Activation of Receptor for Advanced Glycation End Products
A Mechanism for Chronic Vascular Dysfunction in Diabetic Vasculopathy and Atherosclerosis

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Abstract—Receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules and engages diverse ligands relevant to distinct pathological processes. One class of RAGE ligands includes glycoxidation products, termed advanced glycation end products, which occur in diabetes, at sites of oxidant stress in tissues, and in renal failure and amyloidoses. RAGE also functions as a signal transduction receptor for amyloid β peptide, known to accumulate in Alzheimer disease in both affected brain parenchyma and cerebral vasculature. Interaction of RAGE with these ligands enhances receptor expression and initiates a positive feedback loop whereby receptor occupancy triggers increased RAGE expression, thereby perpetuating another wave of cellular activation. Sustained expression of RAGE by critical target cells, including endothelium, smooth muscle cells, mononuclear phagocytes, and neurons, in proximity to these ligands, sets the stage for chronic cellular activation and tissue damage. In a model of accelerated atherosclerosis associated with diabetes in genetically manipulated mice, blockade of cell surface RAGE by infusion of a soluble, truncated form of the receptor completely suppressed enhanced formation of vascular lesions. Amelioration of atherosclerosis in these diabetic/atherosclerotic animals by soluble RAGE occurred in the absence of changes in plasma lipids or glycemia, emphasizing the contribution of a lipid- and glycemia-independent mechanism(s) to atherogenesis, which we postulate to be interaction of RAGE with its ligands. Future studies using mice in which RAGE expression has been genetically manipulated and with selective low molecular weight RAGE inhibitors will be required to definitively assign a critical role for RAGE activation in diabetic vasculopathy. However, sustained receptor expression in a microenvironment with a plethora of ligand makes possible prolonged receptor stimulation, suggesting that interaction of cellular RAGE with its ligands could be a factor contributing to a range of important chronic disorders. (Circ Res. 1999;84:489-497.)

Key Words: glycoxidation ■ diabetes ■ nuclear factor-κB ■ inflammation ■ amyloid

Studies of proinflammatory cytokines have provided vascular biologists with important insights into the pathogenesis of the acute inflammatory response, either locally or in sepsis. Rapid production of mediators such as tumor necrosis factor-α and other cytokines/chemokines induced leukocyte adherence to the vessel wall and activation, a diminished vascular barrier function, an altered vasomotor tone, and a pronounced shift in hemostatic mechanisms favoring procoagulant events.1–3 One of the key facets of this acute host response derives from its limited duration; levels of cytokines/chemokines quickly decay, and evidence of cellular activation recedes. Much less is known about mechanisms underlying the pathogenesis of chronic vascular lesions in which cellular activation and dysfunction occur over years. The best-studied example of sustained vascular perturbation results from elevated levels of plasma lipids/lipoproteins which, by themselves, induce atherosclerosis in genetically manipulated mice.4–7

Diabetes provides a distinct model of chronic vascular disease in which disordered glucose homeostasis triggers abnormalities eventuating in dysfunction of virtually every organ, deriving, in part, from vascular perturbation. Although superimposition of other risk factors, such as hyperlipemia or hypertension, adds to the complex atherogenic milieu, diabetes by itself is a well-recognized independent cardiovascular risk factor.8–12 In fact, up to 80% of deaths in patients with diabetes are closely associated with vascular disease. The impact of diabetic complications in economic terms is emphasized by the consumption by diabetic patients of the largest share of the health care...
dollar in the United States compared with any other single disease.

Several candidate mechanisms contributing to perturbation of vascular properties in diabetes have been proposed. Two such mechanisms that have received wide attention include the polyol pathway and diacylglycerol-mediated activation of protein kinase C. Elevated flux of metabolites through the polyol pathway results in excess generation of sorbitol, decreased myoinositol uptake, and diminished Na/K ATPase activity and has been suggested as a means of globally perturbing cellular functions in the setting of hyperglycemia. However, the most striking benefit of aldose reductase inhibitors studied to date has been in models of neuropathy, and their efficacy in the clinical setting has been less than expected to date. Hyperglycemia-mediated activation of protein kinase C, resulting in recruitment of effector mechanisms causing increased vascular permeability and ameliorating retinopathy, has been demonstrated. The apparent success of selective inhibitor protein kinase C II in preventing early changes in vascular barrier function and retinopathy leaves us now poised to meet the challenge of demonstrating efficacy in models of chronic organ system dysfunction most characteristic of diabetes. The proposed association of sustained activation of protein kinase C with diabetes will require especially careful analysis, as it contrasts with the well-known short-term activation of protein kinase C isoforms well known to be followed by a state refractory to subsequent stimulation. This Mini Review focuses on another mechanism proposed to contribute to diabetic vasculopathy, the accumulation of glycoxidation products that occur in the extracellular space and within cells of the vessel wall.

**Glycoxidation Products: Hyperglycemia, Molecular Turnover, and Oxidant Stress**

Although virtually any polypeptide can be subject to glycoxidation posttranslational modification over time, 3 factors have been recognized as critical to the formation of glycoxidation products: the extent of hyperglycemia, the turnover of substrates for glyoxidation, and the pro-oxidant nature of the tissue microenvironment. In the setting of hyperglycemia, nonenzymatic glycoxidation results from the interaction of aldoses, such as glucose, with free amino groups on polypeptides or lipids. Formation of early glycation end products, such as Schiff bases and Amadori products, the best-known of which is hemoglobin A1c, is reversible. Further molecular rearrangements, often involving oxidation, eventuate in the formation of advanced glycation end products (AGEs). Recent studies from Dr Michael Brownlee’s laboratory have emphasized the accelerated time course for intracellular glycoxidation in an environment with elevated levels of glucose, resulting in high concentrations of intracellular aldose phosphates, which enhance glycation compared with aldoses alone. Such intracellular glycoxidation has been shown to alter properties of critical growth factors, such as fibroblast growth factor. Once formed, AGE-protein adducts, such as carboxymethyl-lysine and pentosidine linked to polypeptides, are quite stable and, in fact, virtually irreversible. Although there are many other AGE-related chemical structures likely to be present in deposits of AGEs found in the vasculature and other tissues (eg, pyrroline that from as a result of glycation alone), carboxymethyl-lysine-protein adducts are the predominant AGEs present in vivo. For example, carboxymethyl-lysine epitopes are increased at sites of atherosclerotic lesions, especially in patients with concomitant diabetes. Pentosidine, a key AGE cross-link, has also been identified at pathological sites, and cross-link–induced increased tissue rigidity could have important functional and architectural effects on mechanical properties of tissues. Probably the most relevant recent insights in AGE biology concern the association of AGEs with sustained oxidant stress; thus, situations in which the local redox potential has been shifted to favor oxidant stress, as at inflammatory loci or, more generally, in patients with renal failure, AGE formation is substantially enhanced. Taken together, these findings indicate that although AGEs may have been previously considered to be restricted to diabetes, it is now recognized that oxidation alone can lead to AGE formation and AGEs have been identified in atherosclerotic tissues without diabetes (see references above). The association of AGEs with factors other than hyperglycemia is underscored by their occurrence in amyloïdoses, in which tissue accumulation of amyloidogenic polypeptides occurs. Dialysis-related amyloidosis occurs in patients on long-term hemodialysis and results from the accumulation of amyloid largely composed of β2-microglobulin in joint tissues. Clinically, this disorder is characterized by periartricular soft-tissue swelling, diffuse destructive arthropathy, and subchondral bone erosions and cysts. Study of β2-microglobulin from patient-derived tissues has demonstrated the abundance of an AGE-modified form likely due to the concerted effects of delayed macromolecular turnover and renal failure in driving glycoxidation. In Alzheimer disease, AGE modification of paired helical filament tau and of amyloid β peptide (Aβ) has also been observed in patients without diabetes or renal insufficiency, consistent with AGE modification of macromolecules the turnover of which has been delayed by other pathological processes. In each of these situations, glycoxidation superimposed on amyloid deposition is likely to increase the pathogenicity of the amyloid by virtue of the ability of AGEs to form cross-links, to generate reactive oxygen species, and to interact with cellular receptors (see below). Because the formation of AGEs appears to be virtually irreversible, their accumulation in tissues imparts, likely, a chronic, long-term “memory” for previous perturbations and could render the host response to future challenges quite different from that observed in normal tissues.

**Cellular Interactions of AGEs: Central Role of Receptor for AGE (RAGE)**

Experiments with AGEs prepared in vitro, made by incubating a variety of proteins (especially albumin) with high concentrations of aldoses/aldose phosphates, or more limited studies with AGEs derived from in vivo sources, have demonstrated a spectrum of altered cellular properties. For example, in the endothelium, AGEs diminish vascular barrier function, enhance expression of vascular cell adhesion molecule-1 (VCAM-1), quench nitric oxide, and alter the
balance of cellular coagulant properties, in part through induction of procoagulant tissue factor. These cellular effects of AGEs are largely mediated by their specific engagement of cell surface molecules. Our studies have led to the identification, cloning, and analysis of RAGE, the best-characterized signal transduction receptor for AGEs. The type A macrophage scavenger receptor and other polypeptides potentially present on the cell surface (p60/p90/galectin-2) have also been identified as possible AGE binding sites. The role of these other AGE binding sites in AGE-mediated cellular activation remains to be determined. However, the scavenger receptor may exert its effect principally at the level of endocytotic clearance of AGEs, and it is possible that p60/p90/galectin-2 recognizes different AGE structures compared with RAGE. Because of the incomplete information available on these binding sites in AGE biology, it is premature to make detailed comparisons between these molecules and RAGE, and this issue will not be discussed further in this brief review.

RAGE is a member of the immunoglobulin superfamily of cell surface molecules, and its extracellular portion, including 332 amino acids, comprises 1 V-type domain followed by 2 C-type domains. Structural determinants in the receptor mediating binding of AGEs are harbored in the N-terminal V domain. Among immunoglobulin superfamily members, the RAGE sequence is most homologous to MUC 18 and the neural cell adhesion molecule. After the extracellular region, there is a single transmembrane-spanning domain and a short, highly charged cytosolic tail at the C terminus. The latter portion of RAGE is most homologous to the B cell activation marker CD20 and probably binds signal transduction molecules in the cytoplasm to recruit cellular effector mechanisms once ligand occupies the receptor. Placement of RAGE in the immunoglobulin superfamily members suggested that RAGE might participate in the host response to environmental perturbation, as do cell adherence molecules, rather than solely functioning as a scavenger of modified polypeptides. Consistent with this concept, the RAGE gene is on chromosome 6 in the major histocompatibility complex between genes for class II and class III. Furthermore, analysis of the RAGE promoter shows putative nuclear factor-κB (NF-κB) sites, along with an interferon-γ response element and nuclear factor-interleukin 6 DNA binding motif. We have analyzed the 3 NF-κB sites and found 2 of them to be active and involved in the regulation of RAGE expression.

Studies on human and rodent tissues have shown a characteristic pattern of RAGE expression. During development, the receptor is present at high levels, especially in the central nervous system. This led us to seek a ligand for RAGE of which the expression would be enhanced during development, an unlikely time for AGE formation. Amphoterin, a protein associated with basement membranes and abundant in the developing central nervous system, was found to bind RAGE. On amphoterin-coated matrices, RAGE mediates neurite outgrowth in primary cultures of rat cortical neurons. Furthermore, expression of RAGE and amphoterin in the developing rat brain is closely coordinated both temporally and spatially. Although these results do not establish a cause-effect relationship for amphoterin-RAGE interaction in the nervous system, they do suggest a role for RAGE under physiological conditions, rather than the unlikely possibility that RAGE participates solely in pathological events.

As animals mature, RAGE expression decreases to low levels in a range of cells, including endothelium, smooth muscle cells, mononuclear phagocytes, pericytes, neurons, cardiac myocytes, hepatocytes, and Muller and bipolar ganglion cells of the retina. However, when particular pathological processes intervene, RAGE expression increases, and receptor upregulation can be sustained, apparently over years. A striking feature of pathological lesions characterized by an abundance of RAGE-expressing cells is the almost invariable association with sites of accumulated RAGE ligands. For example, in diabetic vasculature, cells expressing high levels of RAGE are often proximal to areas in which AGEs are abundant. A similar relationship has been shown in affected vasculature from patients with Alzheimer disease, in which RAGE functions as a cell surface receptor for Aβ-mediated cellular perturbation under conditions in which low (nanomolar) levels of amyloidogenic material are present.

In the Alzheimer disease brain, RAGE is expressed in the vasculature in proximity to deposits of Aβ compared with age-matched controls (Figure 1). A molecular basis for these immunohistological findings is suggested by the results of our initial experiments analyzing regulatory elements in the RAGE promoter; NF-κB sites 1 and 2 are likely to have an important role in ligand-associated upregulation of RAGE. We have found that engagement of RAGE by AGEs or Aβ results in activation of NF-κB, thereby triggering a positive feedback loop in which increased RAGE expression enhances the capacity of the cell for subsequent binding of
AGEs/Aβ. These events perpetuate another cycle of increased receptor expression and cellular perturbation. Such a mechanism stands out in sharp contrast to the acute and self-limited host response to a burst of tumor necrosis factor-α production after exposure of cells to lipopolysaccharide.²

As a first test of the involvement of the AGE-RAGE axis in vascular perturbation, the effect of RAGE on barrier function of diabetic vasculature was studied. Increased vascular leakage is a well-known feature of diabetic microvasculature⁶⁻⁷² and is mirrored in rodent models of diabetes.⁹,³³ Induction of diabetes using the β-cell toxin streptozotocin has been shown to decrease insulin levels, resulting in a state analogous to type I or insulin-dependent diabetes. By 10 to 11 weeks after administration of streptozotocin, diabetic animals display increased vascular leakage, as demonstrated by several methods, including the tissue-blood isotope ratio.⁴⁷,⁷³ Increased vascular permeability in our diabetic rats was most evident in intestine, skin, and kidney, in which albumin leakage was increased 2.8-fold, 3-fold, and 2.8-fold, respectively, compared with nondiabetic controls (Figure 2). Blockade of AGE-RAGE interaction was accomplished using a truncated soluble form of the receptor, which we have termed sRAGE, composed of only the extracellular domain (V-C-C).⁴⁷ In vitro, we have found that sRAGE binds up AGES and prevents their activation of the cell surface receptor.³⁷,⁴⁷ Furthermore, sRAGE, either that prepared from tissues or that produced using the baculovirus expression system, was especially suitable for these and longer-term experiments: (1) it was conveniently produced and purified to homogeneity in a lipopolysaccharide-free form; (2) its half-time for elimination from the plasma was 22 hours, allowing once-daily intraperitoneal administration; and (3) sRAGE occurs in normal plasma and, thus, is not a foreign species that incites an immune response (even after several months of parenteral administration). However, the low levels of RAGE in normal and diabetic plasma (picogram range) are insufficient to antagonize AGE-RAGE interaction. Diabetic rats were treated with a single dose of sRAGE, 2.25 or 5.15 mg/kg, resulting in plasma levels of sRAGE corresponding to 10 to 30 and 40 to 60 μg/mL, respectively. Vascular permeability studies were then performed using the tissue-blood isotope ratio (Figure 2); sRAGE at the lower dose completely blocked vascular leakage in intestine and skin and largely prevented it in the kidney (≈60%). The higher dose of sRAGE suppressed hyperpermeability completely in intestine and skin and by ≈90% in kidney. These data emphasize the contribution of a reversible component of diabetic vascular dysfunction to hyperpermeability in streptozotocin-treated diabetic rats at 10 weeks. Furthermore, since administration of sRAGE reversed vascular leakage, antagonism of AGE-RAGE interaction may be a relevant strategy for preventing vascular dysfunction.

![Figure 2](image-url) "Effect of sRAGE infusion on vascular permeability in streptozotocin-treated rats assessed using the tissue-blood isotope ratio (TBIR) method. Results are presented as mean±SEM, and data were analyzed by 1-way ANOVA followed by Dunnett’s test to compare diabetic sRAGE-treated and vehicle-treated rats. Data for each organ were analyzed separately. *P<0.05; **P<0.01. Reproduced with permission from Wautier JL et al. J Clin Invest. 1996;97:238–243 (Reference 47)."

![Figure 3](image-url) "Schematic depiction of a 2-hit model of vascular perturbation in which interaction of RAGE with its ligands resets the baseline, resulting in a state of cellular activation on which other stimuli are superimposed. M₁ (red) indicates macrophage."

**RAGE and a Two-Hit Model of Vascular Perturbation**

These data suggested a possible fundamental difference between normal tissues and those tissues with abundant RAGE ligands, such as AGES or Aβ. We have encapsulated these considerations into a 2-hit model of vascular perturbation (Figure 3). AGE-rich tissues are populated by cells expressing high levels of RAGE and are subject to sustained AGE-RAGE interaction resulting in chronic cellular activation (see below). The latter constitutes a chronic, underlying “first” stimulus/hit in our model. We hypothesize that in AGE-enriched tissues of diabetes, for example, superimposition of a second stress, such as accumulated lipoproteins in atherosclerosis, results in an exaggerated, chronic inflammation and accelerated atherosclerosis, which are typical of diabetes. We believe this concept can be extrapolated to the pathogenesis of other pathological conditions related to diabetic complications, as well as to consequences of amyloid angiopathy. Impaired wound healing in diabetes extends this concept to the host response to acute tissue damage, including the presence of a foreign body and introduction of bacterial pathogens. In contrast to the rapid and transient triggering of repair mechanisms in normal tissues, AGE-RAGE interaction introduces a sustained and upwardly spiraling inflammatory component preventing normal tissue repair from reaching completion. Cerebral amyloid angiopathy involves vascula-
tured rich in Aβ and AGEs with high levels of RAGE-bearing cells subject to repeated episodes of ischemia. The likely impact of sustained RAGE activation in this setting is enhanced severity of vascular and cerebral damage, as mechanisms to limit the destructive host inflammatory response are suppressed and repair cannot be consummated.

The hypothesis outlined in Figure 3 led us to develop a model system in which AGE-RAGE interaction was blocked in a setting of ongoing vascular perturbation in an AGE-rich environment. Accelerated atherosclerosis in patients with diabetes provided an excellent arena to test this concept in view of its clinical significance and the known role of additional risk factor(s), other than hyperglycemia, hypertension, and hyperlipidemias. However, small-animal models mirroring macrovascular disease associated with diabetes have been difficult to develop. For example, alloxan-induced diabetic rabbits displayed protection from diet-induced atherosclerosis probably as the result of accumulated large triglyceride-rich lipoproteins in the plasma that could not enter the vessel wall. The optimal choice would be to use a rodent model, allowing exploitation of genetically manipulated mice. However, previous studies have shown only enhanced fatty streak formation in diabetic BALB/c versus strain-matched euglycemic controls. For this reason, we turned to atherosclerosis-prone mice, initially animals with homozygous deletion of the apolipoprotein E (apoE) gene, which had been backcrossed 10 times into the C57BL/6J background to increase genetic homogeneity. Induction of diabetes was accomplished using streptozotocin. Within 7 weeks of streptozotocin administration, diabetic apoE-null animals showed atherosclerosis of increased severity compared with euglycemic apoE controls. Methylene blue-stained preparations of mouse aortas demonstrated abundant lesions at aortic branch points and the lesser curvature versus controls (Figure 4A and 4B). Quantitative analysis of lesion formation showed ≈5.3-fold increased lesion area in diabetic versus euglycemic apoE-null mice (Figure 5A). Microscopic examination of vascular tissues displayed not only a more rapid time course for development of vascular lesions in diabetic animals but also more complex lesions (fibrous caps, extensive monocyte, smooth muscle infiltration, etc) and atherosclerosis extending distally in the aorta and major arteries. Increased expression of RAGE and the presence of AGEs in the vessel wall, especially at sites of vascular lesions in diabetic apoE-null animals, was evident. Although to a lesser extent than in diabetic animals, nondiabetic apoE-null mice also showed increased expression of the receptor and deposition of AGEs compared with wild-type C57BL/6J. These data are consistent with the previously noted occurrence of AGEs in vascular lesions from atherosclerosis-prone hyperlipidemic rabbits and in human vascular samples in the absence of diabetes. The pro-oxidant environment present in lipid-rich vascular lesions infiltrated by inflammatory effector cells would appear sufficient to drive AGE formation in these settings, as described above. It is important to emphasize that vascular changes in streptozotocin-treated animals were not due to toxic effects of this drug other than induction of diabetes. About 5% of mice receiving streptozotocin did not become diabetic, although they were treated with the same lot and dose of drug at the same time. Atherosclerosis in these animals was identical to that in apoE-null mice treated with vehicle alone; ie, it was not accelerated as in the diabetic animals. Further support for the generality of our observations concerns recent extension of our findings to other genetically manipulated atherosclerosis-prone mice and crossbreeding studies into the genetically diabetic db/db background.

In view of the increased expression of RAGE in the vasculature of diabetic apoE-null animals, along with the presence of AGEs, we used sRAGE to interrupt the cycle of AGE engagement of RAGE potentially underlying sustained cellular activation. Animals were treated with several concentrations of sRAGE over a 6-week period after the induction of diabetes, and dose-dependent reduction in lesion formation to baseline (ie, vascular lesions observed in euglycemic apoE-null mice) was observed (Figure 5A). Analysis of aortas showed a striking suppression of lesions in samples from diabetic apoE-null mice treated with sRAGE compared with controls (Figure 5B and 5C). Lesions that did form in animals receiving sRAGE appeared largely arrested at the fatty streak stage; complex lesions were much less abundant than in untreated/vehicle-treated diabetic apoE-null animals. Unexpectedly, the tissue AGE burden in mice treated with sRAGE was also substantially diminished (Figure 5D). This may represent attenuated formation of AGEs and/or accelerated clearance of AGEs after formation of sRAGE-AGE complexes. Such complexes could be detected by immuno-precipitation in the plasma of animals treated with sRAGE. Diminished AGE formation might be related to sRAGE-mediated suppression of local oxidant stress by blocking RAGE-induced cellular activation (see below). This would be consistent with the diminished susceptibility to copper-induced oxidation of LDL retrieved from sRAGE-treated diabetic apoE-null mice compared with LDL obtained from control diabetic apoE-null mice.

An important facet of the analysis of diabetic apoE-null animals concerned the effect of diabetes and sRAGE treatment on the lipoprotein profile and hyperglycemia. Induction of diabetes in mice caused a ≈2-fold increase in total cholesterol, which was mainly due to elevated levels of chylomicrons/VLDL and IDL/LDL, as determined by ultracentrifugation, whereas triglycerides were unchanged. Treat-
ment of animals with sRAGE had no effect on their cholesterol or triglyceride levels and also resulted in no change in glycemia or hemoglobin A1c. These studies delineate a lipid- and glycemia-independent factor in diabetic atherosclerosis due, at least in part, to AGE-RAGE interaction.

RAGE-Induced Cellular Activation: Induction of Oxidant Stress

RAGE functions as a signal transduction receptor mediating binding of AGEs and other ligands (see other sections of this review) to the cell surface and activating intracellular signal transduction mechanisms. One pathway of RAGE-dependent cellular perturbation includes activation of p21ras, followed by activation of mitogen-activated protein (MAP) kinases and nuclear translocation of the transcription factor NF-κB, resulting in transcription of target genes. Binding of AGEs to RAGE increases levels of GTP-bound p21ras within 10 minutes. Depletion of endogenous intracellular glutathione with l-buthionine-(S,R)-sulfoximine enhanced p21ras activation consequent to AGE-RAGE interaction, consistent with an oxidant stress–mediated mechanism. The MAP kinases extracellular signal–regulated kinases (ERKs) 1 and 2 are targets of activated p21ras in cells exposed to AGEs; ERK1 and ERK2 activation peaked within 15 to 20 minutes. The close relationship of p21ras activation to activation MAP kinases was indicated by the inhibition of ERK1 and ERK2 activation in the presence of the farnesyl transferase inhibitor α-hydroxyfarnesylphosphonic acid. Furthermore, when wild-type p21ras was substituted for a mutant in which cysteine present at residue 118, known to be a target of reactive free radicals in p21ras, was replaced by serine, AGE-RAGE–dependent ERK1 and ERK2 activation was blocked. Each of these events was closely tied to AGE binding to RAGE, as blockade of the receptor with either anti-RAGE IgG or excess sRAGE prevented NF-κB activation. Additional evidence to support the role of RAGE as a signaling molecule comes from our recent data indicating that expression of a dominant negative form of RAGE (a mutant lacking the cytosolic tail) prevents AGE-induced cellular activation (A.M. Schmidt, S.D. Yan, and D. Stern, unpublished observation, 1998). Furthermore, the principal AGE ligand of RAGE, carboxymethyl-lysine adducts, appears to be biologically inert in the absence of the...
receptor; it does not generate reactive oxygen species, it is not fluorescent, it does not form cross-links, etc. Similar observations concerning RAGE-dependent signal transduction have been made when Aβ is the RAGE ligand.

Expression of NF-κB-regulated genes is observed in pathological samples in which RAGE and its ligands are present at high levels. For example, increased expression of VCAM-1 and heme oxygenase type I have been noted in diabetic tissues. Infusion of AGEs into rodents enhances expression of these cell stress markers in a RAGE-dependent manner and is closely correlated with NF-κB activation. In Alzheimer disease, Aβ engagement of RAGE on neurons leads to expression of macrophage colony-stimulating factor (M-CSF), the expression of which is also subject to regulation by NF-κB. Accordingly, Aβ applied to neuron-like cells induces activation of NF-κB and expression of M-CSF in a RAGE-dependent manner. The potential contribution of these mediators to pathogenesis of chronic tissue injury can be easily suggested; increased expression of VCAM-1 in diabetic vasculature would enhance mononuclear phagocyte mediators to pathogenesis of chronic tissue injury can be in a RAGE-dependent manner. The potential contribution of these mediators to pathogenesis of chronic tissue injury can be easily suggested; increased expression of VCAM-1 in diabetic vasculature would enhance mononuclear phagocyte adherence to the vessel wall promoting atherogenesis. In Alzheimer disease, Aβ engagement of RAGE on neurons leads to expression of macrophage colony-stimulating factor (M-CSF), the expression of which is also subject to regulation by NF-κB. Accordingly, Aβ applied to neuron-like cells induces activation of NF-κB and expression of M-CSF in a RAGE-dependent manner. The potential contribution of these mediators to pathogenesis of chronic tissue injury can be easily suggested; increased expression of VCAM-1 in diabetic vasculature would enhance mononuclear phagocyte adherence to the vessel wall promoting atherogenesis.

Hypothesis

These data lead us to hypothesize that activation of RAGE occurs in diverse circumstances using a repertoire of ligands. In the setting of hyperglycemia, oxidant stress, renal failure, and amyloidosis, glycoxidation causes formation and deposition of AGEs in the tissues and vasculature. RAGE expression is enhanced, and the prolonged proximity of AGEs to cells expressing RAGE sets the stage for sustained cellular activation. Carboxymethyl-lysine adducts, produced by glycoxidation, by oxidation alone, or in acute inflammation, may be an important component of RAGE activation in each of these settings. Alzheimer disease is another situation in which cells expressing high levels of RAGE, neurons, microglia, and cells of the vasculature are closely apposed to ligand, Aβ, over long periods. In addition, we have identified an inflammatory cytokine-like molecule, termed EN-RAGE, which is elaborated by polymorphonuclear leukocytes at inflammatory loci and which induces activation of RAGE-bearing cells. In contrast to other host response systems in which a negative feedback loop terminates cellular activation, ligand engagement of RAGE appears to recruit cellular effector mechanisms such as activation of NF-κB, thereby enhancing receptor expression and perpetuating cellular perturbation in an ascending spiral. Taken together, these data suggest that intercepting the vicious cycle of ligand-RAGE interaction will interrupt cellular activation, potentially having a profound effect on a range of chronic disorders. However, definitive proof that RAGE-dependent mechanisms actually underlie the pathogenesis of human diseases must await more direct experiments in mice, in which expression of RAGE has been genetically manipulated, and in a range of higher species and humans after the development of low molecular weight RAGE inhibitors.

Although it is tempting to speculate that RAGE is optimally designed for molecular mischief in pathological states, it is essential to note that RAGE is likely to have important roles in discrete subsets of homeostasis as well. In adult rodents, antagonism of RAGE by administration of soluble receptor for up to 6 months has no adverse effects. This is consistent with the low levels of RAGE observed in tissues of mature animals in the absence of pathological processes. However, in development, high levels of RAGE are present in particular organ systems, especially the central nervous system. As noted previously, neurons expressing high levels of RAGE also produce amphoterin, another RAGE ligand. RAGE-dependent neurite outgrowth suggests a possible role of the receptor cell matrix interactions during normal development. Generation of RAGE knockout mice and targeted overexpression of dominant negative RAGE constructs should provide insights into such physiological functions of RAGE.

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