HMGB1 action on cells is mediated via RAGE. As a response to HMGB1 stimulation, macrophages from RAGE KO mice produced significantly lower amounts of TNF, IL-1 $\beta$ , and IL-6. However, cytokine production was not totally abrogated in RAGE<sup>-/-</sup> macrophages, although there was a significant difference from that of wild-type macrophages. In addition, phosphorylation of p38, p44/42, or SAPK/JNK kinases was similar to that of wild-type macrophages and macrophages from IL-1 RI KO mice. These data clearly indicate that RAGE is a major receptor for HMGB1 but that HMGB1 also exerts important effects via different receptors such as Toll-like receptor (TLR)-2 and TLR-4 [68].

Park and colleagues [68] showed that interactions of HMGB1 with TLR-2 and TLR-4 represent early events after macrophage exposure to HMGB1. In contrast, they found only scant evidence of binding between HMGB1 and RAGE in their experiments. Recently, Yu and colleagues [69] demonstrated that neutralizing antibodies against TLR-4, but not TLR-2 or RAGE, dose-dependently attenuated HMGB1-induced IL-8 release in human whole blood. The interaction of HMGB1 and TLR-4 seems to be important in liver ischemia/reperfusion also [70]. Interestingly, the N-terminal domain of thrombomodulin sequesters HMGB1, preventing HMGB1 from binding to RAGE [63]. Furthermore, a soluble form of this N-terminal domain of thrombomodulin protected mice from LPS-induced lethal shock. This survival benefit was observed in both wild-type and RAGE KO mice. Importantly, lethality in RAGE KO mice was only 50%, as compared with 100% in wild-type mice, after administration of a high dose of LPS. Neutralizing HMGB1 reduced the lethality in RAGE KO mice to zero, further confirming that HMGB1 exerts its deleterious effects not only via RAGE.

*In vivo*, administration of blocking antibodies to HMGB1 resulted in an improved survival in rodents subjected to high-dose LPS [6]. In an animal model of LPS-induced acute lung injury (ALI), the administration of anti-HMGB1, notably both before and after endotoxin administration, reduced the typical signs of lung damage in acute inflammation, such as neutrophil accumulation and lung edema [71]. These results were supported by Ueno and colleagues [72], who found that concentrations of HMGB1 were increased in plasma and lung epithelial lining fluid of patients with ALI. Extracellular HMGB1 may play a key role in the pathogenesis of clinical and experimental ALI. However, it is also expressed in healthy airways, which suggests that it plays a physiologic role in the

lung as well [72]. Data have shown that anti-HMGB1 antibodies protect against sepsis in an animal model of cecal ligation and puncture (CLP), even when antibody administration is delayed by 24 hours [73]. These studies indicate that anti-HMGB1 antiserum may be a new, potential therapeutic target, as survival improved greatly in LPS- and CLP-treated mice [6,73]. The observation that administration of blocking antibodies to HMGB1 protected mice from lethal septicemia strongly suggests that the engagement of cell surface receptors such as RAGE by HMGB1 might play an important role in mediating the pathogenic effects of HMGB1 [6].

Wang and colleagues [74] demonstrated that nicotinic stimulation prevents activation of the NF- $\kappa$ B pathway and inhibits HMGB1 secretion through a specific nicotinic anti-inflammatory pathway. In conclusion, acetylcholine seems to be the first known inhibitor of HMGB1 released from human macrophages. Nevertheless, it has not yet been formally proven that direct interaction of HMGB1-RAGE contributes to sepsis lethality, and other interactions such as HMGB1-TLR-2 and -TLR-4 are also important.

### Potential clinical perspectives

Engagement of RAGE by its ligands results in sustained NF- $\kappa$ B activation [14] in all cell types studied so far, particularly mononuclear phagocytes and vascular endothelium [75]. Sustained cellular activation leads to cellular dysfunction and tissue destruction. When sRAGE used as a decoy, RAGE-neutralizing antibodies, and a dominant-negative receptor have been used, RAGE has been shown to be involved in different chronic disease models.

RAGE also has a critical role in acute inflammation. A resulting deleterious inflammatory response after ischemia/reperfusion of the liver has been associated with RAGE engagement in mice. The problem of ischemia/reperfusion is clinically relevant for liver transplantation or resection. In an animal model of total hepatic ischemia, blocking RAGE by administering sRAGE increased survival and caused fewer histological alterations in treated animals, which is in line with a decrease in RAGE-induced signaling and activation of transcription factors [76]. Furthermore, blocking RAGE significantly increased survival after massive liver resection [77].

We have clarified the role of RAGE in sepsis, DTH, and autoimmune encephalomyelitis (EAE) [75]. Several studies investigating the role of RAGE in inflammatory diseases used sRAGE to bind extracellular potential RAGE ligands [26,43,78]. However, not only does sRAGE scavenge the ligands and prevent them from interacting with RAGE, but these ligands may be able to engage further receptor types and transduce completely different signaling pathways. To overcome this problem, RAGE KO mice were studied. In the setting of EAE, which served as a model to test the role of RAGE in the adaptive immune response, no differences between wild-type and RAGE KO mice could be detected

[75]. Interestingly, RAGE transgenic mice showed significantly enhanced clinical EAE scores. In addition, RAGE KO mice and wild-type species developed the same inflammatory response in a second model (DTH). At first glance, these results seemed to be in contrast to previous data from DTH experiments with sRAGE, in which the inflammatory response in mice pretreated with sRAGE was lower [26]. The application of sRAGE in wild-type and RAGE KO mice, however, reduced the inflammatory response in mice in the DTH model [75]. These findings support the concept that the effects of sRAGE in DTH were not caused mainly by preventing ligand engagement of the cell-bound RAGE [75]. Thus, RAGE ligands exert their effects in these diseases through different receptors. Likely candidates to bind one or more of these ligands are known AGE receptors AGE-R1, AGE-R2, AGE-R3, and CD36 as well as newly identified AGE-binding proteins such as *N*-glycans, ezrin, and megalin [14].

In contrast to the minor role of RAGE in adaptive immunity, the most interesting finding was that RAGE KO mice were protected from lethal septic shock as compared with wild-type controls. In a CLP model, largely dependent on the innate immune response, RAGE KO mice were also better protected from lethal septic shock than were wild-type controls (80% versus 20%). To confirm the critical role of RAGE in sepsis and to exclude artifacts by gene deletion, RAGE KO mice were crossed into tie2-RAGE mice overexpressing RAGE in the vasculature. Mortality in these mice was similar to that in wild-type mice.

To test whether blocking of RAGE signaling pathways by sRAGE might be a therapeutic option, we injected sRAGE into wild-type mice, which resulted in an improved survival (40% versus 17%) compared with untreated control animals. NF- $\kappa$ B activation was more strongly induced in lungs of wild-type mice than in RAGE KO mice, suggesting a main contributing role in reducing mortality in RAGE KO mice and one that fits the high RAGE expression in the lung. In conclusion, these findings show that RAGE KO mice have, at least in part, a normal adaptive immune system. In contrast, the PRR RAGE displays a central role in the innate immune system, with an impact on perpetuation of the immune response [75].

In addition, we found that RAGE serves as a novel counter-receptor for the leukocyte  $\beta$ 2 integrin Mac-1 (CD11b/CD18) and (to a lesser extent) p150,95 (CD11c/CD18), being directly involved in leukocyte recruitment *in vitro* and *in vivo* [33]. This leukocyte recruitment via RAGE is enhanced in the presence of S100 proteins. Thus, RAGE acts as an endothelial adhesion receptor, promoting leukocyte recruitment and subsequent inflammation by direct binding of leukocytes, and enhances expression of VCAM-1 and ICAM-1 after engagement of RAGE [14,26]. These findings are consistent with the fact that histological examination showed

fewer inflammatory cells adherent to the peritoneum of RAGE KO mice after CLP as compared with that of wild-type mice [75]. Remarkably, RAGE<sup>-/-</sup> seems to have a moderate proinflammatory phenotype because C-reactive protein, basal NF- $\kappa$ B activation, and cytokine levels were slightly increased. In conclusion, the crucial role of RAGE in experimental sepsis not only provides strong evidence for its perpetuating role in the innate immune response, but may open further opportunities to develop novel approaches for treating septicemia.

A number of studies, including clinical investigations, have shown that genetic variants of RAGE might be of further interest [79,80]. These variants are found on coding/translational sequences as well as in the transcriptional regulatory elements. Hofmann and colleagues [81] found that the variant form of RAGE enhances binding and cytokine production compared with wild-type animals. What kind of cellular consequences these genetic polymorphisms have and what clinical relevance these polymorphisms will have remain to be determined in further studies.

## Conclusion

This review summarizes the current knowledge on RAGE, a new inflammation-perpetuating receptor, which plays a pivotal role in sepsis. It is involved in signal transduction from pathogen substrates to cell activation during the onset of inflammation and perpetuates the immune response. Targeting this receptor might attenuate hyperinflammation. Essentially, understanding the basic signal transduction of RAGE may offer new diagnostic and therapeutic options in patients with sepsis.

## Competing interests

The authors declare that they have no competing interests.

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