Senescence-dependent impact of anti-RAGE antibody on endotoxemic liver failure

Angela Kuhla · Mandy Hauke · Kai Sempert ·
Brigitte Vollmar · Dietmar Zechner

Received: 7 August 2012 / Accepted: 27 December 2012 / Published online: 15 January 2013
© American Aging Association 2013

Abstract Aging often restricts the capacity of the immune system. Endotoxemia is characterized by an immune response initiated by a group of pattern recognition receptors including the receptor for advanced glycation end products (RAGE). The aim of this study was to clarify to which extent RAGE and its signaling pathways such as the so called mitogen-activated protein kinase (MAPK) pathways can contribute to the perpetuation of inflammation in the aging organism. We used senescence-accelerated-prone (SAMP8) and senescence-accelerated-resistant (SAMR1) mice and studied them at the age of 2 and 6 months. Livers of SAMP8 mice had significantly higher malondialdehyde concentrations and a modest reduction of glyoxalase-I expression. Consequently, the abundance of highly modified advanced glycation end products was increased in the liver and plasma of these mice. After galactosamine/lipopolysaccharide-induced acute liver injury, significant activation of the MAPK cascade was observed in both mouse strains. Administration of an anti-RAGE antibody diminished p42/44-phosphorylation as well as tissue injury in SAMP8 mice, whereas the identical treatment in SAMR1 mice leads to a significant increase in p42/44-phosphorylation and intensified liver injury. This observation suggests that

dependent on the senescence of the organism, anti-RAGE antibody can have differential effects on the progression of endotoxemic liver failure.

Keywords Senescence · Oxidative stress · Advanced glycation end products · RAGE blockade · Inflammation

Abbreviations

PRRs	Pattern recognition receptors
RAGE	Receptor for advanced glycation end products
AGE	Advanced glycation end products
SAMP8	Senescence-accelerated-prone mouse
SAMR1	Senescence-accelerated-resistant mouse

Introduction

Endotoxemia and sepsis still represent an important clinical and economic challenge for intensive care units. Severe complications like multiorgan failure with high mortality and the lack of specific diagnostic tools continue to hamper the development of improved therapies of sepsis (Bopp et al. 2008). Fundamental questions regarding the cellular and molecular pathogenesis of experimental and clinical sepsis remain unresolved. However, it is known that the innate immune response during sepsis is initiated through a group of pattern recognition receptors (PRRs) including receptor for

A. Kuhla · M. Hauke · K. Sempert · B. Vollmar ·
D. Zechner (✉)
Institute for Experimental Surgery, University of Rostock,
Schillingallee 69a,
18057 Rostock, Germany
e-mail: dietmar.zechner@uni-rostock.de

advanced glycation end products (RAGE), which recognize pathogen-associated molecular patterns (PAMPs) (Bopp et al. 2008) and danger-associated molecular patterns (DAMPs). A typical PAMP is lipopolysaccharide (LPS), whereas advanced glycation end products (AGEs) are typical DAMPs. Both can bind to RAGE (Neeper et al. 1992; Yamamoto et al. 2011) which results in the rapid induction of pro-inflammatory intracellular signaling cascades including phosphorylation of mitogen-activated protein kinases (MAPKs) (Lin et al. 2009) and activation of NF- κ B signaling. As recently published, RAGE may form oligomers when bound to ligands, and the induced clustering of the receptor can influence subsequent signaling events (Srikrishna et al. 2010; Zong et al. 2010). However, a truncated isoform of RAGE, i.e., soluble RAGE (sRAGE) and an endogenous secretory RAGE, spanning the extracellular ligand-binding domain, have been reported to exert potent anti-inflammatory properties by acting as a decoy for RAGE ligands (Yonekura et al. 2003). Indeed, sRAGE has been shown to prevent or to reverse RAGE signals in experimental models of diabetic atherosclerosis (Park et al. 1998; Bucciarelli et al. 2002), wound healing (Goova et al. 2001), and amyloidosis (Hou et al. 2002). Not only sRAGE but also anti-murine RAGE IgG (F(ab)2 fragments) is capable of reducing an inflammatory response (Susa et al. 2009). Furthermore, Zhang et al. (2008) and our group (Kuhla et al. 2010) demonstrated that blockade of RAGE has potent anti-inflammatory properties, as indicated by an attenuation of endotoxin- or galactosamine/lipopolysaccharide-induced liver injury.

Due to the fact that AGEs are markedly elevated not only in inflamed liver tissue (Sebekova et al. 2002) but also recruit and accumulate upon increased oxidative stress which represents a main mechanism involved in biological aging (Ramasamy et al. 2005), we studied the contribution of AGE/RAGE in liver injury during aging. Therefore, we used the senescence-accelerated-prone mouse (SAMP8) and the corresponding control mouse, i.e., the senescence-accelerated-resistant mouse (SAMR1).

Material and methods

Animal model

Female SAMP8 and SAMR1 mice were provided by Harlan (Harlan Laboratories, Rosdorf, Germany) and

were used at the age of 2 and 6 months. Animals were kept on water and standard laboratory chow ad libitum. All animals received humane care according to the German legislation on protection of animals and the Guide for the Care and Use of Laboratory Animals (NIH publication 86-23 revised 1985).

Galactosamine/lipopolysaccharide-induced liver injury and experimental groups

For induction of acute liver failure, we used 6-month-old SAMR1 and SAMP8 mice ($n=14$) because these mice revealed higher oxidative stress than the 2-month-old mice. Mice were injected with galactosamine (G, 720 mg/kg BW ip; Sigma-Aldrich, Taufkirchen, Germany) and lipopolysaccharide (L, 10 μ g/kg BW ip, *Escherichia coli* serotype 0128:B12; Sigma-Aldrich) (Eipel et al. 2004; Kuhla et al. 2008) and were studied 6 h later. Time-matched sham-treated animals with application of equivalent volumes of 0.9 % saline were performed and designated as NaCl ($n=14$).

To verify the contribution of RAGE as a PRR in G/L-induced acute liver failure, additional animals were pretreated with mouse anti-RAGE antibody (abRAGE) (10 μ g ip, R&D Systems, Wiesbaden-Nordenstadt, Germany) or equivalent volumes of 0.9 % saline 12 h prior to exposure to G/L ($n=14$).

Sampling and assays

All animals were exsanguinated by puncture of the vena cava inferior for immediate separation of plasma, followed by harvesting of liver tissue. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured spectrophotometrically as indicators for hepatocellular disintegration and necrosis.

Measurement of plasma malondialdehyde (MDA), serving as an indicator of lipid peroxidation and oxidative stress, was performed using the MDA-586 method according to the manufacturer's instructions (OxisResearchTM, Portland, OR, USA). Cellular redox environment in plasma of SAMR1 and SAMP8 mice was assessed by measuring the ratio of glutathione (GSH) to glutathione disulfide (GSSG) by using the GSH/GSSG-412 assay according to the manufacturer's instructions (OxisResearchTM).

Harvested liver tissue was processed for isolation of proteins. For this purpose, liver tissue was homogenized in lysis buffer (10 mM Tris pH7.5, 10 mM NaCl,

Fig. 1 Analysis of plasma MDA (a) and representative Western blot as well as densitometric analysis of glyoxalase-I (21 kDa; b) in livers of 2- and 6-month-old SAMR1 ($n=14$) and SAMP8 mice ($n=14$). Signals were corrected with that of β -actin. Values are given as means $\pm$ SEM; ANOVA, post hoc pairwise comparison tests: * $p<0.05$ versus 2 months old mice of the identical genetic background; # $p<0.05$ versus SAMR1 mice

0.1 mM EDTA, 0.5 % Triton-X 100, 0.02 % NaN₃, and 0.2 mM PMSF, protease inhibitor cocktail), incubated for 30 min on ice, and centrifuged for 15 min at 10,000 $\times$ g. Protein contents were assayed by bicinchoninic acid method (Pierce, Biotechnology) with bovine serum albumin (BSA) (Pierce, Biotechnology) as standard.

Western blot analysis

On 12 % SDS gels, 40 μ g protein of liver tissue or plasma was separated and transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Eschborn, Germany). After blockade with 2 % BSA (Santa Cruz Biotechnology, Santa Cruz, CA, USA), membranes were incubated overnight at 4 °C with a mouse monoclonal anti-AGE (1:1,000; clone No. 6D12, TransGenic Inc., Kobe, Japan) which recognizes AGE-human serum albumin, AGE-BSA, AGE-hemoglobin, AGE-Collagen, AGE-Lys-derivatives (AGE- α -Tos-Lys, AGE- α -Tos-Lys-o-Me), AGE-monoamino carboxylic acids (AGE- β -alanine, AGE- γ -aminobutyric acid, AGE- ϵ -aminocaproic acid); a rabbit polyclonal anti-RAGE antibody (1:500; abcam, Cambridge, UK), which also detects sRAGE; a rabbit anti-p44/42 MAPK (Erk1/2) antibody (1:1,000; Cell Signaling, Danvers, USA); a rabbit anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody (1:1,000; Cell Signaling); and a rabbit polyclonal anti-cleaved caspase-3 antibody (1:1,000; Cell Signaling). As secondary antibody, a peroxidase-linked rabbit anti-mouse antibody (AGE; 1:10,000; Sigma) or a goat anti-rabbit antibody (RAGE/sRAGE, anti-p44/42 MAPK, anti-phospho-p44/42, 1:5,000; cleaved caspase-3, 1:2,000) was used. Protein expression was visualized by means of luminol-enhanced chemiluminescence (ECL plus; Amersham Pharmacia Biotech, Freiburg, Germany) and digitalized with ChemiDoc™ XRS System (Bio-Rad Laboratories, Munich, Germany). Signals were densitometrically assessed (Quantity One; Bio-

Rad Laboratories) and normalized to the β -actin signals (mouse monoclonal anti- β -actin antibody; 1:20,000; Sigma). The relative plasma AGE-levels were given per 40 μ g protein.

Histology of liver tissue

For hematoxylin & eosin (H&E) staining and immunohistochemical analysis of RAGE-positive liver cells, liver tissue was fixed in 4 % phosphate-buffered formalin for 2–3 days and then embedded in paraffin. From the paraffin-embedded tissue blocks, 4- μ m sections were put on glass slides and stained with H&E. For histomorphometric analysis of necrotic tissue images of 20 random low-power fields ($\times 10$ magnification, Olympus BX 51, Hamburg, Germany) were acquired with a Color View II FW camera (Color View, Munich, Germany) and evaluated by means of an image analysis system (Adobe Photoshop, Adobe Systems, Uxbridge, UK). The quotient of the focal necrosis surface to the total liver section area was assessed and given in percent. For immunohistochemical analysis, 4- μ m thin sections on poly-L-lysine-covered glass slides were treated with a goat polyclonal anti-RAGE antibody (R&D Systems) and a DAB chromogen Universal LSAB kit (System-HRP; DakoCytomation, Dako, Hamburg, Germany). The sections were counterstained with hemalaun and analyzed with a light microscope (Olympus BX51).

Statistical analysis

All data are expressed as means $\pm$ SEM. Statistical differences were determined using ANOVA, followed by post hoc pairwise comparison tests for analysis between either strains or stages of life. Data were considered significant if $p < 0.05$. Statistical analysis was performed using the SigmaStat software package (Jandel Scientific, San Rafael, CA, USA).

Results

Enhanced oxidative stress as a precondition for glycation

To evaluate the potential impact of age-related oxidative stress for glycation, we first determined the plasma MDA levels (Fig. 1a). While at the age of 2 months, both mouse strains showed comparable values of MDA; MDA values were found significantly increased in the SAMP8 mice at the age of 6 months when compared to age-matched SAMR1 mice (Fig. 1a). SAMP8 versus SAMR1 mice also revealed higher accumulation of GSSG and, thus, a slightly lower ratio of GSH to GSSG at the age of 2 and 6 months (2 months, 8.5 ± 1.2 vs. 6.9 ± 0.8 ; and 6 months, 10.1 ± 1.1 vs. 7.5 ± 1.1).

Furthermore, the expression of glyoxalase-I, an enzyme that is necessary for the detoxification of

Fig. 2 Representative Western blot and densitometric analysis of plasma AGE (a) and of hepatic AGEs (60 kDa; b) of 2- and 6-month-old SAMR1 ($n=14$) and SAMP8 mice ($n=14$). Hepatic AGE signals were corrected with that of

β -actin. Plasma AGE-levels were given per 40 μ g protein. Values are given as means $\pm$ SEM; ANOVA, post hoc pairwise comparison tests: # $p < 0.05$ versus SAMR1 mice

AGE precursor compounds, increased modestly with age in SAMR1 mice, whereas in SAMP8 mice, the expression was slightly reduced by 34 % in the 6-month-old SAMP8 mice compared to age-matched SAMR1 mice (Fig. 1b).

Senescent-dependent hepatic AGE and RAGE levels

To further examine whether increased oxidative stress and decreased glyoxalase-I expression aggravates the protein glycation process, we assessed the plasma AGE-levels and hepatic expression of AGE and RAGE. Plasma AGE levels were significantly increased in both 2- and 6-month-old SAMP8 mice when compared to age-matched SAMR1 mice (Fig. 2a). Western blot analysis of highly modified AGEs in the liver showed multiple bands ranging from 12 to >60 kDa (Fig. 2b, right panel). For analysis, we chose the 60-kDa band which represents modified human serum albumin. The senescent-accelerated phenotype in SAMP8 mice was associated with a significant increase of AGE as well as RAGE in liver tissue when compared with age-matched SAMR1 mice (Figs. 2b and 3a). In both mouse strains, immunohistochemical analysis revealed that RAGE was mainly expressed in endothelial cells, Kupffer cells, as well as in some hepatocytes (Fig. 3b).

Hepatic RAGE expression and activation of MAPK cascade upon G/L and abRAGE treatment

The treatment with G/L caused a modest increase in RAGE expression, whereas the expression of this receptor was slightly reduced by administration of abRAGE (Fig. 4a). However, both treatments had a strong impact on RAGE downstream signaling pathways, as indicated by significant changes of p42/44-phosphorylation (Fig. 4b). The treatment with G/L caused a significant increase in p42/44-phosphorylation in both mouse strains. Treatment with abRAGE before G/L challenge moderately reduced the phosphorylation of p42/44 in SAMP8 mice, whereas SAMR1 mice demonstrated a significantly increased activation of the MAPK cascade (Fig. 4b).

G/L-induced liver

G/L exposure caused significant liver injury, as given by increased AST and ALT levels in both mouse

Fig. 3 Analysis of RAGE expression by Western blot and immunohistochemistry Representative Western blot as well as densitometric analysis of RAGE (50 kDa; **a**) in livers of 2- and 6-month-old SAMR1 ($n=14$) and SAMP8 mice ($n=14$). Signals were corrected with that of β-actin. Representative light microscopic images of RAGE positive cells at a 400-fold magnification (**b**). Values are given as means ± SEM; ANOVA, post hoc pairwise comparison tests: [#] $p<0.05$ versus SAMR1 mice

strains when compared with NaCl-treated controls (Fig. 5a, b). Accordingly, H&E histopathology of G/L-exposed livers showed severe necrosis compared to livers of NaCl-exposed mice of both mouse strains (Fig. 5c, d). Necrotic tissue injury was characterized by a disruption of the general architecture, microvascular disintegration, and parenchymal cell death (Fig. 5d). Liver injury-associated transaminase release was more pronounced in SAMP8 than in SAMR1 mice (Fig. 5a and b). In line with this, quantitative analysis revealed that 79 % of the liver tissue showed characteristic necrotic injury in G/L-treated SAMP8 mice in contrast to 36 % in G/L-treated SAMR1 mice ($p=0.067$; Fig. 5c). The observed impact of G/L exposure and genotype on liver injury is consistent with the observed activation of the MAPK signaling cascade.

Modulation of liver damage by abRAGE

Application of abRAGE reduced AST activity slightly from ~1,400 to ~800 U/L (Fig. 5a) and ALT activity from ~1,000 to ~500 U/L in SAMP8 mice (Fig. 5b). Administration of this antibody also reduced the percentage of necrotic tissue and the cleaved caspase 3 level as marker of apoptotic cells in SAMP8 mice (Figs. 5c, d and 6). Surprisingly, administration of abRAGE in SAMR1 mice caused higher ALT and AST activities and aggravated both liver necrosis and apoptosis (Figs. 5a–d, and 6). Thus, only the SAMP8 mice benefited from the application of abRAGE, whereas treatment with the same antibody significantly aggravated liver injury in SAMR1 mice.

Discussion

The present study shows that senescence-induced AGE accumulation in SAMP8 mice enhances the

Fig. 4 Representative Western blot and densitometric analysis of RAGE (50 kDa; **a**), and of phospho-p42/44 (42/44 kDa; **b**) in livers of 6-month-old SAMR1 ($n=21$) and SAMP8 animals ($n=21$). Signals were corrected with that of β -actin. Animals were injected with either saline (NaCl; $n=14$), G/L (G: 720 mg/kg, L: 10 μ g/kg BW ip) (G/L; $n=14$), or additionally pretreated (–6 h) before G/L challenge with RAGE antibody (10 μ g ip; abRAGE + G/L; $n=14$). Analysis was performed 6 h after G/L challenge. Values are given as means $\pm$ SEM; ANOVA, post hoc pairwise comparison tests: $^{\#}p<0.05$ versus SAMR1 mice, $^{\$}p<0.05$ versus NaCl, $^{\&}p<0.05$ versus G/L

Fig. 5 Plasma AST (**a**) and ALT activities (**b**), histomorphometric analysis of necrosis (**c**), and representative H&E stained images of liver tissue (**d**, original magnification $\times 100$) in 6-month-old SAMR1 ($n=21$) and SAMP8 animals ($n=21$). Animals were injected with either saline (NaCl; $n=14$), G/L (G: 720 mg/kg, L: 10 μ g/kg BW ip) (G/L; $n=14$), or additionally

pretreated (-6 h) before G/L challenge with RAGE antibody (10 μ g ip; abRAGE + G/L; $n=14$). Analysis was performed 6 h after G/L challenge. Values are given as means $\pm$ SEM; ANOVA, post hoc pairwise comparison tests: § $p<0.05$ versus NaCl, & $p<0.05$ versus G/L

vulnerability to G/L-induced inflammatory liver injury and that administration of anti-RAGE antibodies can reduce liver injury in SAMP8 mice. Senescence also increased RAGE expression and the phosphorylation of p42/44 in the liver. These observations are consistent with other studies describing that activation of

RAGE causes a rapid induction of pro-inflammatory intracellular signaling cascades such as phosphorylation of p42/44 MAPKs (Bopp et al. 2008) leading to an increased RAGE expression and a perpetuation of inflammation (Bierhaus et al. 2005; Reynolds et al. 2008). Furthermore, it has been reported that the

Fig. 6 Representative Western blot and densitometric analysis of cleaved caspase-3 (17/19 kDa) in livers of 6-month-old SAMR1 ($n=21$) and SAMP8 animals ($n=21$). Signals were corrected with that of β -actin. Animals were injected with either saline (NaCl; $n=14$), G/L (G: 720 mg/kg, L: 10 μ g/kg BW ip) (G/L; $n=14$), or additionally pretreated (-6 h) before G/L challenge with RAGE antibody (10 μ g ip; abRAGE + G/L; $n=14$). Analysis was performed 6 h after G/L challenge. Values are given as means $\pm$ SEM; ANOVA, post hoc pairwise comparison tests: $^{\#}p<0.05$ versus SAMR1

expression of RAGE is upregulated in many organ lesions emphasizing the pathophysiological role of RAGE in inflammation (Basta et al. 2002).

It has been shown that sRAGE and F(ab)₂ fragments block ligand binding to RAGE and thereby can inhibit distinct pathophysiological mechanisms (Park et al. 1998; Taguchi et al. 2000). We evaluated if administration of an anti-RAGE antibody can inhibit G/L-induced liver failure in SAMP8 and SAMR1 mice. In fact, RAGE blockade by anti-RAGE antibodies inhibited the activation of MAPK and therefore decreased necrotic and apoptotic tissue damage in livers of SAMP8 mice. It is important to mention that in the present study, RAGE blockade did not completely restore hepatic tissue integrity. One explanation might be that not only RAGE but also TLRs participate as PRRs in

the innate immune response upon G/L exposure (Bierhaus et al. 2005). Surprisingly, the administration of identical antibodies in G/L-exposed SAMR1 mice lead to an activation of MAPK and increased liver injury. This seems to be a contradiction to the observation in SAMP8 mice, but might be explained by the following hypothesis (Fig. 7a–d). Since increased AGE accumulation was specifically detected in SAMP8 mice, but both mouse strains were treated with the newly identified RAGE agonist LPS (Yamamoto et al. 2011), the absence or presence of AGE might influence the impact of the anti-RAGE antibody. As recently published, RAGE forms oligomers when bound to ligands, and the induced clustering of the receptor can influence subsequent signaling events (Srikrishna et al. 2010; Zong et al. 2010). After treatment with LPS, this ligand binds to RAGE, but may not cause clustering of the receptor and thus induce a moderate activation of downstream signaling pathways and moderate tissue damage (Fig. 7a). When the bivalent anti-RAGE antibody is administered, the antibody does not compete for binding with the small molecule LPS, but might be able to bind two receptors and thus induces a moderate level of receptor clustering (Fig. 7b). The clustering of RAGE enhances the activation of downstream signaling pathways (Srikrishna et al. 2010; Zong et al. 2010) and increases tissue damage (Fig. 7b). SAMP8 mice have higher AGE concentration. Both ligands, LPS and AGE bind to RAGE (Neeper et al. 1992; Yamamoto et al. 2011). Due to multiple glycosylations of AGEs, AGEs support clustering of the receptor and induce together with LPS a strong activation of downstream signaling pathways and increased tissue damage (Fig. 7c). When the anti-RAGE antibody is administered, the antibody competes for binding with AGEs and blocks oligomerisation of the receptor (Fig. 7d). This reduces the activation of downstream signaling pathways and tissue damage.

The regulation of receptor clustering and signaling by antibodies has been widely observed. For example, binding of the antibody Apomab can cluster and thereby activate the proapoptotic receptor death receptor5 (Adams et al. 2008). Apomab and many other activating antibodies have also an

Fig. 7 Hypothesis on regulation of receptor clustering and signaling by abRAGE. In SAMR1 mice, treatment with LPS fails to cluster RAGE but induces a moderate level of signal transduction (**a**), whereas additional application of abRAGE induces clustering of the receptor and thus enhances signaling (**b**). In SAMP8, AGEs cluster the RAGE receptor and together with LPS induce a high level of signal transduction (**c**). The applied abRAGE competes with AGEs for binding to the receptor and thus reduces receptor clustering and signaling in SAMP8 mice (**d**)

effect in vivo and some of these antibodies are currently evaluated in clinical studies for specific therapies (Jin et al. 2008; Wiezorek et al. 2010). It has also been well-recognized that a protein that binds to a receptor can cause both activation and inhibition of distinct signal transduction pathways. For example, the linker protein Dap12 has been shown to potentiate as well as to attenuate the activation of leukocytes. It was suggested that DAP12 can have stimulatory or inhibitory effects dependent on the magnitude of receptor clustering (Turnbull and Colonna 2007).

Due to RAGE's fundamental role in the inflammatory response, it is widely expected that inhibition of this receptor might be a therapeutic target

for distinct diseases, such as Alzheimer, multiple sclerosis, pneumonia, and sepsis (Christaki et al. 2012; Deane et al. 2012; Ramasamy et al. 2009). The feasibility of this concept has been verified in several preclinical studies mainly using anti-RAGE antibodies (Huang et al. 2012; Lutterloh et al. 2007; Susa et al. 2009; van Zoelen et al. 2009). In addition, the clinical relevance of RAGE blockage for the treatment of Alzheimer disease was also evaluated by a phase II clinical study using the small inhibitory molecule PF-04494700 (ClinicalTrials.gov identifier: NCT00566397). Our study describes the ambivalent impact of an anti-RAGE antibody and thereby emphasizes how important it is to evaluate the effect of an inhibitor in multiple animal models.

Acknowledgments The authors cordially thank Berit Blendow, Doris Butzlaff, Dorothea Frenz, Maren Nerowski, and Eva Lorbeer-Rehfeld (Institute for Experimental Surgery, University of Rostock) for their excellent technical assistance.

References

- Adams C, Totpal K, Lawrence D, Marsters S, Pitti R, Yee S, Ross S, Deforge L, Koeppen H, Sagolla M, Compaan D, Lowman H, Hymowitz S, Ashkenazi A (2008) Structural and functional analysis of the interaction between the agonistic monoclonal antibody Apomab and the proapoptotic receptor DR5. *Cell Death Differ* 15:751–761
- Basta G, Lazzerini G, Massaro M, Simoncini T, Tanganelli P, Fu C, Kislinger T, Stern DM, Schmidt AM, De Caterina R (2002) Advanced glycation end products activate endothelium through signal-transduction receptor RAGE: a mechanism for amplification of inflammatory responses. *Circulation* 105:816–822
- Bierhaus A, Humpert PM, Morcos M, Wendt T, Chavakis T, Arnold B, Stern DM, Nawroth PP (2005) Understanding RAGE, the receptor for advanced glycation end products. *J Mol Med (Berl)* 83:876–886
- Bopp C, Bierhaus A, Hofer S, Bouchon A, Nawroth PP, Martin E, Weigand MA (2008) Bench-to-bedside review: the inflammation-perpetuating pattern-recognition receptor RAGE as a therapeutic target in sepsis. *Crit Care* 12:201
- Bucciarelli LG, Wendt T, Qu W, Lu Y, Lalla E, Rong LL, Goova MT, Moser B, Kislinger T, Lee DC, Kashyap Y, Stern DM, Schmidt AM (2002) RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. *Circulation* 106:2827–2835
- Christaki E, Lazaridis N, Opal SM (2012) Receptor for advanced glycation end products in bacterial infection: is there a role for immune modulation of receptor for advanced glycation end products in the treatment of sepsis? *Curr Opin Infect Dis* 25:304–311
- Deane R, Singh I, Sagare AP, Bell RD, Ross NT, LaRue B, Love R, Perry S, Paquette N, Deane RJ, Thiagarajan M, Zarcone T, Fritz G, Friedman AE, Miller BL, Zlokovic BV (2012) A multimodal RAGE-specific inhibitor reduces amyloid β -mediated brain disorder in a mouse model of Alzheimer disease. *J Clin Invest* 122:1377–1392
- Eipel C, Borden R, Nickels RM, Menger MD, Vollmar B (2004) Impact of leukocytes and platelets in mediating hepatocyte apoptosis in a rat model of systemic endotoxemia. *Am J Physiol Gastrointest Liver Physiol* 286:G769–G776
- Goova MT, Li J, Kislinger T, Qu W, Lu Y, Bucciarelli LG, Nowygrod S, Wolf BM, Caliste X, Yan SF, Stern DM, Schmidt AM (2001) Blockade of receptor for advanced glycation end-products restores effective wound healing in diabetic mice. *Am J Pathol* 159:513–525
- Hou FF, Jiang JP, Guo JQ, Wang GB, Zhang X, Stern DM, Schmidt AM, Owen WF Jr (2002) Receptor for advanced glycation end products on human synovial fibroblasts: role in the pathogenesis of dialysis-related amyloidosis. *J Am Soc Nephrol* 13:1296–1306
- Huang W, Liu Y, Li L, Zhang R, Liu W, Wu J, Mao E, Tang Y (2012) HMGB1 increases permeability of the endothelial cell monolayer via RAGE and Src family tyrosine kinase pathways. *Inflammation* 35:350–362
- Jin H, Yang R, Ross J, Fong S, Carano R, Totpal K, Lawrence D, Zheng Z, Koeppen H, Stern H, Schwall R, Ashkenazi A (2008) Cooperation of the agonistic DR5 antibody apomab with chemotherapy to inhibit orthotopic lung tumor growth and improve survival. *Clin Cancer Res* 14:7733–7740
- Kuhla A, Eipel C, Siebert N, Abshagen K, Menger MD, Vollmar B (2008) Hepatocellular apoptosis is mediated by TNF α -dependent Fas/FasLigand cytotoxicity in a murine model of acute liver failure. *Apoptosis* 13:1427–1438
- Kuhla A, Hettwer C, Menger MD, Vollmar B (2010) Oxidative stress-associated rise of hepatic protein glycation increases inflammatory liver injury in uncoupling protein-2 deficient mice. *Lab Invest* 90:1189–1198
- Lin L, Park S, Lakatta EG (2009) RAGE signaling in inflammation and arterial aging. *Front Biosci* 1:1403–1413
- Lutterloh EC, Opal SM, Pittman DD, Keith JC Jr, Tan XY, Clancy BM, Palmer H, Milarski K, Sun Y, Palardy JE, Parejo NA, Kessimian N (2007) Inhibition of the RAGE products increases survival in experimental models of severe sepsis and systemic infection. *Crit Care* 11:R122
- Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, Elliston K, Stern D, Shaw A (1992) Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 267:14998–15004
- Park L, Raman KG, Lee KJ, Lu Y, Ferran LJ Jr, Chow WS, Stern D, Schmidt AM (1998) Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat Med* 4:1025–1031
- Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM (2005) Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. *Glycobiology* 15:16R–28R
- Ramasamy R, Yan SF, Schmidt AM (2009) RAGE: therapeutic target and biomarker of the inflammatory response—the evidence mounts. *J Leukoc Biol* 86:505–512
- Reynolds PR, Kasteler SD, Cosio MG, Sturrock A, Huecksteadt T, Hoidal JR (2008) RAGE: developmental expression and positive feedback regulation by Egr-1 during cigarette smoke exposure in pulmonary epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 294:L1094–L1101
- Sebeková K, Kupcová V, Schinzel R, Heidland A (2002) Markedly elevated levels of plasma advanced glycation end products in patients with liver cirrhosis—amelioration by liver transplantation. *J Hepatol* 36:66–71
- Srikrishna G, Nayak J, Weigle B, Temme A, Foell D, Hazelwood L, Olsson A, Volkmann N, Hanein D, Freeze HH (2010) Carboxylated N-glycans on RAGE promote S100A12 binding and signaling. *J Cell Biochem* 110:645–659
- Susa Y, Masuda Y, Imaizumi H, Namiki A (2009) Neutralization of receptor for advanced glycation end-products and high mobility group box-1 attenuates septic diaphragm dysfunction in rats with peritonitis. *Crit Care Med* 37:2619–2624
- Taguchi A, Blood DC, del Toro G, Canet A, Lee DC, Qu W, Tanji N, Lu Y, Lalla E, Fu C, Hofmann MA, Kislinger T, Ingram M, Lu A, Tanaka H, Hori O, Ogawa S, Stern DM, Schmidt AM (2000) Blockade of RAGE-amphoterin

- signaling suppresses tumour growth and metastases. *Nature* 405:354–360
- Turnbull IR, Colonna M (2007) Activating and inhibitory functions of DAP12. *Nat Rev Immunol* 7:155–161
- van Zoelen MA, Schmidt AM, Florquin S, Meijers JC, de Beer R, de Vos AF, Nawroth PP, Bierhaus A, van der Poll T (2009) Receptor for advanced glycation end products facilitates host defense during *Escherichia coli*-induced abdominal sepsis in mice. *J Infect Dis* 200:765–773
- Wiezorek J, Holland P, Graves J (2010) Death receptor agonists as a targeted therapy for cancer. *Clin Cancer Res* 16:1701–1708
- Yamamoto Y, Harashima A, Saito H, Tsuneyama K, Munesue S, Motoyoshi S, Han D, Watanabe T, Asano M, Takasawa S, Okamoto H, Shimura S, Karasawa T, Yonekura H, Yamamoto H (2011) Septic shock is associated with receptor for advanced glycation end products ligation of LPS. *J Immunol* 186:3248–3257
- Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ, Li H, Yasui K, Takeuchi M, Makita Z, Takasawa S, Okamoto H, Watanabe T, Yamamoto H (2003) Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem J* 370:1097–1109
- Zhang H, Tasaka S, Shiraishi Y, Fukunaga K, Yamada W, Seki H, Ogawa Y, Miyamoto K, Nakano Y, Hasegawa N, Miyasho T, Maruyama I, Ishizaka A (2008) Role of soluble receptor for advanced glycation end products on endotoxin-induced lung injury. *Am J Respir Crit Care Med* 178:356–362
- Zong H, Madden A, Ward M, Mooney MH, Elliott CT, Stitt AW (2010) Homodimerization is essential for the receptor for advanced glycation end products (RAGE)-mediated signal transduction. *J Biol Chem* 285:23137–23146