Receptor for Advanced Glycation Endproducts and Implications for the Pathogenesis and Treatment of Cardiometabolic Disorders: Spotlight on the Macrophage

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Abstract—The receptor for advanced glycation endproducts (RAGE) interacts with a unique repertoire of ligands that form and collect in the tissues and circulation in diabetes mellitus, aging, inflammation, renal failure, and obesity. RAGE is expressed on multiple cell types linked to tissue perturbation in these settings. This brief review focuses on the role of RAGE in monocytes/macrophages and how RAGE ligand engagement on these cells mediates seminal changes in monocyte/macrophage migration, oxidative stress, cholesterol efflux, and pro- versus anti-inflammatory cues that signal to tissue damage. Studies using mice devoid of Ager (gene encoding RAGE) or pharmacological antagonists of RAGE are protective in animal models of diabetes mellitus, atherosclerosis, and high-fat diet–induced obesity, in least in part through key roles in monocytes/macrophages. RAGE signal transduction requires the interaction of RAGE cytoplasmic domain with the formin, DIAPH1 and novel antagonists of this interaction show significant promise in attenuation of the maladaptive effects of RAGE ligands in cellular and in vivo models. Finally, this brief review discusses evidence for RAGE axis perturbation in human monocytes/macrophages and how tracing RAGE activity in these cells may identify target engagement biomarkers of RAGE antagonism for future clinical trials. (Arterioscler Thromb Vasc Biol. 2017;37:00-00. DOI: 10.1161/ATVBAHA.117.307263.)

Key Words: advanced glycosylation end product-specific receptor ■ atherosclerosis ■ diabetes mellitus ■ inflammation ■ obesity

Receptor for Advanced Glycation Endproducts: A Multiligand Receptor of the Immunoglobulin Superfamily

Receptor for advanced glycation endproducts (RAGE), a member of the immunoglobulin superfamily of cell surface molecules, was originally discovered for its ability to bind to and transduce the biological effects of advanced glycation endproducts (AGEs), which accumulate in settings such as hyperglycemia, aging, inflammation, renal failure, and oxidative stress. AGEs are an heterogeneous group of modified molecules, which form particularly on lysine and arginine amino acid residues. Among the AGEs, one of the most prevalent species found in human subjects with diabetes mellitus, the carboxymethyl lysine (CML) AGEs are signal transduction ligands of RAGE. The key to the biology of RAGE lies in its diverse ligand families. The discovery that RAGE bound non-AGE ligands linked the receptor indelibly to mechanisms important in the inflammatory response. Beyond AGEs, RAGE is also a signal transduction receptor for molecules such as members of the S100/calgranulin family, high mobility group box-1, Mac-1, and lysophosphatidic acid. Hence, RAGE and its ligands accumulate in the nondiabetic state and enhance degrees in diabetes mellitus, at least in part on account of the potent effects of hyperglycemia on stimulating increased formation and accumulation of RAGE ligand AGEs, AGES, and other ligands. Through RAGE, ligands comprise a unique family that imbues chronic inflammatory stress as their chief biological action, for example, through sustained activation of the proinflammatory transcription factor nuclear factor-κB. Furthermore, RAGE action downregulates mRNA transcript and protein and activity levels of Glo1 (or glyceraldehyde-1). Glo1, which detoxifies the pre-AGE intermediate, methylglyoxal, is a defense against increased AGE production. The finding that RAGE downregulates Glo1 underscores the possibility that RAGE is at the center of a feedforward loop regulating both ligand activity and ligand levels. In this context, the finding that RAGE sustains chronic inflammatory cues stimulated by its ligands provides further fuel for sustaining production and accumulation of proinflammatory RAGE ligands, which may be escorted into inflammatory sites through S100/calgranulin-bearing or high mobility group box-1–bearing immune cells.

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Adding to the complexity of this axis is the consideration that some of the ligands of RAGE, such as high mobility group...
box-1, also bind to toll-like receptors. Experiments testing the effects of massive hepatic injury induced by 85% hepatectomy suggest that whereas mice devoid of the toll-like receptor signaling effector, Myd88, displayed accelerated mortality after this injury compared with wild-type controls, mice devoid of Ager (gene encoding) displayed improved survival when compared with either wild-type or Myd88 null mice. Strikingly, mice doubly devoid of Myd88 and Ager displayed comparable mortality to the mice devoid of Myd88, thereby suggesting that RAGE expression, unlike toll-like receptor signaling through MYD88, was not essential for the innate response to massive liver injury.

RAGE is expressed on multiple cell types implicated in cardiovascular disease, such as vascular cells (endothelial cells and smooth muscle cells [SMCs]) and immune cells such as monocytes/macrophages, T and B lymphocytes, and neutrophils. This brief review, focusing on RAGE and monocytes/macrophages in cardiometabolic disorders, will detail the evidence that points to roles for RAGE not only in recruitment of these key cells in atherosclerosis and obesity but also to their expression of proinflammatory mediators and reduced expression of molecules linked to cholesterol efflux. Novel insights into RAGE signal transduction through the formin DIAPH1, and how such signaling may be pharmacologically antagonized, will be discussed. Finally, the evidence in human monocytes/macrophages linking the RAGE axis to cellular perturbations that may contribute to both the development and biomarking of cardiometabolic pathobiology will be presented.

**RAGE and Atherosclerosis**

**RAGE and Atherosclerosis in Human Subjects**

Evidence from human subjects places RAGE in atherosclerotic plaques, particularly, but not limited to the diabetic state. Cipollone et al evaluated atherosclerotic plaques from 60 patients undergoing carotid endarterectomy and reported that the diabetic plaques had significantly more macrophages, CD3+ lymphocytes, SMCs, and HLA-DR+ cells compared with the nondiabetic plaques. In parallel, the diabetic plaques demonstrated more RAGE expression (particularly in macrophages and SMCs), activated nuclear factor-kB, higher cyclooxygenase 1/microsomal prostaglandin E-synthase 1 expression, and higher levels of matrix metalloproteinases and gelatinolytic activity. Lower collagen content and higher lipid and oxidized low-density lipoprotein content were also noted in the diabetic plaques.

In other studies, the expression of RAGE and plaque characteristics in diabetic versus nondiabetic subjects were examined in the coronary vasculature of subjects who experienced sudden cardiac death. Atherosclerotic plaques from type 2 diabetic subjects revealed larger mean necrotic cores and greater total and distal plaque load compared with the nondiabetic lesions. The intimal staining for macrophages, T lymphocytes, and HLA-DR status was significantly greater in the diabetic than in the nondiabetic plaques. Expression of RAGE and RAGE ligand S100A12, especially in macrophages and in apoptotic SMCs, was significantly greater in the diabetic lesions.

Prompted by these observations in human subjects, mechanistic studies in animal models were performed to test the role of RAGE in atherosclerosis, particularly in the context of its role in immune cell recruitment and activity.

**RAGE, Murine Models, and Atherosclerosis**

Studies in atherosclerosis-prone mice have provided definitive evidence for roles for RAGE in the pathogenesis of nondiabetic and diabetic accelerated atherosclerosis. In both mice devoid of Apoe or the Ldlr, deletion of Ager resulted in lower mean atherosclerotic lesion areas, particularly in Apoe null mice. In these studies, a prominent underlying mechanism was the reduction in lesion levels of Mcp1 and reduced macrophages or T-lymphocyte content per lesion area in the atherosclerosis-prone mice globally devoid of Ager. Evidence of generalized reductions in inflammatory and oxidative stress was noted on deletion of Ager in these settings. In other studies, mice expressing cytoplasmic domain-deleted RAGE in endothelial cells (driven by the preproendothelin 1 promoter) displayed reduced atherosclerosis in the nondiabetic Apoe null mouse background, in parallel with significant reductions in Vcam1 in the vasculature.

In distinct studies, mice were treated with soluble RAGE (sRAGE); sRAGE is composed of the extracellular ligand-binding domains of RAGE. Administration of sRAGE to diabetic mice resulted in suppression of early initiation and progression of diabetic atherosclerosis. Prominent effects on downregulation of inflammatory and oxidative stress responses were observed in sRAGE- versus vehicle-treated animals. Beneficial effects of sRAGE were also noted in nondiabetic Apoe null mice, but the extent of benefit was lower, consistent with the premise that although the hyperlipidemic environment of the nondiabetic state stimulates the generation of RAGE ligands, the hyperglycemia of diabetes greatly accelerates formation of RAGE ligands compared with those generated in the nondiabetic state.

The role of myeloid RAGE was definitively tested using bone marrow transplantation strategies. First, these concepts were tested in nondiabetic mice devoid of Apoe. Seven- and 23-week-old Apoe null mice were lethally irradiated and reconstituted with bone marrow from Apoe null mice expressing or devoid of Ager. After 16-week standard chow diet in the mice irradiated at age 7 weeks, there were no differences in atherosclerotic lesion area vis-à-vis RAGE. However, in the older mice, lesions in the brachiocephalic arteries were significantly smaller in the mice receiving Ager null bone marrow versus control bone marrow. In parallel, plaques were more stable and expressed significantly lower levels of Vcam1, Lcam1, and Mcp1 versus the plaques of mice reconstituted with Ager-expressing bone marrow.
In diabetes mellitus, streptozotocin-induced Apoe null diabetic mice were lethally irradiated and reconstituted with Apoe null/Ager null or control Ager-expressing Apoe null bone marrow and mice were euthanized at either 10 or 20 weeks. In both groups of mice, deletion of Ager resulted in reduced atherosclerotic lesion areas and lower levels of Vcam1. Of note, the authors determined in distinct bone marrow transplantation studies that non–bone marrow–derived cells also contributed to diabetic atherosclerosis via RAGE. In these model systems, deletion of Ager or administration of sRAGE had no effect on levels of glucose or on total levels of cholesterol or triglyceride, thereby suggesting that unique RAGE-dependent mechanisms distinct from typical risk factors affect atherosclerosis. Hence, these data suggested that vascular cell RAGE contributes to upregulation of adhesion molecules and chemokines, processes by which RAGE may contribute to the development of atherosclerosis in nondiabetic and diabetic mice. Definitive studies using bone marrow transplantation strategies implicated both myeloid and nonmyeloid cell RAGE directly in atherosclerosis in murine models, in both non–diabetes mellitus and diabetes mellitus. Evidence of overall reduced macrophage content per lesion area was demonstrated, in parallel with reduced inflammation and oxidative stress. The possible contributions of RAGE in macrophage retention/stasis, proliferation, and fate (death) in the atherosclerotic lesion remains to be determined.

In addition to testing how RAGE affects diabetic atherosclerosis, the role of RAGE in uremia-associated atherosclerosis has also been addressed. Uremic conditions are linked to highly increased accumulation of RAGE ligand AGEs. In animal models, performance of the 5/6 nephrectomy in Apoe null mice resulted in accelerated atherosclerosis in mice expressing Ager but not in mice devoid of Ager. RAGE ligands serum amyloid A and S100B increased significantly in the uremic environment and on stimulation of SMCs with these ligands, prominent increases in production of reactive oxygen species were observed in a RAGE-dependent manner. In other studies, a neutralizing anti-RAGE IgG was used in Apoe null mice subjected to the 5/6 nephrectomy. Compared with the treatment with isotype control, anti-RAGE antibodies resulted in lower atherosclerotic lesion areas. Treatment with anti-RAGE antibodies had no effect on a panel of inflammatory markers in the lesions but had a dramatic effect on reduction of oxidative stress. In the section to follow, recent data implicating RAGE in the mechanisms regulating cholesterol metabolism will be discussed.

RAGE, Macrophages, Cholesterol Efflux, and Reverse Cholesterol Transport

Macrophage cholesterol efflux in human subjects with diabetes mellitus is impaired and such impairment is observed in animal models of diabetes mellitus as well. Links to RAGE were uncovered by the finding that in murine bone marrow–derived macrophages from diabetic mice devoid of Ager, cholesterol efflux to ApoA1 and high-density lipoprotein was higher than that observed in diabetic bone marrow–derived macrophages from wild-type mice. In vivo, macrophage reverse cholesterol transport to plasma, liver, and feces was reduced in diabetic macrophages through RAGE. The underlying mechanism was traced to RAGE ligand-mediated downregulation of the key cholesterol transporters, Abca1 and Abcg1.

In vitro, using RAGE-expressing THP1 cells, and RAGE-overexpressing HEK cells, the RAGE ligand CML-AGE suppressed ABCG1 and ABCA1 promoter luciferase activity and transcription of ABCG1 and ABCA1 through peroxisome proliferator–activated receptor-γ–responsive promoter elements. Interestingly, the effects of RAGE ligands/RAGE were not dependent on the liver X receptor elements. Plasma levels of high-density lipoprotein were lower in diabetic C57BL/6 mice devoid of Ager than in wild-type mice of the same genetic background. Laser capture microdissected CD68(+) macrophages from atherosclerotic plaques of Ldlr null mice devoid of Ager versus the RAGE-expressing Ldlr null mice displayed higher levels of Abca1, Abcg1, and Pparγ mRNA transcripts versus Ager-expressing Ldlr null mice independently of glycemia or plasma levels of total cholesterol and triglycerides. Given that peroxisome proliferator–activated receptor-γ exerts beneficial effects on macrophage inflammation, these considerations highlighted possible roles for RAGE in downregulation of peroxisome proliferator–activated receptor-γ–dependent mechanisms in macrophages. Although not the focus of this review, it is important to note that extensive evidence links RAGE and its ligands to microvascular or small-vessel disease in settings such as diabetes mellitus, aging, and Alzheimer disease.

In addition to important roles for macrophages in inflammation, oxidative stress and cholesterol metabolism in atherosclerosis, macrophages are also tightly linked to metabolic regulation of insulin sensitivity, particularly in high-fat feeding. The section to follow explores how RAGE contributes to metabolic dysfunction in obesity and the possible links to macrophage functions.

RAGE and High-Fat Diet–Induced Obesity

Macrophages exert complex effects in adipose tissue in obesity, not only depending on the adipose tissue depot but also on the species. Whereas correlations between numbers of adipose tissue macrophages with obesity and insulin resistance have been noted in mice, the findings in the human obesity are less clear. Furthermore, markers of polarization do not seem to reliably reflect the effects of macrophages on systemic glucose and insulin sensitivity. For example, M2-type markers such as CD206 and CD163 have been associated with insulin resistance. More recently, to link macrophage properties to systemic immunometabolism, an emerging theme relates to how macrophage fatty acid oxidation and handling may regulate inflammatory signatures.

RAGE, Adipose Tissue, Obesity, and Human Subjects

The study of macrophage RAGE in obesity and systemic metabolism is a work in progress. Recently, Gaens et al examined subcutaneous and visceral adipose tissue from lean versus obese male human subjects. Compared with the lean subjects, the obese subjects displayed evidence of glucose and insulin-related metabolic dysfunction, as the obese subjects...
demonstrated significantly higher levels of fasting glucose and fasting insulin and lower glucose infusion rates. These authors performed immunohistochemical staining to localize RAGE ligand CML-AGE and RAGE in adipose tissue. Whereas only scant immunoreactivity for CML-AGE epitopes was noted in human lean subcutaneous adipose tissue, much more readily detectable CML-AGE was noted in obese tissue. RAGE expression in adipose tissue was also significantly higher in obese than in lean subjects, particularly in visceral versus subcutaneous adipose tissue. Both CML-AGE and RAGE epitopes in adipose tissue localized to adipocytes, CD68+ macrophages, and CD31+ endothelial cells. Experiments were performed in mice to determine the mechanistic inferences from these findings.

**RAGE, Obesity, and Murine Models**

Wild-type and homozygous *Ager* null male mice were fed a high-fat diet (60%) versus standard low-fat chow. In the wild-type mice, even before the appearance of insulin resistance or obesity, metabolic tissues displayed significantly higher levels of RAGE ligands, CML-AGE, and high mobility group box-1. Surprisingly, despite equal food consumption in mice fed the high-fat diet, compared with the wild-type mice, *Ager* null mice displayed significantly lower body mass; by DEXA scanning, this was accompanied by a significantly lower lean and fat mass in the high-fat-fed *Ager* null mice. Metabolic phenotyping identified that the *Ager* null mice fed the high-fat diet were more glucose and insulin sensitive versus the wild-type mice, as indicated by higher VCO2 consumption and higher VCO2 production. Interestingly, in the hyperinsulinemic euglycemic clamp studies, this was confirmed by hyperinsulinemic euglycemic clamp studies. In addition, the *Ager* null mice displayed significantly higher energy expenditure versus the wild-type mice, as indicated by higher VO2 consumption and higher VCO2 production. Interestingly, in the hyperinsulinemic euglycemic clamp studies, even on the low-fat diet, mice devoid of *Ager* were more insulin sensitive than the corresponding wild-type mice.

A key question to address was whether there were correlations between the expression of RAGE, macrophage content and profile, and insulin sensitivity in the visceral (perigonadal) adipose tissue in low-fat or high-fat feeding. In mice fed the low-fat diet, there were no significant differences in *Emr1* (F4/80) mRNA transcripts between wild-type and *Ager* null mice; by immunohistochemistry, significantly lower numbers of F4/80+ and CD11c+ cells populated this tissue in *Ager* null versus the wild-type mice on the low-fat diet. Although *Emr1* mRNA transcripts were significantly higher in wild-type high-fat–fed versus low-fat–fed mice, they were significantly lower in *Ager* null visceral adipose tissue. By immunohistochemistry, on high-fat diet, there were significantly lower F4/80+ and CD11c+ cells in the *Ager* null versus wild-type visceral adipose tissue. By real-time quantitative polymerase chain reaction, levels of *Tnfa*, *Cd209d*, *Cd163*, *Emr1*, and *Nos2*, normalized to *Emr1* transcripts, were either unchanged or higher in *Ager* null than in wild-type visceral adipose tissue. In contrast, levels of *Cd163*, *Cd209d*, *Arg1*, and *Cd209e* were all significantly higher in the *Ager* null than in wild-type visceral adipose tissue.

Lethal irradiation of wild-type mice and reconstitution with *Ager* null versus wild-type bone marrow resulted in partial protection from high-fat diet–induced obesity, in parallel with improved glucose tolerance. Interestingly, macrophage content and inflammatory gene expression profiles in mice reconstituted with *Ager* null bone marrow closely paralleled those in the global *Ager* null mice. Taken together, these considerations led us to propose that myeloid/macrophage RAGE seems to play important roles in the response to high-fat feeding—both in terms of regulation of body mass, adipose tissue macrophage content, and systemic metabolism. The precise mechanisms remain to be identified and are under active investigation. Whereas earlier studies focused on visceral adipose tissue, ongoing work is probing how RAGE expression, in macrophages and adipocytes, affects obesity and the metabolic response to high-fat feeding in distinct adipose tissue depots of subcutaneous and brown adipose tissue.

**RAGE and Signal Transduction**

**RAGE and DIAPH1: Biology**

RAGE activates diverse signaling cascades in a range of cell types; until recently, the proximate mechanisms governing RAGE signaling have been elusive. Experiments in a yeast 2-hybrid assay revealed that the cytoplasmic domain of RAGE binds to the formin DIAPH1 and that DIAPH1 is required for RAGE ligand–mediated activation of Rac1 or Cdc42 and cellular migration. Formins play key roles in actin cytoskeleton dynamics, cellular migration, cytoskeleton, signaling (particularly through the RHO GTPases), and regulation of serum response factor activities. In transformed cells, RNAi–knockdown of DIAPH1 suppressed RAGE ligand–mediated activation of Rac1 or Cdc42 and cellular migration. In SMCs, RNAi knockdown of DIaph1 or global deletion of *Diaph1*–blocked RAGE ligand–mediated signaling through AKT and suppressed SMC migration.

In vascular cells and macrophages, it was shown that deletion of *Ager* protected these cells from hypoxia-stimulated upregulation of Egr1 and its sequelae on inflammatory and prothrombotic gene expression. The mechanistic link to RAGE was illustrated by experiments revealing that exposure of cells to hypoxia resulted in rapid generation of RAGE ligand AGEs and that blockade of AGEs (using anti-AGE IgG) or aminoguanidine prevented hypoxia-stimulated upregulation of *Egr1* in human THP1 macrophage–like cells. In THP1 cells, treatment with AGEs directly upregulated *Egr1* in a manner blocked by RNAi–knockdown of DIAPH1. In peritoneal macrophages obtained from *Diaph1* null mice, a highly significant reduction in hypoxia-stimulated upregulation of Egr1 was observed when compared with the wild-type cells. These considerations indicated that RAGE ligand AGEs and hypoxia require DIAPH1 in macrophages to upregulate EGR1. Given the profound tissue-damaging effects of EGR1 in hypoxic stress, identification of roles for RAGE/DIAPH1 in these processes in macrophages and likely other cell types may highlight novel strategies to mitigate tissue damage in hypoxia and ischemia. Hence, it was essential to discern the precise mechanisms accounting for the interaction between the cytoplasmic tail of RAGE and DIAPH1.

**RAGE and DIAPH1: Physical Interaction**

Shekhtman et al used a range of techniques, particularly NMR spectroscopy, to discover that the first half of the cytoplasmic
domain of RAGE was ordered and that this was the region of the molecule that bound the FH1 (formin homology 1) domain of DIAPH1. They identified that the RAGE cytoplasmic domain possesses an unusual α-turn, which was required for the interaction with DIAPH1. Mutation of amino acid residues R5/Q6 in human RAGE cytoplasmic domain to alanine caused the disruption of the interaction with DIAPH1.

Critically, when these amino acids in the RAGE cytoplasmic domain were mutated to alanine residues in SMCs, activation of Akt and cellular migration and proliferation triggered by the incubation of these cells with RAGE ligand S100B were blocked compared with the control constructs. Importantly, the mutated R5/Q6 (to alanine residues) construct in SMCs displayed no reduction in cellular migration or proliferation triggered by a non-RAGE ligand, PDGF. Hence, these experiments provided further support for the RAGE–DIAPIH1 interaction in cellular signaling.

Recent experiments by Shekhtman et al illustrated that RAGE forms constitutive homodimers through its extracellular VC1 and C2 domains and that on ligand engagement, the molecular dimension of RAGE increases, thereby allowing its cytoplasmic domain to recruit DIAPH1 and to initiate signaling. In that study, mutations of DIAPH1 were prepared, which indicated that full-length DIAPH1 is required for RAGE signal transduction stimulated by RAGE ligands such as S100B.

RAGE and DIAPH1: Discovering Antagonists of RAGE Signaling

A high-throughput binding assay was developed to screen a >58,000 small-molecule library for inhibitors of the RAGE-DIAPH1 interaction. Through a series of experiments including binding assays and NMR spectroscopy, 13 small molecules were identified that blocked RAGE–DIAPH1 binding and suppressed the effects of RAGE ligands on signal transduction, that is, phosphorylation of Akt and ERK1/2 in cultured cells. In SMCs and in THP1 macrophage-like cells, these compounds suppressed RAGE ligand-mediated functions such as migration and production of inflammatory mediators. Critically, in SMCs, these compounds had no effect to suppress migration to non-RAGE ligand PDGF. Ex vivo, these compounds were tested for their effects on blocking ischemia/reperfusion injury in the isolated diabetic reperfused heart and many of them prevented the loss of left ventricular developed pressure, a marker of heart function. Finally, when RAGE ligand CML-AGE was infused into wild-type mice, upregulation of inflammatory mediators was observed in liver and kidney and prevented, at least in part, by certain of the compounds under study.

To date, 2 lead series of molecules have been illuminated from the original 13. Structure activity relationship and small-molecule refinement studies are underway to optimize these lead series for further development with an eye toward therapeutics. Although ultimate clinical trials testing in diabetes mellitus and its complications is the long-term goal for these compounds in human subjects, it is acknowledged that clinical trials in diabetic complications are long in duration and often complicated by factors such as placebo effect or the beneficial effects of rigorously adhered to standard-of-care efforts to lower blood glucose and attenuate other risk factors (such as hyperlipidemia, hypertension, or reduce/eliminate smoking). For this reason, it is plausible that given the strong evidence linking RAGE to nondiabetic immune/inflammatory disorders, a step-wise approach to clinical trial testing of the RAGE hypothesis may be more practical, especially given that exacerbations of such immune/inflammatory disorders may be shorter in duration and more limited in scope, thereby accelerating the timeline for establishing efficacy, or not, of RAGE antagonism in the human subject.

Irrespective of the strategy for further development of these agents, an important challenge is to identify target engagement biomarkers to track RAGE activity in readily accessible tissues in the human subject, such that interim analyses may inform successful blockade of RAGE activity—or not—in RAGE signaling target engagement. Toward this end, 2 readily accessible sources of trackable material include plasma/serum and peripheral blood monocytes or monocyte-derived macrophages. What is the evidence supporting the use of these tissue sources to biomark the effectiveness of RAGE signaling inhibitors?

RAGE, Peripheral Blood Monocytes, and Human Subjects

Accruing evidence suggests that tracking RAGE expression in peripheral blood monocytes or monocyte-derived macrophages may reflect activity of this axis in disorders in which RAGE ligands accumulate. Su et al assessed AGER mRNA levels in monocytes from the peripheral blood of type 2 diabetic versus nondiabetic human subjects (50 subjects per group). They reported a statistically significant increase in levels of AGER in the diabetic versus nondiabetic monocytes, which paralleled an increase in serum AGE levels and was inversely correlated with levels of soluble RAGE. Tam et al studied RAGE protein levels by Western blot in 53 diabetic and 52 control subjects and reported that monocyte full-length RAGE expression was significantly higher in the diabetic group but that a splice form of RAGE, called RAGEv1 or endogenous secretory (es)RAGE was lower. They reported an inverse association between monocyte RAGE expression and serum sRAGE but not serum esRAGE levels.

Others asked whether RAGE expression in myeloid cells tracked with tissue activities in disease. Sunahori et al examined synovial tissue from rheumatoid arthritis subjects and reported that AGER mRNA expression was higher in this tissue from rheumatoid arthritis versus osteoarthritis subjects and that CD68 lining macrophages in the synovial tissue expressed high levels of RAGE expression. Furthermore, they showed that RAGE expression could be augmented on normal monocytes by incubation with rheumatoid arthritis synovial tissue cell culture supernatants or by treatment by cytokines, especially IL1B. Uhle et al reported that severe trauma or surgical intervention resulted in a downregulation of monocytic RAGE expression by flow cytometry compared with the admission values and a trend toward lower levels of monocyte RAGE when compared with healthy controls. These authors showed that post-trauma, 2 phases of release of RAGE ligands occurred and they speculated that their interaction with RAGE contributed to modulation of immune responses after severe
injuries. Evidence that the changes in RAGE expression in such settings is not spurious but, rather, that specific cues in distinct environments may result in alterations in monocyte RAGE expression was evident from studies by Stew et al who showed that there are no differences in monocyte cell surface RAGE expression between tuberculosis patients with or without type 2 diabetes mellitus.

In other studies, researchers treated monocytes or monocyte-derived macrophages from diabetic or nondiabetic human subjects with RAGE ligands and showed that (1) treatment of monocytes with AGE-BSA increased expression of CD36 and production of reactive oxygen species, which was blocked by RNAi-knockdown of AGER; (2) treatment of monocytes/macrophages with RAGE ligands AGES or S100B or incubation with high glucose increased generation of reactive oxygen species, which was blocked by anti-RAGE, but not by control IgGs; and (3) treatment of THP1 macrophage-like cells with RAGE ligand S100B increased phosphorylation of pleckstrin and consequent phosphorylation of protein kinase C substrates, which was suppressed by RNAi-knockdown of PLEC. These authors showed that phosphorylation of pleckstrin was increased in diabetic mononuclear phagocytes compared with control, thereby linking RAGE ligands to mechanisms that regulate activity of protein kinase C.

**RAGE and sRAGEs**

There are 2 forms of sRAGEs detectable in human subjects; the first is total sRAGE, which reflects cell surface cleaved material by the actions of matrix metalloproteinases or ADAM10; and the second is esRAGE, which results from a splice variant of RAGE. Although the precise source(s) of sRAGEs is not fully clarified, it is plausible that vascular cells and circulating immune cells, such as monocytes, account for detectable sRAGEs in circulation. A recent literature search (PubMed) revealed that >500 articles have been published in which levels of sRAGE and esRAGE have been tested in a variety of disorders known to be linked to the accumulation of RAGE ligands. Whether monitoring levels of sRAGEs may be of value in biomarking target engagement is yet to be determined. However, it is notable that therapeutic interventions such as dexamethasone; statins, telmisartan, curcumin, vitamin D, and rosiglitazone; physical exercise; and bariatric surgery may modulate levels of sRAGEs.

**Summary and Perspectives:**

**Heading Into the Future**

Much of the past and current study on RAGE in monocytes/macrophages and other key cell types linked to cardiovascular disorders has focused on probing roles for the receptor and its signaling axis in initiation and progression of diabetic (and nondiabetic) atherosclerosis and in diet-induced obesity, insulin resistance and type 2 diabetes mellitus. Key future directions include the testing of RAGE/DIAPH1 in regression of diabetic atherosclerosis and in established obesity or weight loss paradigms, as these are equally and highly relevant clinical milieus that need to considered in clinical studies.

The Figure indicates the known effects of RAGE ligands on monocyte/macrophage properties. Specifically, evidence...
for important roles for RAGE in monocyte/macrophage recruitment, cholesterol efflux, and reverse cholesterol transport, oxidative stress, and inflammation stimulate us to keep digging to discover what are the precise biochemical and molecular pathways thatunderlie experimental findings. Lead pathways for investigation include those that regulate glucose and lipid (fatty acid) metabolism and addressing whether energy preferences mediate key RAGE actions in these cells in immunometabolic milieu. Finally, the unexpected, but intriguing finding that mice devoid of Ager are protected from high-fat diet–induced obesity and its metabolic consequences, spurs us to address the unresolved question on RAGE’s natural functions. No doubt that RAGE actions impart considerable maladaptive consequences, leading to chronic disease, especially in cardiometabolic disorders. Perhaps, however, the silver lining is the ability of the RAGE to combat starvation stress by conservation of energy. Answers to these questions will help to unravel the complex biology of RAGE, understand its value through evolution and to establish its utility or not as a target for therapeutic intervention in chronic cardiometabolic and immune/inflammatory diseases.

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Schmidt RAGE, Macrophages, Obesity, and Atherosclerosis


