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Soluble Levels of Receptor for Advanced Glycation Endproducts (sRAGE) and Coronary Artery Disease The Next C-Reactive Protein?

Barry I. Hudson, Evis Harja, Bernhard Moser, Ann Marie Schmidt

The Receptor for advanced glycation endproducts (RAGE) is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules.¹ Although first described as a receptor for AGEs, the products of nonenzymatic glycation and oxidation of proteins/lipids, later studies indicated that RAGE was also a signal transduction receptor for proinflammatory S100/calgranulins and amphoterin (or high mobility group box 1 [HMGB1]) and amyloid- β -peptide and β -sheet fibrils.²⁻⁴ Additionally, RAGE is a counter-receptor for Mac-1.⁵ These ligands may be generated and accumulate in diverse settings, such as diabetes, renal failure, neurodegeneration, autoimmunity/inflammatory milieu, and aging. RAGE ligands may synergize in tissues primed by genetic and/or environmental triggers to amplify perturbation. For example, in the case of Mac-1, it was demonstrated that in the presence of S100, the RAGE-Mac-1 interaction was augmented.⁵ Thus, RAGE ligands may be pieces of a scaffold that, together, magnify inflammatory mechanisms in the tissues. If left unchecked, RAGE-mediated sustained inflammation, coupled with failure of regenerative mechanisms, may lead to irreversible tissue injury.¹

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Ligand-RAGE Axis: Breaking the Cycle

These concepts have been tested in rodent models of exaggerated neointimal expansion, the hallmark of atherosclerosis and restenosis, triggered by chronic hyperglycemia or acute physical stress. In apolipoprotein E-null mice rendered diabetic with streptozotocin, blockade of RAGE was accomplished using a soluble form of the receptor, soluble (s) RAGE, generated and purified from a baculovirus expression system as a decoy for the families of RAGE ligands. Administration of sRAGE to diabetic apolipoprotein E-null mice suppressed early acceleration of atherosclerosis as well as prevented progression of disease in diabetic mice with established atherosclerosis.⁶⁻⁷ In parallel, blockade of RAGE significantly decreased vascular expression of adhesion molecules, prothrombotic species such as tissue factor, and

diminished antigen/activity of matrix metalloproteinases (MMPs). In addition, vascular levels of phosphorylated p38 MAP kinase and activated NF- κ B, a key signaling molecule and transcription factor linked to the inflammatory response, respectively, were reduced in sRAGE-treated mice.⁷⁻⁸ Importantly, these studies provided the first clue that RAGE-dependent inflammatory and tissue-perturbation mechanisms were not limited to the diabetic state. Administration of sRAGE to *euglycemic* apolipoprotein E-null mice with established atherosclerosis suppressed progression.⁷

In other studies, administration of sRAGE to diabetic rats subjected to carotid artery balloon injury diminished neointimal expansion, in parallel with decreased proliferation of smooth muscle cells in the expanding neointima.⁹ As in the case of lipid-driven atherosclerosis, these findings were not limited to the diabetic state. Acute denudation of the femoral artery in *euglycemic* C57BL/6 mice rapidly upregulated RAGE as well as ligands for the receptor in the injured vessel wall, such as AGEs and S100/calgranulins.¹⁰ Key roles for the ligand-RAGE axis were shown by suppression of neointimal expansion in the presence of sRAGE. The target of sRAGE was indeed RAGE, as acute arterial injury in wild-type mice treated with antibodies to RAGE, in homozygous RAGE-null mice, or in transgenic mice expressing signal transduction mutant RAGE specifically in smooth muscle cells was significantly decreased compared with vehicle-treated animals or injured littermates.¹⁰

Taken together, these studies indicate that in rodent models, upregulation of the ligand-RAGE axis contributed to glucose-, lipid- and/or physical stress-induced neointimal expansion.

From Rodents to Humans?

Evidence is accruing that RAGE is in the right place and time to contribute to atherosclerosis and restenosis in human subjects. Cipollone and colleagues showed that RAGE was expressed in nondiabetic and diabetic human atherosclerosis, and to enhanced degrees in diabetes. Further, RAGE colocalized with cox-2, type 1/type 2 microsomal prostaglandin (PG) E₂, and MMPs in the diabetic atherosclerotic plaques.¹¹ When semi-quantified by using Western blot, levels of RAGE in the diabetic plaques rose in parallel with the increase in glycosylated hemoglobin, pre-AGE species.¹¹

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Falcone and colleagues take the next step in linking RAGE to human atherosclerosis. In two distinct populations of age-matched Italian male subjects without diabetes, these authors show that endogenously lower levels of sRAGE were

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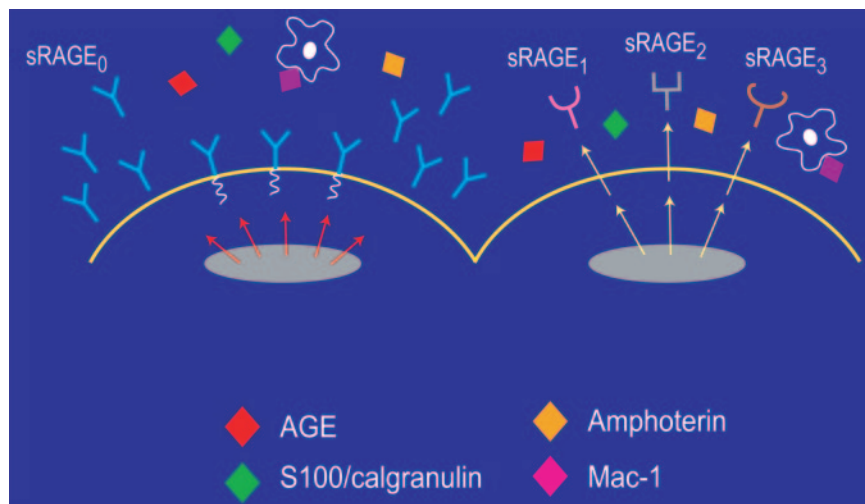


Figure 1. Model for the potential sources of sRAGE. There are a number of potential means by which sRAGE may be generated. First, it is possible that yet to be delineated mechanisms may cleave sRAGE from the endogenous full-length cell surface receptor. Second, multiple discrete novel splice variants of RAGE may generate diverse sRAGEs. The assay used by Falcone and colleagues in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* does not distinguish these possibilities. Although this cartoon suggests that endothelial cells may be a principal source of sRAGE, it is possible that other cells such as smooth muscle cells or circulating inflammatory cells may generate sRAGEs. Key roles for sRAGEs, as decoys for the cell surface receptor RAGE, may serve as biomarker and/or endogenous protection factor in diseases characterized by upregulation of RAGE ligands.

associated with *enhanced* risk of coronary artery disease, as detected by angiography.¹² The link between levels of sRAGE and coronary artery disease was “dose-dependent,” as meticulous statistical analysis suggested that individuals with the very lowest levels of sRAGE displayed the greatest overall risk for disease. The chief limitation of this study is that using antibodies generated generically to RAGE, the authors were unable to distinguish whether the species detected by this assay represented soluble RAGEs, perhaps cleaved/released from full-length endogenous receptor on the cell surface by molecules such as MMPs, and/or novel splice variants of RAGE^{13–15} (Figure 1). Although increased cell surface RAGE in diseased milieu might suggest that levels of sRAGE generated directly from this species should be *higher* in disease states, not lower, our present lack of understanding of the mechanisms by which sRAGE might be generated from full-length cell surface receptor does not allow us to make conclusions at this time. Further, future studies must delineate that levels of sRAGE are not lower in the diseased population because of sRAGE binding to circulating ligands, thereby diminishing antibody binding to the sRAGE species. This possibility is unlikely, however, given that a polyclonal antibody is included within the sandwich ELISA for detection of sRAGE.

Furthermore, these studies do not delineate the specific cellular source(s) of sRAGE. In addition to endothelial cells, it is possible that smooth muscle cells and circulating inflammatory cells such as monocytes and lymphocytes, for example, may be generators of sRAGE. A further consideration arises from these possibilities: if there are distinct sRAGE species, is it possible that their affinity for the ligands of the receptor may differ? Irrespective of such caveats, the work of Falcone and colleagues mandates that antibodies and corresponding assays for detection of discrete splice variants of RAGE be developed to best address these hypotheses.

RAGE & the Lipid Connection?

One of the most intriguing findings in the work of Falcone and colleagues is that even after correction for levels of lipids, the chief risk factor driving atherosclerosis in nondiabetic subjects, levels of sRAGE remained associated with the

presence of coronary artery disease.¹² These authors found that in the subgroup of subjects with LDL cholesterol levels <130 mg/dL (3.4 mmol/L), the most-recently recommended highly stringent goal for lipid control, multivariate-adjusted odds ratios for coronary artery disease in the first (lowest), second, and third quartiles of sRAGE levels were highly significantly increased by 10.256, 3.005, and 3.039, respectively, versus the fourth (highest) quartile of sRAGE level.¹² The power of this observation is further propelled by the statistical analyses. Even after correction for the presence of confounding factors, presumably factors such as tobacco use or hypertension, levels of sRAGE remained an independent determinant of coronary artery disease among subjects with normal/near normal LDL.

RAGE & Disease: Is it Really a Multiple Hit Model?

Based on overwhelming evidence that the ligands for the receptor such as AGEs and inflammatory S100/calgranulins, amphoterin, and Mac-1 are linked to upregulation of RAGE and RAGE-related settings such as diabetes and immune/inflammatory diseases, it was predicted that RAGE was not one of the proximate causes of atherosclerosis or diabetes or autoimmunity, but, rather, a magnification factor set in motion particularly in settings of basal ligand accumulation and receptor upregulation. These concepts are depicted in Figure 2. The finding of Falcone and colleagues that even in the absence of basal dysfunction such as hyperglycemia or hyperlipidemia, reduced levels of sRAGE remained correlated with coronary artery disease suggests that an additional possibility must be considered. Is it plausible that innate characteristics of RAGE and its regulation, distinct from environmental factors, contribute importantly to risk for atherosclerosis? Thus, as shown in Figure 2, it is possible that basal upregulation of the ligand/RAGE axis *itself* sets the stage for the development of vascular inflammation. In this alternate view, is it possible that genetic variants of full-length receptor or its novel splice forms hold, at least, part of the key? To date, intriguing studies suggest that a particular promoter allele (–374 A) of the gene encoding *RAGE* is associated with lesser degrees of macrovascular disease in

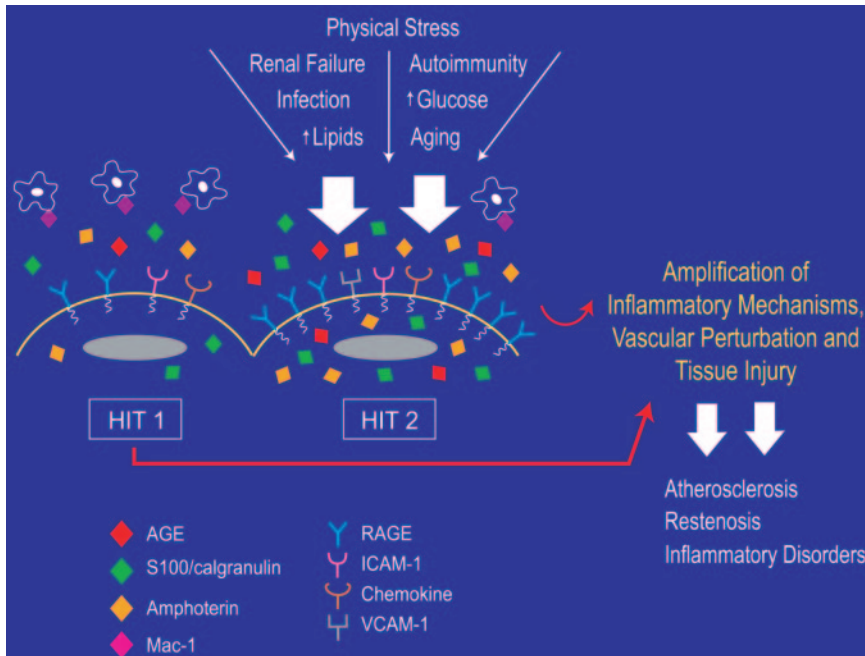


Figure 2. RAGE and cellular stress: initiating factor and/or amplifier of inflammatory mechanisms and tissue injury? In the multiple hit model, certain disease states are characterized by basally enhanced generation of RAGE ligands (AGEs, S100/calgranulins, amphoterin, and/or Mac-1), in parallel with increased expression of RAGE (hit one). On super-imposed stresses (hit two), such as infection, renal failure, hyperglycemia, physical stress, hyperlipidemia, aging, or autoimmunity, further generation of RAGE ligands activates signal transduction mechanisms, via RAGE, that lead to upregulation of inflammatory and pro-thrombotic pathways. If left unchecked, these processes amplify inflammation and tissue destruction. In the alternate view, basal upregulation of the ligand–RAGE axis itself, as a single hit, contributes innately to the development of vascular inflammation and tissue injury. Experiments must be performed to dissect the contribution of each facet of the RAGE axis in vascular and other forms of inflammatory disorders.

diabetic human subjects.^{16–17} Additionally, Falcone and colleagues, in a distinct report, have recently extended these observations to subjects without diabetes. They reported that subjects with the –374 T/A or A/A genotypes displayed decreased severity of coronary atherosclerosis, as determined by angiography.¹⁸ Although two other smaller studies failed to find such an association with incidence of macrovascular disease,^{19–20} these findings strongly suggest that genetic factors may indelibly link RAGE to propensity to coronary artery disease. Large scale studies, especially those in nondiabetic subjects, are critically needed to better address the potential predictive value of this variant.

Soluble RAGE: Biomarker or Endogenous Protection Factor?

Certainly, none of the available evidence allows us to conclude “cause or effect” from these observed low levels of sRAGE in association with higher risk for coronary artery disease in nondiabetic human subjects. Yet, attention to the criteria for study entry by Falcone and colleagues reveals that they *excluded* individuals on lipid-lowering therapy. By excluding such subjects, they likely removed from consideration individuals benefiting from the potent antiinflammatory effects of at least certain of these agents. Even in this setting, low sRAGE levels still reflected higher incidence of coronary artery disease. These considerations suggest that measurement of sRAGE might be a powerful complement to assessment of high sensitivity C-reactive protein, itself critically associated with innate and adaptive inflammatory responses, including those associated with atherosclerosis.²¹

The work of Falcone and coworkers sets the stage for testing novel concepts in the biology of RAGE. Specifically, future studies must address the relationship of endogenous sRAGE to macro- and microvascular complications in human types 1 and 2 diabetes. Further, given that RAGE may play key roles in other inflammatory diseases, such as those

triggered by immune stimuli,^{2,22} measurement of sRAGE in such settings may highlight disease activity and/or the response to therapeutic intervention. Is sRAGE the next CRP? How do levels of sRAGE correlate with other known indices of acute stress and vascular perturbation, such as circulating levels of interleukin (IL)-6, serum amyloid-A, soluble adhesion molecules, or soluble thrombomodulin? Long-term prospective clinical studies in both male and female subjects will be required to test this hypothesis.

Lastly, if the findings of Falcone and colleagues are borne out in such large-scale prospective trials, then sRAGE might, perhaps, be more than just a biomarker. Is it possible that efforts to burgeon endogenous production of sRAGE might provide a therapy for atherosclerosis and other inflammatory disorders linked to the ligand/RAGE axis? Alternatively, is it possible that bolstering levels of sRAGE pharmacologically may impart benefit in human subjects? Indeed, administration of sRAGE to mice and rats predisposed to exaggerated neointimal expansion significantly limited atherosclerosis and restenosis in these species. The importance of the work of Falcone and colleagues is that it takes the next steps in building the bridge from rodent to human in the biology of RAGE. The particular stepping stone shaped by these authors may set the stage for critical studies to address the question: sRAGE, biomarker or endogenous therapy? However it turns out, valuable insights into RAGE as cause or effect—or both—stand to be uncovered.

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