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Commentary

Soluble RAGE: Therapy and biomarker in unraveling the RAGE axis in chronic disease and aging

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ABSTRACT

The multi-ligand Receptor for Advanced Glycation Endproducts (RAGE) is implicated in the pathogenesis and progression of chronic diseases such as diabetes and immune/inflammatory disorders. Recent studies are uncovering the precise mechanisms by which distinct RAGE ligands bind the extracellular (soluble) domain of the receptor at the V-, C1- and/or C2-immunoglobulin like domains. Experiments using soluble RAGE in animals as a ligand decoy have illustrated largely beneficial effects in reducing vascular and inflammatory stress and, thereby, preventing long-term tissue damage in models of diabetes and immune/inflammatory disorders. Measurement of soluble RAGE levels in the human, both "total" soluble RAGE and a splice variant-derived product known as endogenous secretory or esRAGE, holds promise for the identification of potential therapeutic targets and/or biomarkers of RAGE activity in disease. In this article, we review the evidence from the rodent to the human implicating RAGE in the diverse disease states in which its ligands accumulate.

1. Introduction

The Receptor for Advanced Glycation Endproducts (RAGE) is a ubiquitous receptor present on epithelial, neuronal, vascular and inflammatory cells, usually expressed at low levels in homeostasis and to increased degrees at sites of stress or injury [1]. A notable exception is the lung, in which relatively high basal levels of RAGE expression have been identified relative to other tissues [2].

The multi-ligand nature of RAGE places this receptor in the midst of chronic disease states, such as diabetes, aging, inflammation, neurodegeneration, amyloidoses, and tumors. Experimental evidence using pharmacological antagonists of RAGE and genetically modified mice suggests that blocking RAGE halts progression of chronic inflammation and cell stress. In animal models, administration of the ligand-binding extracellular domain of RAGE, soluble RAGE, suppresses immediate and chronic inflammatory stresses thereby thwarting tissue injury [1].

Although there is one gene encoding RAGE, *AGER* [2], it is now clear that in human and murine systems, multiple splice variants of this gene may be detected. One such variant in the human subject encodes for "endogenous secretory (es) RAGE," also known as RAGE_v1 according to the Human Gene Nomenclature Committee, a naturally occurring soluble form of the receptor lacking the membrane and intracellular domains [3,4]. Although forms of soluble RAGE are readily detected and quantified in human plasma/serum by ELISA, detectable amounts of soluble RAGE have not been identified to date in the murine circulation [5]. In human subjects, studies suggest that the absolute levels of soluble RAGE may correlate with chronic disease states and their severity, and that soluble RAGE levels may be mutable consequent to therapeutic interventions [6].

In this review, we will discuss the state of RAGE, soluble RAGE and implications for the therapy and biomarking of chronic diseases and innate aging.

2. RAGE: extracellular domain and ligand binding

RAGE is a member of the immunoglobulin superfamily. The extracellular domain of RAGE is composed of one variable (V)-type immunoglobulin (Ig) domain followed by two distinct constant (C)-type domains [2]. Recent evidence deduced from various biochemical techniques suggests that the V and C1 RAGE domains are not independent of each other but that they form an integrated structural unit required for at least some of the ligand binding properties [7]. Further, it was suggested by that work that the C2 domain of RAGE is attached to the VC1 unit via a flexible linker but that it functions as a fully independent unit. Experimental evidence indicates that the various ligands of RAGE may preferentially

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Table 1
The families of RAGE ligands. This table details specific members of three of the classes of RAGE ligands, advanced glycation endproducts (AGEs), advanced oxidation protein products (AOPPs), selected members of the S100/calgranulin family, HMGB1, amyloid-β peptide and their likely sites of binding to the extracellular domain of RAGE and selected references for the reader.

Ligand	Ligand family	Extracellular domain(s) of RAGE/interaction	Selected references
CML-AGE (carboxy-methyl lysine)	AGEs	V-type domain	[8]
Pronyl glycine	AGEs	Not studied	[9]
AGE peptides	AGEs	V-type domain	[10,12]
AOPPs advanced oxidation protein products	AOPPs	Not studied	[13]
HMGB1	HMGB1	V-type domain	[14,16]
S100A12	S100/calgranulins	V- and C1-type domains	[18,19]
S100B	S100/calgranulins	V-type domain	[18,19]
S100A6	S100/calgranulins	C2-type domain	[18]
Amyloid-β oligomers	Amyloid-β and β-sheet fibrils	V-type domain	[20–22]
Amyloid-β aggregates	Amyloid-β and β-sheet fibrils	C1-type domain	[20–22]

interact with one or more of these individual domains (Table 1 and Fig. 1).

Of these diverse ligands of RAGE, the first to be identified were the advanced glycation endproducts or AGEs, the products of nonenzymatic glycation and oxidation of proteins and lipids. AGEs form in varied settings from diabetes, aging, inflammation to neurodegeneration [1]. AGEs are highly heterogeneous; of the known AGEs, carboxy-methyl lysine AGEs (CML-AGE) and possibly pronyl glycine (a food-derived AGE) are specific AGEs that bind RAGE [8,9]. Based on available evidence, it is likely that the AGEs and specific AGE peptides bind to RAGE via its V-type Ig domain [10–12]. In addition to AGEs, advanced oxidation protein products (AOPPs) also bind RAGE and trigger signal transduction [13]. The specific sites to which they bind the extracellular RAGE domain have yet to be identified.

Amphoterin or high mobility group box-1 (HMGB1) protein binds to RAGE. These ligands are involved in a range of cell stress mechanisms, such as induction of cellular migration, neurite outgrowth and differentiation, and up-regulation of the inflammatory response, at least in part via RAGE [14–17]. Available evidence suggests that amphoterin/HGMB1 binds to the V-type RAGE domain [14].

The S100/calgranulin family of polypeptides is a diverse family of molecules that contains at least 21 members. S100/calgranulins are calcium-binding proteins which generally function as dimers; this family of molecules has a wide range of functions, both inside and outside the cell [18]. Members of the family that bind RAGE were first identified as S100A12 and S100B [19], but subsequently others including S100A1, S100A2, S100A5, S100A6, S100A4, S100A7, S100A8A9, S100A11, S100A13 and S100P have been documented to bind RAGE [18]. Of these S100/calgranulins known to bind RAGE, S100B, S100A1, S100A2, S100A5, S100A6, S100A12, interact with the V-type domain; S100A12 also interacts with C1 domain; and S100A6 interacts with the C2-type Ig domain [18].

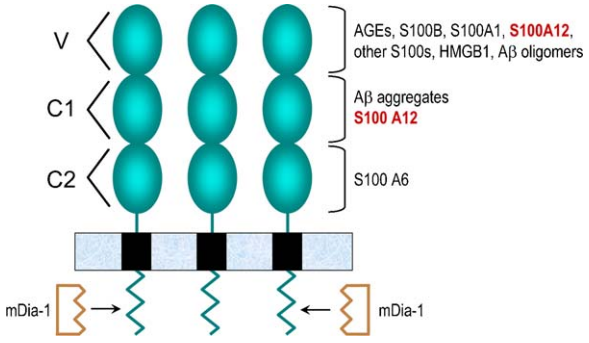

Fig. 1. RAGE domains and its ligands. RAGE is a member of the immunoglobulin superfamily. The extracellular domain of RAGE is composed of one V-type followed by two C-type immunoglobulin domains. Recent evidence has suggested that although the majority of ligands tested to date bind to the V-type Ig domain, the C1- and C2-domains also contribute to the binding of certain ligands. Following the extracellular domain is a single, transmembrane spanning domain followed by a short, charged cytoplasmic domain. Recent evidence suggests that the binding of the RAGE cytoplasmic domain to mDia-1 (diaphanous-1) is important in the transduction of signal stimulated by RAGE ligand binding to RAGE.

Amyloid-β peptide and β-sheet fibrils are ligands of RAGE implicated in Alzheimer's disease and amyloidoses [20]. Whereas Aβ aggregates bind the C1-Ig-type RAGE domain, Aβ oligomers (believed to be the more pathogenic species) bind to the V-type domain of RAGE [21]. These findings suggest the possibility that the more aggregated ligand species are those most apt to bind to RAGE and stimulate signaling, particularly consequent to interaction with the V-type Ig domain. In fact, our recent work suggested that soluble S100B and soluble Aβ were less able to activate NF-κB and increase Vascular Endothelial Growth Factor (VEGF) expression in cultured cells compared to oligomeric forms of these species [22].

3. RAGE: intracellular domain and signal transduction

The cytoplasmic domain of RAGE is linked to the extracellular domain by a single transmembrane domain. The intracellular domain is short (<50 amino acids) and highly charged [2]. Studies in cultured cells and *in vivo* using tissue-targeted transgenic mice reveal that the cytoplasmic domain of RAGE is essential for RAGE ligand-triggered signal transduction, as deletion of the cytoplasmic domain of RAGE blocks ligands from inducing signaling and modulating gene expression. However, exposure of cells bearing the RAGE cytoplasmic domain-deleted RAGE does not affect the actions of non-RAGE ligands, such as TNF-α or platelet-derived growth factor [19,23]. How the cytoplasmic domain exerts such a strong influence on RAGE action has been unclear until recently.

As the RAGE cytoplasmic domain lacks endogenous tyrosine kinase activity, one mechanism by which it exerts its effects on signal transduction and modulation of gene expression is via docking with intracellular molecules to bridge phosphorylation/activation events required to initiate signaling. Ishihara et al. suggested that the cytoplasmic domain of RAGE (particularly its component most close to the transmembrane domain) interacted directly with ERK (extracellular signal-regulated kinase) [24]. However, no functional consequences of the proposed interaction were shown in that study, hence, it is possible that the interaction is not required for RAGE ligand-stimulated signaling.

In a distinct study, we used a yeast-two-hybrid assay and probed a lung library (tissue type from which RAGE and four of its ligand families were identified) with a construct encoding the human RAGE cytoplasmic domain. Our findings revealed that the cytoplasmic domain of RAGE binds diaphanous-1 (mDia, or mammalian diaphanous-1) and that the interaction was direct [25]. Beyond binding, however, mDia-1 is required for RAGE ligand action based on the following evidence. First, RNA interference

(RNAi)-mediated knock down of mDia-1 in RAGE-expressing cells suppressed RAGE ligand-stimulated cellular migration, but had no effect on a general stimulus, fetal bovine serum. Second, the activation of two central signaling pathways important for transducing the effects of RAGE ligands on cellular migration, cdc42 and rac-1, was reduced in cells after knock down of mDia-1 compared to scramble small interference (si)RNAs [25]. As mDia-1 is important for both signal transduction and for the actions of certain Rho GTPases, it is conceivable that at least certain of RAGEs signaling effects are dependent on mDia-1. Studies are in progress to clarify the specific settings in which RAGE signaling is dependent on this cytoplasmic domain binding partner.

4. Soluble RAGE: pharmacology and findings in animal models

The soluble extracellular domain of RAGE has been prepared for experimentation in *in vitro* and *in vivo* model systems. *In vitro*, soluble RAGE added to cultured cells blocked the effects of RAGE ligands on expression of inflammatory markers, cellular migration and proliferation, and cytotoxicity [23,26–28]. These promising studies set the stage for rigorous testing of these concepts *in vivo*. Thus, the key test of the ligand decoy capacities of soluble RAGE was established in animal models of disease. In the sections to follow, we review the evidence that in RAGE ligand-enriched environments, administration of soluble RAGE exerted protection against the adverse complications of diabetes and inflammation.

4.1. Diabetes

The micro- and macrovascular complications of diabetes may be modeled in animals such as mice and rats. Although there are acknowledged limitations to rodent models, they nevertheless provided a feasible means to test chronic administration of soluble RAGE *in vivo* on fundamental endpoints in atherosclerosis, early retinal injury, nephropathy, cardiac dysfunction, and neuropathy, as examples [29,30].

Accelerated atherosclerosis is a leading cause of morbidity and mortality in diabetes [31,32]. The mechanisms underlying accelerated diabetic atherosclerosis have been studied in mouse models. Induction of type 1 or type 2 diabetes in atherosclerosis-vulnerable apolipoprotein E (apoE) null mice resulted in increased atherosclerotic lesion area and vascular inflammation [33,34]. RAGE and its ligands were expressed in the atherosclerotic plaques and administration of soluble RAGE to diabetic mice resulted in a highly significant reduction in atherosclerosis in parallel with reduced expression of adhesion molecules, chemokines, cytokines and tissue factor in the aortas of soluble RAGE-treated mice versus vehicle. Importantly, as RAGE ligands such as oxidized low density lipoprotein (LDL)-bearing AGE epitopes accumulate in non-diabetic atherosclerosis-prone vasculature as well, but to lesser degrees than that observed in diabetes, soluble RAGE also reduced atherosclerosis in non-diabetic apoE null mice [34,35]. In diabetic and non-diabetic mice, although soluble RAGE exerted significant effects on reduction of atherosclerosis, this was not accompanied by changes in glucose, cholesterol or triglyceride levels. Evidence supporting that the chief target of soluble RAGE was RAGE itself was established in genetically modified apoE null mice bred into the homozygous RAGE null background. In both the non-diabetic and diabetic states, apoE null mice devoid of RAGE displayed significant reduction in atherosclerosis in parallel with suppression of vascular inflammation and endothelial dysfunction [26,36]. Recently, in a distinct model of atherosclerosis (LDL receptor null mice), breeding into the RAGE null background also reduced atherosclerosis in non-diabetic mice [37].

Diabetic retinopathy is a significant complication in diabetes given the increased risk for severe visual loss or blindness. Although mouse and rat models of diabetes do not develop frank proliferative retinopathy with microaneurysms, they do develop early vascular and retinal neuronal dysfunction that is quantifiable using both pathological and electrophysiological endpoints. In order to enhance potential RAGE-dependent actions in the development of early retinopathy in animal models, two strategies to date have been implemented. In the first, type 2 diabetic (db/db) mice were bred into the apoE null background based on the hypothesis that induction of hyperlipidemia (and, thereby, predilection for the development of macrovascular disease in diabetes) would accelerate vascular dysfunction. Compared to db/db mice or apoE null mice, the apoE null/db/db mice displayed increased acellular capillaries and pericyte ghosts in retinal digests, and early inner retinal neuronal dysfunction based on prolonged latencies of oscillatory potentials and the b-wave in electroretinograms [38]. AGEs and RAGE expression were greatest in the mice which were both hyperglycemic and hyperlipemic. To test the role of RAGE, soluble RAGE was administered by intraperitoneal injection. Compared to vehicle treatment, treatment with soluble RAGE attenuated neuronal dysfunction (as assessed by electroretinograms) and suppressed the development of acellular capillaries and pericyte ghosts [38]. These data were the first to show in an animal model that soluble RAGE might beneficially impact early vascular and neuronal dysfunction in the diabetic retina.

In other studies, a transgenic approach was utilized to augment RAGE expression in endothelial cells. Kaji et al. developed a human RAGE-expressing transgenic mouse under control of the flk-1 promoter. When these mice were made type 1 diabetic with streptozotocin, a significant induction of retinal RAGE expression was noted in retinal vessels (greater than that in wild-type mice with or without diabetes and in human RAGE-transgenic mice without diabetes) [39]. Compared to wild-type diabetic mice, diabetic transgenic mice displayed greater degrees of blood-retinal barrier breakdown, leukostasis and retinal expression of VEGF and Intercellular Adhesion Molecule-1 versus all other groups. Administration of soluble RAGE for 14 consecutive days to diabetic wild-type and RAGE-transgenic mice resulted in highly significant reductions in blood-retinal barrier breakdown, leukostasis and retinal expression of ICAM-1 [39]. Taken together, these data provide support for beneficial effects of soluble RAGE in prevention of early vascular and neuro-retinal abnormalities that characterize diabetes.

Diabetic nephropathy is a leading cause of end-stage renal failure and attempts to identify optimal prevention and treatment strategies are of urgent importance. Although angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are a mainstay of therapy in subjects with established albuminuria, recent studies have reported that treatment of type 1 diabetic subjects with these agents prior to development of overt renal complications has no substantive effect on preventing the primary endpoint, defined as change in the fraction of glomerular volume occupied by mesangium in kidney-biopsy specimens [40]. These studies underscore the importance of developing novel strategies for the prevention of diabetic kidney disease. RAGE is expressed in the kidney at low levels in health and to accelerated degrees in diabetes, particularly in the glomerular epithelial cell or podocyte [41]. Similar findings were made in type 2 diabetic (db/db) mouse kidney [42]. To test the role of RAGE, soluble RAGE was administered to db/db mice from the onset of diabetes to age 27 weeks. Compared to vehicle-treated mice, soluble RAGE-treated animals displayed diminished albuminuria, mesangial expansion and thickening of the glomerular basement membrane [42]. Further, podocyte expression of VEGF was lower in soluble RAGE-treated versus vehicle-treated mice. Additional studies using anti-RAGE antibodies and RAGE null mice illustrated the beneficial

effects of RAGE blockade or deletion on diabetes-associated renal dysfunction [42–45].

In diabetes, the development of myocardial dysfunction is in part independent of accelerated atherosclerosis and associated ischemic events but presents a major risk to subjects for the development of heart failure. RAGE is expressed in the diabetic heart in multiple cell types, such as vascular, infiltrating inflammatory cells and cardiomyocytes [30]. To test if RAGE and its ligands contributed to cardiac injury consequent to ischemia/reperfusion, studies were performed in both rat and mouse models in the absence and presence of diabetes. Administration of soluble RAGE to animals protected against *ex vivo*-induced ischemia/reperfusion injury in the isolated perfused heart [46] and *in vivo*, transient ischemia/reperfusion-induced ligation of the left anterior descending coronary artery resulted in reduced infarct volume and echocardiographic evidence of left ventricular dysfunction which was largely prevented by treatment with soluble RAGE [47]. Of note, studies in RAGE null mice hearts supported that deletion of RAGE exerted similar and marked degrees of protection in ischemia/reperfusion injury to the heart and implicated both AGE and HMGB1 ligands in the RAGE-dependent cardiac injury [46–49]. Interestingly, S100 ligands (S100A8 and S100A9) of RAGE mediate depressed cardiac contractility in the mouse heart via RAGE secondary to endotoxemia [50]. Taken together, administration of soluble RAGE may represent a specific therapy for diabetes and endotoxin-mediated cardiac dysfunction by virtue of its ability to trap pathogenic ligands.

Lastly, diabetic neuropathy is one of the long-term painful and debilitating complications of diabetes. RAGE is expressed in human diabetic peripheral nerve and its expression co-localized with that of activated NF-κB p65 and Interleukin-6 (IL-6) in sural nerve biopsies [51]. In diabetic mice, chronic administration of soluble RAGE largely prevented functional sensory deficits associated with long-term hyperglycemia [51]. It is important to note that RAGE-dependent action in the peripheral nervous system is complex, in that in non-diabetic mice, administration of soluble RAGE consequent to acute crush injury to the sciatic nerve results in impaired regeneration, as assessed by impaired motor and sensory conduction velocities and decreased myelinated fiber densities versus vehicle treatment [52].

Taken together, in rodent models of diabetes, chronic administration of soluble RAGE protects against macro- and microvascular complications in the great vessels, heart, kidney, retina and peripheral nerve. In all settings, to date, distinct studies in RAGE null mice supported that a chief target of soluble RAGE is RAGE itself. In chronic diabetes, administration of soluble RAGE (or RAGE deletion) does not affect levels of glucose or lipids, suggesting that RAGE action is downstream of the primary insults in diabetes and vascular disease, thereby identifying soluble RAGE and RAGE antagonists as attractive targets for complementary therapies in diabetes.

4.2. Immune/inflammatory disease

The discovery of non-AGE ligands of RAGE including the S100/calgranulins and HMGB1 opened the doors to fundamental insights into the natural functions of RAGE. When S100(A12) was first identified as a RAGE ligand, the signature of this molecule and its family members implicated RAGE in inflammation, even in the absence of diabetes [19]. In the first test of these concepts, non-diabetic mice were subjected to delayed type hypersensitivity (DTH) reactions using methylated bovine serum albumin to systemically sensitize and locally challenge mice in the footpad. Administration of soluble RAGE to these animals resulted in dose-dependent reductions in inflammation "scores" as measured by physical assessment of redness and swelling and by histological evaluation of edema and inflammatory cell infiltration into the challenged footpad. In parallel, nuclear extracts from soluble RAGE-treated DTH mice revealed decreased activation of NF-κB [19]. In other studies, mice deficient in IL-10 which develop spontaneous inflammatory bowel disease demonstrated reduced intestinal inflammation and cytokine production upon treatment with soluble RAGE versus vehicle [19].

Based on these findings, soluble RAGE has been tested in other model settings of immune/inflammatory disease such as human rheumatoid arthritis (RA). RAGE is highly expressed in joint tissue macrophages in RA [53]. In addition to tissue localization of RAGE to human RA synovium, a polymorphism of the *RAGE* (*AGER*) gene, the glycine82serine or G82S, has been found to be in linkage disequilibrium with HLA-DR4 commonly linked to RA and type 1 diabetes [54]. Expression of the G82S allele in cultured cells increased S100B ligand affinity and increased production of cytokines and matrix metalloproteinases (MMPs) compared to the wild-type allele [55]. To address these concepts in an animal model, bovine collagen type II-induced arthritis was induced in mice. Compared to vehicle-treated mice, soluble RAGE administration reduced joint swelling and erythema, and at the biochemical level suppressed cytokine and MMP activation [55]. Of note, in recent studies, roles for RAGE in osteoarthritis have been suggested by *in vitro* studies. In cultured chondrocytes, RAGE-induced S100A11 collagen X production consequent to p38 MAPK signaling was illustrated by suppression of these processes in the presence of soluble RAGE (and blocking RAGE antibodies) [56].

In addition to macrophages, RAGE is expressed in T and B lymphocytes and dendritic cells [57,58], thereby suggesting roles for RAGE in adaptive immune responses. Chen et al. transferred diabetogenic splenocytes from NOD mice into NOD/*scid* recipients and found that administration of soluble RAGE prolonged the time to development of diabetes compared to vehicle treatment [57]. In the soluble RAGE-treated murine islets, levels of IL-1β and TNF-α were significantly reduced, together with increased islet expression of IL-10 and transforming growth factor-β. Although the precise role of RAGE in T lymphocyte biology is under active study at this time, evidence using soluble RAGE and RAGE null mice points to roles for this receptor in T lymphocyte priming and in early events leading to Th1 differentiation of T lymphocytes [59,60].

A potential caveat deduced from these studies reflects the possible contributions of RAGE to essential host defense mechanisms in response to massive injury, sepsis and infections. To date, the majority of the evidence using soluble RAGE (and other anti-RAGE agents and RAGE null mice) suggests that RAGE action drives exaggerated inflammation in massive injury, as administration of soluble RAGE enhances survival in murine models of massive liver injury [58] and hemorrhagic shock [61]. In cecal ligation and puncture-mediated sepsis in mice, administration of soluble RAGE or RAGE deletion improves survival with no apparent RAGE-dependent differences in the ability to clear pathogenic bacteria [62,63]. In fact, in other studies, RAGE action was found to be deleterious to the host in influenza A viral pneumonia and in pneumococcal pneumonia [64,65]. To date, one published study suggests important roles for RAGE in bacterial defense. When abdominal sepsis was induced in mice via direct intraperitoneal injection of *Escherichia coli*, RAGE deletion or anti-RAGE IgGs resulted in increased bacterial outgrowth in the peritoneal cavity and systemically, in parallel with increased liver damage, cytokine release and activation of coagulation [66]. The reasons for the discordant results among these studies have yet to be elucidated.

Taken together, in animal models of diabetes and inflammatory responses, blockade of ligand-RAGE interaction using soluble RAGE

is effective in suppressing vascular and inflammatory cell activation, thereby reducing the manifestations of diabetes and its complications and immune diseases. It is important to note, however, that to date no studies have reported on the treatment of large animals, such as dogs, rabbits, cows, sheep, swine or non-human primates with soluble RAGE or other forms of RAGE antagonism. Although this was difficult to accomplish with soluble RAGE, now that small molecule antagonists are available (60), critical testing of the RAGE hypothesis in large animal models may be feasible.

Interestingly, mice and rats do not display measurable quantities of soluble RAGE. Yet, the same is not true in human subjects. Multiple studies are appearing in the literature linking levels of soluble RAGE in human plasma/serum to disease. In the section to follow, we will review these findings and their implications in selected disorders.

5. Soluble RAGE in human circulation – mediator or biomarker or both?

As discussed above, there are two known forms of soluble RAGE in human plasma; the first is generated from the actions of ADAM10 (A Disintegrin And Metallopeptidase 10) and MMPs presumably on cell surface RAGE [67,68], and the second is an alternatively spliced pre-mRNA form of the receptor known as endogenous secretory or esRAGE (or RAGE_v1) [3,4] (Fig. 2). The latter contains a novel stretch of amino acids in the C2-Ig domain, thereby presenting a unique sequence for the generation of esRAGE-specific antibodies [4]. Since the first description of detectable levels of soluble RAGE in human plasma, a number of publications have appeared linking levels of soluble or esRAGE to the status of disease state and/or severity in multiple disorders. In this review, we will discuss findings in diabetes and inflammation, as well as the intriguing "response" of soluble RAGE levels to therapeutic interventions.

5.1. Diabetes and soluble RAGE

Multiple studies in subjects with type 1 or type 2 diabetes have been published illustrating associations between levels of soluble forms of the receptor and the status of diabetes. In a study of 30 European patients with type 2 diabetes, levels of soluble RAGE were significantly lower than controls and correlated with the presence of microvascular complications and elevated levels of CML-AGE ligand in plasma [69]. Particular attention has been paid to the relationship between soluble RAGE levels and evidence of coronary artery disease in diabetes. In a Japanese population, levels of soluble RAGE were significantly higher in subjects with type 2 diabetes than in non-diabetic subjects and were positively associated with the presence of coronary artery disease [70]. In another group of Japanese subjects with type 1 diabetes, levels of esRAGE but not soluble RAGE were inversely correlated with carotid intima-media thickness, leading the authors to speculate that esRAGE and soluble RAGE are distinct markers [71]. Yet, in a follow-up study, these same authors showed that levels of esRAGE and soluble RAGE negatively correlated with progression of intima-media thickness, independently of conventional risk factors [72]. Studies in the EURODIAB Prospective Complications Study examined type 1 diabetes subjects and found that levels of soluble RAGE tended to be higher in subjects with cardiovascular disease than without, and that these associations may be partly linked to endothelial and renal dysfunction and low-grade inflammation [73]. In fact, in that study, the authors reported that as the severity of albuminuria increased, so did levels of soluble RAGE.

Other studies in diabetes have attempted to link soluble RAGE levels to the degree of inflammation evident in the subjects. Nakamura et al. showed that circulating levels of AGEs and soluble RAGE were independent determinants of the level of MCP-1 in type 2 diabetes [74]. This same author group also showed that serum levels of soluble RAGE were positively correlated with levels of

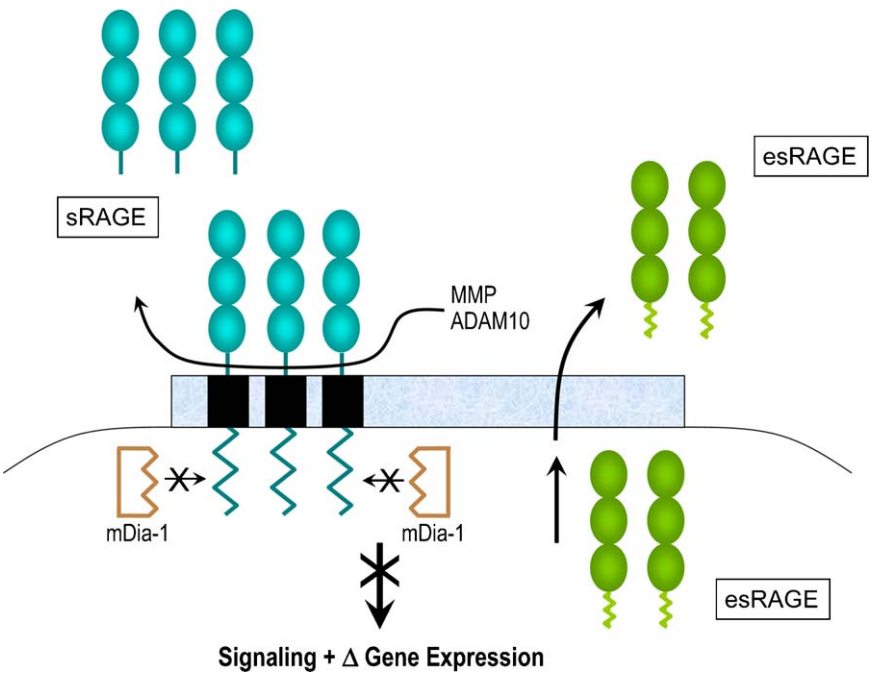

Fig. 2. Breaking the cycle of RAGE signaling – soluble RAGE(s) and blocking RAGE – mDia-1 interaction. The binding of ligands to RAGE stimulates cell signaling pathways at least in part through mDia-1. In order to block the adverse consequences of ligand-RAGE binding, maximizing levels of soluble RAGE or esRAGE may be a key strategy. Soluble RAGE may be formed by the action of MMPs or ADAM10 cleaving RAGE from the cell surface, or from an alternatively spliced form of RAGE known as esRAGE. Endogenous secretory RAGE contains unique sequences within the C2-type domain that facilitate its unique identification compared to cell surface-cleaved soluble RAGE. The cytoplasmic domain of RAGE binds to mDia-1 and recent studies suggest that in transformed cells, mDia-1 is essential for ligand-stimulated cellular migration. Taken together, these studies suggest there may be multiple means to block the action of RAGE ligands–RAGE interaction in vascular and cellular pathobiology.

monocyte chemoattractant protein-1 and TNF-α in type 2 diabetic subjects [75].

Thus, it is somewhat curious that in distinct studies soluble RAGE levels may correlate positively or negatively with diabetic complications. What might underlie these findings? First, it is possible that subtle differences in age of the subjects may be responsible. No study to date has prospectively monitored soluble RAGE levels in individual subjects. Second, it is possible that there are strong ethnic determinants of soluble RAGE modulated at least in part by distinct RAGE polymorphisms. Third, it is plausible that subjects had concomitant disorders which independently affected soluble RAGE levels. Fourth, it is possible that ligand burden, even with equivalent degrees of hyperglycemia, varied significantly among subjects, perhaps because of polymorphisms in genes such as glyoxalase. Fifth, changes in renal function may result in altered soluble RAGE levels. Gohda et al. reported that levels of esRAGE were increased in subjects with type 2 diabetes (Japanese population) and decreased renal function [76]; in other studies it was shown that levels of soluble RAGE but not esRAGE were higher in subjects with type 2 diabetes and albuminuria in a European population [77]. In subjects not selected for diabetic state, levels of soluble RAGE in a Czech population correlated positively with increasing serum creatinine; the highest levels of soluble RAGE were detected in subjects with end-stage renal disease [78].

In summary, multiple factors may impact on levels of soluble RAGE, including gene polymorphisms of the *AGER* (*RAGE*) gene [6]. Future studies are required to rigorously dissect the relationships between soluble RAGE and esRAGE in subjects with diabetes. In other work, links of soluble RAGE levels to inflammatory disorders have been shown.

5.2. Inflammatory diseases

Studies of RAGE in RA suggest that soluble RAGE levels may be modified in this disorder. In human subjects with RA, levels of soluble RAGE were negatively associated with serum levels of C-reactive protein (CRP), high density lipoprotein (HDL), history of vasculitis and the RAGE G82S polymorphism [79]. Of note, in that study, levels of various S100 proteins also correlated positively with RA as well. Pullerits et al. reported that soluble RAGE levels were reduced in subjects with RA [80].

Soluble RAGE has been measured in children with Kawasaki disease and with juvenile idiopathic arthritis. In both disease settings, children with active disease displayed decreased levels of soluble RAGE especially in subjects with Kawasaki disease [81]. Soluble RAGE was found to correlate negatively with the level of S100A12. Of note, those authors found that when subjects with Kawasaki disease were treated with intravenous immunoglobulin, the S100A12:soluble RAGE ratio changed, suggesting that the ratio might be a measure of the response to therapy. In this context, a number of studies are appearing suggesting that indeed medications may alter soluble RAGE levels. At least one implication of this finding is that studies reporting soluble RAGE in human subjects likely should take into account medication history.

5.3. Soluble RAGE and therapeutic responses

In subjects with RA, treatment with methotrexate resulted in higher levels of soluble RAGE in synovial fluid; in contrast, treatment with agents not known to have "disease-modifying" effects had no impact on soluble RAGE levels [80].

Soluble RAGE also is affected by treatment in diabetic subjects. Treatment of type 2 diabetic subjects with metabolic control (metformin, gliclazide or insulin) caused soluble RAGE levels to increase in parallel with reductions in glycosylated hemoglobin [82]. In studies from a Chinese population, treatment of type 2 diabetic subjects with thiazolidinediones, but not sulfonylureas, resulted in increased circulating levels of soluble RAGE and esRAGE [83]. In that study, levels of glycemic control were similarly improved by metabolic control.

The responsiveness of soluble RAGE levels to interventions was also noted in subjects with type 1 diabetes. Treatment of such subjects with the angiotensin-converting enzyme inhibitor perindopril resulted in increases in plasma soluble RAGE [84].

Statin therapy has also been shown to modulate soluble RAGE levels in subjects with hypercholesterolemia. Intriguingly, although treatment for 8 weeks with atorvastatin caused a significant rise in soluble RAGE levels, in parallel with reduced LDL cholesterol levels, the same degree of changes in soluble RAGE levels was not observed in subjects treated with a different agent, pravastatin, despite the fact that LDL cholesterol levels were also reduced by that therapy [85].

Lastly, the response of soluble RAGE levels to adenosine treatment was studied in normal volunteers. When subjects were treated with adenosine versus placebo, subsequent treatment with *E. coli*-derived endotoxin caused an increase in soluble RAGE levels in the placebo- but not adenosine-treated groups [86]. Interestingly, compared with the above-noted studies, here, the response of soluble RAGE to pharmacological treatment was measured in the *acute* and not chronic setting, and the individuals were apparently normal healthy volunteers and not those known to be burdened with chronic illness.

6. Summary

Recent insights into the precise nature of RAGE ligands and their interaction with RAGE V-, C1, and/or C2 domains have suggested mechanisms by which the diverse ligands of RAGE may exert distinct responses in signal transduction and response to stress. *In vivo*, however, the concept of "one ligand-one disease" is not likely to be realistic, as we propose that in diabetes, for example, although elevated levels of glucose may trigger rapid development of AGEs, the consequent inflammatory response appears to drive release and action of S100/calgranulins and HMGB1. In primary immune disorders, there is evidence that inflammatory and oxidative stress may drive generation of AGEs, even in euglycemia, mediated in part by the actions of the myeloperoxidase pathway (reviewed in [6]). Thus, it is plausible that soluble RAGE may represent a therapy for disease states in which the ligands of RAGE accumulate.

Yet, it is essential to ask the question, are there hazards to long-term treatment with soluble RAGE or RAGE antagonism in general? Indeed, the ligands of RAGE may interact with distinct non-RAGE receptors such as toll receptors and CD36. To what extent blocking access of those ligands to such receptors might be disadvantageous is not yet clarified. Indeed, in only two cases to date, is there evidence that soluble RAGE or RAGE-blocking approaches exerted deleterious effects. First, administration of soluble RAGE (or RAGE blocking antibodies) to wild-type mice undergoing acute crush of the sciatic nerve resulted in decreased regeneration, in part to attenuation of the inflammatory response required to stimulate Wallerian degeneration [52]. Second, although in all published cases of pathogen challenge (including sepsis) soluble RAGE or other RAGE blocking agents, or RAGE deletion exerted benefit, in the case of direct intraperitoneal injection of *E. coli*, RAGE deficient mice succumbed more readily due to rapid activation of inflammatory and prothrombotic mechanisms [66]. Hence, the balance of beneficial versus damage-provoking inflammation in the context of RAGE blockade remains to be fully determined.

Lastly, the intriguing finding that mice do not display measurable plasma levels of soluble or esRAGE yet humans do,

has provided insights into soluble RAGE levels – biomarker or mediator or both? We predict that although these levels of soluble RAGE in the human may be much "lower" than those achieved with treatment in the mouse, soluble RAGE may function as an active scavenger of trouble-making ligands in the circulation and, especially, in the microenvironment as a mechanism to thwart disease. In this context, we remain thoroughly fascinated by the findings of Geroldi et al. who were the first to show that highly elevated soluble RAGE levels correlated with extreme longevity in human subjects [87]. These authors showed that compared to heavily disease-burdened young(er) subjects, "healthy centenarians" displayed the highest levels of soluble RAGE. Is elevated soluble RAGE a marker or mediator of ligand trapping in advanced aging, or both? Many questions remain to be addressed, such as whether soluble RAGE or esRAGE display identical affinity for RAGE ligands, and whether their ratios and absolute levels in plasma predict the opportunity to benefit or biomark in health, disease and successful aging. No doubt, as the literature is abounding with studies examining soluble RAGE in the human subject in homeostasis and disease, the answers to these questions are soon to be revealed.

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