RAGE Axis
Animal Models and Novel Insights Into the Vascular Complications of Diabetes

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Abstract—Receptor for AGE (RAGE) is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules. Engagement of RAGE by its signal transduction ligands evokes inflammatory cell infiltration and activation in the vessel wall. In diabetes, when fueled by oxidant stress, hyperglycemia, and superimposed stresses such as hyperlipidemia or acute balloon/endothelial denuding arterial injury, the ligand–RAGE axis amplifies vascular stress and accelerates atherosclerosis and neointimal expansion. In this brief synopsis, we review the use of rodent models to test these concepts. Taken together, our findings support the premise that RAGE is an amplification step in vascular inflammation and acceleration of atherosclerosis. Future studies must rigorously test the potential impact of RAGE blockade in human subjects; such trials are on the horizon. (Arterioscler Thromb Vasc Biol. 2004;24:1-8.)

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The receptor for advanced glycation end products (RAGE) is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules.1,2 Its ability to recognize multiple classes of ligands, such as advanced glycation end products (AGEs), S100/calgranulins, amphoterin, amyloid-β peptide and β-sheet fibrils, and MAC-1,3–8 suggests that the repertoire of RAGE-dependent effects in the tissues may be diverse. In this context, the function of this receptor does not appear to be to degrade/defoxify ligand, but rather, by RAGE cytosolic domain-triggered signal transduction, to propagate immune/inflammatory responses.3,9

Although AGEs are an heterogeneous class of compounds, studies have shown that a particular class of these species, N-carboxymethyl lysine (CML) adducts of proteins/lipids, are specific signal transduction ligands of RAGE.9 CML-modified adducts may be generated by a variety of stimuli. In addition to generation in hyperglycemia, CML adducts may also be generated by activation of the myeloperoxidase pathway.10 Recent studies have shown that peroxynitrite may induce formation of CML by cleavage of an Amadori product and, also, by the generation of reactive α-oxoaldehydes from glucose.11 These considerations highlight the concept that once set in motion, diabetes-associated hyperglycemia and oxidant stress magnify production of a wide array of biochemical species that accumulate to stimulate cellular activation and sustain tissue perturbation.

Furthermore, in diabetes, distinct mechanisms in diabetes may be engaged to further fuel the cycle of AGE generation and oxidant stress.12 One specific example is the polyl pathway. Glucose is reduced to sorbitol by aldose reductase, the central enzyme of this pathway. This results in generation of fructose; fructose is converted into fructose-3-phosphate by the action of 3-phosphokinase. A chief product of this reaction is 3-deoxyglucosone, a key precursor in the generation of multiple types of AGEs, such as CML adducts and others.13,14 It has been shown that in renal failure, plasma levels of 3-deoxyglucosone increase in parallel with increased levels of aldose reductase in erythrocytes.14 Administration of an inhibitor of aldose reductase, epalrestat, to subjects with diabetes resulted in reduced levels of CML adducts and their precursors in erythrocytes and decreased plasma levels of thiobarbituric acid reactive substances.15 These considerations highlight the concept that multiple forces are in play in diabetes to augment generation of tissue injurious biochemical species that signal chronic cellular perturbation.

Indeed, once AGEs are formed, their interaction with RAGE triggers generation of proinflammatory cytokines, adhesion molecules, and chemokines. Attraction of inflammatory cells such as polymorphonuclear leukocytes, mononuclear phagocytes, and T-lymphocytes into the tissue provides a mechanism to sustain the inflammatory response. In the context of RAGE, these cells may harbor proinflammatory S100/calgranulins and amphoterin. Once these molecules are released into the tissue, S100/calgranulins/amphoterin–RAGE interaction may amplify tissue inflammation and injury by autocrine and paracrine pathways.3,16–18

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that RAGE contributes to the acceleration of atherosclerosis, in addition to the increased incidence of cardiac events, such as heart attacks and strokes.19–22 Studies in human subjects indicate that RAGE and its signal transduction ligands are expressed in diabetic vasculature and atherosclerotic plaques.23,24 Cipollone et al showed that compared with nondiabetic human plaques, diabetic plaques were characterized by greater numbers of mononuclear phagocytes, T-lymphocytes, and HLA-DR+ cells, in parallel with increased expression of RAGE, activated NF-kB, cox-2, and matrix metalloproteinases. The extent of RAGE expression was found to correlate with levels of glycosylated hemoglobin.25 Although such studies do not delineate the biochemical/molecular link between the ligand/RAGE axis, inflammation, and atherosclerosis, these experiments nevertheless provide support for the premise that RAGE may participate in augmentation of vascular injury and progression of atherosclerosis.

Studies in murine models are underway to test the premise that RAGE contributes to the acceleration of atherosclerosis in diabetes.

Models of Atherosclerosis in Diabetes

Multiple epidemiologic investigations support the premise that diabetes accelerates atherosclerosis; human subjects with diabetes display a striking acceleration of atherosclerosis, in addition to the increased incidence of cardiac events, such as heart attacks and strokes.19–22 Studies in human subjects indicate that RAGE and its signal transduction ligands are expressed in diabetic vasculature and atherosclerotic plaques.23,24 Cipollone et al showed that compared with nondiabetic human plaques, diabetic plaques were characterized by greater numbers of mononuclear phagocytes, T-lymphocytes, and HLA-DR+ cells, in parallel with increased expression of RAGE, activated NF-kB, cox-2, and matrix metalloproteinases. The extent of RAGE expression was found to correlate with levels of glycosylated hemoglobin.25 Although such studies do not delineate the biochemical/molecular link between the ligand/RAGE axis, inflammation, and atherosclerosis, these experiments nevertheless provide support for the premise that RAGE may participate in augmentation of vascular injury and progression of atherosclerosis.

Studies in murine models are underway to test the premise that RAGE contributes to the acceleration of atherosclerosis in diabetes.

Animal Models and Accelerated Diabetic Atherosclerosis

The first murine model to support the concept that hyperglycemia accelerated atherosclerosis tested the hypothesis that induction of diabetes using multiple low-dose streptozotocin would result in enhanced atherosclerosis in mice fed a Western-type diet.25 In these first studies, wild-type BALB/c and C57BL/6 mice were used. In these animals, genetic manipulation was not used as a means to trigger/enhance the development of atherosclerosis. Rather, dietary modification induced hyperlipidemia. In this study, Kunjathoor et al showed that BALB/c mice fed an atherogenic diet and rendered diabetic with streptozotocin displayed a 17-fold increase in atherosclerotic lesion area compared with citrate-treated (nondiabetic) mice fed the same atherogenic diet. Although the lesions were limited to fatty streaks, histological studies nevertheless revealed the presence of both excess mononuclear phagocytes and smooth muscle cells in the plaques.25 Interestingly, these investigators found that there were no differences in mean atherosclerotic lesion area between diabetic (streptozotocin) versus nondiabetic atherogenic diet-fed C57BL/6 mice.25 These considerations underscore the critical influence that multiple factors may exert on atherosclerosis initiation and progress, such as genetic background, diet, and environment, especially in the setting of superimposed hyperglycemia.

In addition to dietary modulation of lipid profile, genetic modification provides a means to alter lipid profile in mice. To test the role of hyperglycemia in potentially accelerating atherosclerosis, we used mice in which genetic manipulation rendered the animals hyperlipidemic by deletion of apolipoprotein E (apo E/+/−).26–27 Apo E/+/− mice display lesions of multiple phases of atherosclerosis distributed throughout the arterial tree. On normal chow diet, work from the Russell Ross group showed that apo E/+/− mice displayed foam cell lesions as early as 8 weeks of age; by age 15 weeks, advanced lesions were evident.28 Advanced lesions consisted of a fibrous cap containing smooth muscle cells surrounded by a connective tissue matrix covering a necrotic core with foamy macrophages.29 On induction of a Western-type diet, lesions generally were more advanced.28

Based on the observation that apo E/−/− mice developed complex lesions characteristic of advanced human plaques, we sought to test the hypothesis that induction of hyperglycemia in these animals would further accelerate atherosclerosis and lesion complexity. In the first studies, we rendered 6-week-old male apo E/−/− mice diabetic with streptozotocin.29 These apo E/−/− mice were previously backcrossed >10 generations into C57BL/6. After 6 weeks of established diabetes, at age ∼13 to 14 weeks, diabetic apoE−/− mice exhibited discrete lesions at major branch points in the thoracic aorta and at the arch of the aorta. In contrast, age-matched euglycemic apoE−/− mice did not display lesions in the proximal aorta.30 Quantitative morphometric analysis revealed ∼5-fold increase in mean lesion area at the level of the aortic sinus in diabetic apoE−/− mice compared with nondiabetic apoE−/− animals (Figure 1a). Histologic analysis of euglycemic apoE−/− mice revealed typical fatty streaks visualized with oil red O. At this age, the majority of lesions in the nondiabetic animals did not progress to advanced stages. However, in age-matched diabetic apoE−/− mice, larger, more advanced fibrous plaques with a propensity for cap formation were observed (Figure 1b).30 Lesion complexity was defined by the presence of cholesterol clefts, necrosis, or fibrous cap formation.30

In this model, diabetes was followed by a time-dependent increase in plasma total cholesterol.30 After 6 weeks of
diabetes, ~2-fold increase in cholesterol was noted in diabetic apoE<sup>−/−</sup> mice compared with nondiabetic animals, whereas plasma triglyceride levels were unchanged. Fractionation of plasma cholesterol by density gradient ultracentrifugation revealed significant increases in chylomicrons/very-low-density lipoprotein (~2.6-fold) and intermediate-density lipoprotein/low-density lipoprotein (LDL) (~2.5-fold) in age-matched diabetic versus euglycemic apoE<sup>−/−</sup> mice, whereas HDL was more modestly altered (~1.5-fold higher in diabetic compared with control mice). In contrast, density gradient ultracentrifugation showed minimal to no differences in plasma triglyceride profile.

In parallel with elevation of glucose, enhanced formation of AGEs in diabetic apoE<sup>−/−</sup> mice was shown by ~2.3-fold increase in AGE-immunoreactive epitopes in acid-soluble material extracted from kidney tissue after 6 weeks of diabetes, compared with euglycemic apoE<sup>−/−</sup> controls and ~2.5-fold increase in plasma AGEs in diabetic apoE<sup>−/−</sup>/mice compared with nondiabetic control mice. Further studies revealed that aortic lysates retrieved from diabetic apo E<sup>−/−</sup> mice displayed increased levels of the proinflammatory RAGE ligand family, S100/calgranulin epitopes, compared with nondiabetic apo E (0) mice of the same age.

It is of interest that although Kunjathoor et al did not find substantial atherosclerosis in diabetic (streptozotocin) C57BL/6 mice, the trigger to atherosclerosis, dietary-induced hyperlipidemia, differed from the means to induce hyperlipidemia in apo E<sup>−/−</sup> mice. These findings highlight the concept that in addition to genetic background, other influences, such as the degree and nature of the lipid elevation, may importantly influence the development and progression of atherosclerosis in diabetes.

**Accelerated Diabetic Atherosclerosis: The Role of RAGE**

Consequent to the development of the streptozotocin-apo E<sup>−/−</sup> model of complex accelerated atherosclerosis in murine diabetes, the impact of blockade of RAGE was tested. In the first studies, murine-soluble RAGE (sRAGE), the extracellular two-thirds of the receptor that functions to bind ligand and thereby block interaction with and activation of cell surface RAGE, was used. Soluble RAGE was administered once daily by intraperitoneal route to diabetic apo E<sup>−/−</sup> mice, commencing immediately at the time hyperglycemia developed. Compared with diabetic mice receiving mouse serum albumin (MSA), diabetic apo E<sup>−/−</sup> mice treated with sRAGE showed dose-dependent suppression of accelerated atherosclerosis (Figure 1a and 1b, respectively). In addition to diminished atherosclerotic lesion area, lesion complexity was decreased in sRAGE-treated diabetic mice. This facet of the impact of RAGE blockade suggested that antagonism of this axis bore the potential to suppress lesion progression and, possibly, the development of highly inflamed unstable plaques.

Importantly, treatment of diabetic mice with sRAGE did not affect the degree of hyperglycemia or the level of insulinemia compared with diabetic mice treated with murine serum albumin. Total cholesterol and triglyceride were similarly unaffected by treatment with sRAGE, and lipid particle composition was also not changed in diabetic sRAGE-treated apoE<sup>−/−</sup> mice compared with mice receiving murine serum albumin. These data suggested that the beneficial effects of blockade of RAGE were both glycemia-independent and lipid-independent, indicating that factors unique to the diabetic environment were favorably modulated. In this context, we speculate that hyperglycemia and hyperlipidemia fuel the generation of AGES; thus, sRAGE exerts its impact downstream of the traditional risk factors.

Consistent with this premise, levels of kidney and plasma AGE in diabetic mice were suppressed to levels seen in nondiabetic animals by administration of sRAGE in a dose-dependent manner. Similar results were seen on examination of plasma AGES. These findings support the contention that triggers to AGE formation might also be suppressed by RAGE blockade. To begin to address this, LDL was isolated from sRAGE-treated and murine serum albumin-treated mice. Susceptibility to copper-induced oxidation of LDL was diminished compared with diabetic mice treated with murine serum albumin, as assessed by the mean lag time to formation of conjugated dienes.

**Blockade of RAGE: Late Intervention**

To determine the potential impact of RAGE blockade in established atherosclerotic lesions, the streptozotocin-induced diabetes apo E<sup>−/−</sup> model was used. Mice were rendered diabetic with streptozotocin at age 6 weeks and left untreated until age 14 weeks. By age 14 weeks, certain diabetic apo E<sup>−/−</sup> mice were euthanized to establish baseline atherosclerotic lesion area/complexity. Induction of diabetes was associated with increased atherosclerotic lesion area compared with nondiabetic controls at age 14 and 20 weeks (Figure 2b and 2a and 2d and 2c, respectively). In diabetic mice treated with sRAGE from ages 14 to 20 weeks, atherosclerotic lesion area was significantly reduced compared with mice treated with murine serum albumin (Figure 2e and 2d, respectively). Oil red O-stained cross-sections of the aorta through the aortic sinus revealed that compared with nondiabetic mice, diabetic mice displayed larger and more extensive atherosclerotic lesions at 14 weeks (Figure 2f and 2g, respectively) and 20 weeks (Figure 2h and 2i, respectively). In diabetic mice treated with sRAGE, atherosclerotic lesions were smaller than those observed in vehicle-treated diabetic mice at age 20 weeks (Figure 2) and 2i, respectively.

Quantitative morphometric analysis was performed and confirmed that diabetic mice treated with sRAGE at 20 weeks displayed a significant decrease in mean atherosclerotic lesion area compared with murine serum albumin-treated diabetic animals (Figure 3a). Although mean lesion area was increased in diabetic mice at 20 weeks versus 14 weeks, mean lesion area in sRAGE-treated diabetic mice at 20 weeks was not significantly different than that observed in diabetic mice at 14 weeks (Figure 3a). In addition, at age 20 weeks, mean lesion area in sRAGE-treated diabetic mice was significantly reduced compared with lesions in nondiabetic animals of the same age (Figure 3a).

Furthermore, we examined lesion complexity (as defined, fibrous caps, cholesterol caps, or lesion necrosis) in these
In diabetic mice treated with sRAGE from age 14 weeks to 20 weeks, the mean number of complex lesions was significantly reduced compared with diabetic animals treated with murine serum albumin, and lesion complexity was not significantly different in sRAGE-treated diabetic mice at 20 weeks compared with diabetic mice at age 14 weeks (Figure 3b). A trend toward decreased lesion complexity was observed in sRAGE-treated diabetic mice at age 20 weeks compared with nondiabetic animals of the same age, although the results did not achieve statistical significance (Figure 3b). In parallel with stabilization of atherosclerotic lesion area and complexity, RAGE blockade was associated with decreased vascular expression of vascular cell adhesion molecule-1, JE- monocyte chemoattractant peptide-1, cyclooxygenase-2, nitrotyrosine epitopes, matrix metalloproteinase-9 (antigen and activity), and tissue factor epitopes (Figure 3b).

In addition to biochemical and molecular pathways linked to atherosclerosis, we examined collagen content within the atherosclerotic plaques using picro Sirius red staining and polarized light microscopy. Diabetes was associated with a significant increase in extent of collagen per lesion in diabetic mice versus nondiabetic controls at 20 weeks of age. In the presence of RAGE blockade, a significant decrease in collagen content per lesion was noted compared with that seen in diabetic mice treated with murine serum albumin. The extent of collagen deposition in diabetic sRAGE-treated mice was not significantly different than that observed in nondiabetic animals of the same age or diabetic mice at age 14 weeks. We propose that in diabetes, increased numbers of smooth muscle cells and collagen content in diabetic atherosclerotic plaques, the significant increase in numbers of mononuclear phagocytes, and the striking enhancement of proinflammatory mechanisms within the lesions tips the balance between inflammation and lesion stability. Furthermore, especially in diabetic lesions, increased matrix metalloproteinase expression/activity and generation of tissue factor antigen within the plaques enhances the likelihood of plaque progression and instability.

Certainly, these concepts require further testing in distinct species such as pigs or nonhuman primates to address the potential role of RAGE blockade in suppressing plaque instability.

**Diabetes and Atherosclerosis: Other Models to Test the Impact of RAGE**

It is important to note that the effects of RAGE blockade on atherosclerosis were not limited to apo E−/− mice. An additional murine model was used to trigger basal hypercholesterolemia on which to superimpose hyperglycemic stress. When mice deficient in the LDL receptor were rendered diabetic and fed a diet of normal chow, not enriched in fat, increased atherosclerotic lesion area ensued. On administration of sRAGE, accelerated atherosclerosis was attenuated. These findings were in contradistinction to the work of Reavan et al. These investigators found that induction of diabetes by streptozotocin in LDL receptor−/− mice fed a high-fat diet over an extended time course did not result in acceleration of atherosclerosis. We posit that the differences between these 2 study outcomes may be ascribed, at least in part, to the premise that at the aortic root, the degree of atherosclerosis that may be achieved is finite. Thus, especially over long time courses in Western diet-fed mice, differences between diabetic and nondiabetic groups may become less apparent as atherosclerosis in the nondiabetic animals continues to progress.

Furthermore, we recently extended these concepts to murine models of insulin-resistant, type 2 diabetes. To accom-
Diabetes, Atherosclerosis, and Hyperlipidemic Mice: Effect of Distinct Interventions

The murine models of streptozotocin-induced diabetic apo E and LDL receptor−/− mice have also been used by other investigators to test the impact of distinct pathways and interventions. For example, Lin et al showed that dietary restriction of glycotoxins provides anti-atherogenic protection in these animals, without impacting on levels of blood glucose.39 The role of angiotensin-converting enzyme was studied by Candido et al, these investigators showed that administration of perindopril for 20 weeks prevented diabetes-associated atherosclerosis in parallel with reduced lesion area in multiple vascular segments of these mice; these findings provide further support for the AGE hypothesis in vascular injury. In the AGE-restricted group of diabetic apo E−/− mice, immunohistochemistry revealed decreased accumulation of AGEs and AGE receptors including RAGE, in parallel with reduced numbers of inflammatory cells and expression of tissue factor, vascular cell adhesion molecule-1, and JEMonocyte chemoattractant peptide-1 in the vascular lesions.38 Levi et al have shown that treatment of diabetic LDL receptor−/− mice with peroxisome proliferator-activated gamma agonists such as rosiglitazone attenuates atherosclerosis in these animals, without impacting on levels of blood glucose.39 The role of angiotensin-converting enzyme was studied by Candido et al; these investigators showed that administration of perindopril for 20 weeks prevented diabetes-associated atherosclerosis in parallel with decreased blood glucose and levels of triglyceride.41

Thus, taken together, such mouse models of diabetic atherosclerosis provide a template for dissection of molecular pathways linked to the pathogenesis of macrovascular complications of diabetes and, also, the potential impact of therapeutic interventions.

Diabetes and the Problem of Restenosis

Epidemiologic studies indicate that in human subjects with diabetes, exaggerated restenosis commonly accompanies acute arterial injury, such as that induced by therapeutic angioplasty.42–44 Studies further demonstrated that stenting of the treated vessel does not offer full protection from restenosis and coronary events.42,43 Even with the recent development of sirolimus-coated stents, it is not yet clear if diabetic subjects will fully benefit from this novel therapy.45–47

To dissect the biochemical and molecular events linked to enhanced restenosis in diabetic human subjects, it was first necessary to establish animal models to readily address these concepts.

Studies in Rats

The first studies using diabetic rats were performed in obese Zucker rats displaying type 2 insulin-resistant diabetes.48 Interestingly, compared with induction of diabetes (type 1) in Sprague-Dawley rats by streptozotocin in which neointimal hyperplasia was not exaggerated, Park et al showed that obese Zucker rats subjected to carotid balloon injury displayed a >2-fold increase in neointimal area compared with lean Zucker (nondiabetic) rats on day 21 after injury.48 In the intima of these animals, cell proliferation markedly increased, commencing at day 3 and persisting through day 14. This animal model was then used to test the role of RAGE blockade. Administration of sRAGE was begun just before arterial injury and was continued through the first week after balloon injury. Compared with vehicle treatment (albumin), sRAGE-treated rats displayed a significant decrease in neointimal expansion.49 A key finding in this study was the marked reduction in incorporation of bromodeoxyuridine in the smooth muscle cells of the expanding neointima in sRAGE-treated rats. These experiments strongly suggested that RAGE-expressing smooth muscle cells, at least in part, contributed importantly to diabetes-associated enhanced neointimal expansion after acute injury.

Studies in Murine Models

These findings suggested that it was logical to further dissect the role of RAGE in arterial injury. In rat models, however, the inability to use transgenic/knockout approaches limited this approach. Furthermore, direct studies in murine models would facilitate the testing of genetically RAGE-modified mice, thereby supporting the premise that the chief target of sRAGE was the receptor itself. Thus, to use murine models, acute femoral artery endothelial injury was induced according to a previously-established and validated model.50 Guide wire-induced injury to the femoral artery was performed and the impact of both pharmacological and genetic modification of RAGE was tested. RAGE and its ligands, CML-AGEs and S100/calgranulins, were upregulated in the femoral vessel of RAGE−/− mice versus vehicle (murine serum albumin).51 Importantly, homozgyous RAGE−/− mice displayed decreased I/M ratio on day 28 after injury compared with littermate control animals subjected to arterial denudation (Figure 4a through 4c). Numbers of proliferating smooth muscle cells were significantly reduced in RAGE−/− mice versus controls.51

As homozgyous RAGE−/− mice do not express RAGE in any cell type, an additional strategy was used to specifically dissect the role of smooth muscle cell RAGE in modulating neointimal expansion on acute arterial injury. Previous studies showed that deletion of the cytosolic domain of RAGE imparted a dominant-negative (DN) effect; when wild-type RAGE-bearing vascular or mononuclear phagocyte-like cells were transfected with constructs encoding DN RAGE, a DN effect ensued on incubation with RAGE ligands such as S100B or CML-AGE.3,9 Because smooth muscle cells were the principal RAGE-expressing cells in the expanding neointima,51 these concepts were tested in transgenic mice express-
Hyperglycemia and hyperinsulinemia. In parallel, neointimal expansion was reduced by 65% after arterial injury in apo E−/− mice displayed markedly decreased neointimal expansion consequent to femoral artery injury. Homozygous RAGE−/− mice subjected to arterial injury displayed a significant decrease in I/M ratio on day 28 (Figure 4d). Thus, these experiments provided further support for roles for RAGE in neointimal expansion. In addition to pharmacological blockade of RAGE, genetically modified RAGE mice (deletion or interruption of smooth muscle cell signal transduction) displayed decreased injury-triggered neointimal expansion.

However, a limitation of inducing arterial injury in C57BL/6 mice is that there is little evidence of inflammatory cell influx into the injured vessel wall in wild-type animals. To address this concept, femoral artery injury was induced in apo E−/− mice based on the premise that basal vascular perturbation secondary to hyperlipidemia would augment the response to arterial injury, including migration of inflammatory cells. We found that I/M ratios were higher, reflecting enhanced neointimal expansion in apo E−/− mice vessels 28 days after injury versus C57BL/6 mice (∼2-fold higher I/M ratio). In euglycemic apo E−/− mice, administration of sRAGE diminished I/M ratio on day 28. In parallel, numbers of mononuclear phagocytes infiltrating the injured artery were also reduced. Thus, apo E−/− mice may provide a superior system to test the role of smooth muscle cell and mononuclear phagocyte RAGE in the response to acute arterial injury.

In addition, these studies highlighted the role of RAGE-dependent Jak/Stat signaling in smooth muscle cells. In femoral vessels after injury, although increased phosphorylation of ERK 1/2, protein kinase B (akt), and Jak2/Stat3 were observed versus sham treatment, sRAGE appeared to only exert its effects on Jak2/Stat3 signaling.

**Diabetes and Restenosis: Other Models and Distinct Interventions**

Diabetic rodent models of arterial injury have also been tested for the impact of distinct interventions on neointimal expansion. Phillips et al used Western diet-fed apo E−/− mice to show that these mice develop insulin-resistant diabetes; administration of rosiglitazone prevented the development of hyperglycemia and hyperinsulinemia. In parallel, neointimal expansion was reduced by 65% after arterial injury in Peroxisome proliferator-activated gamma agonist (rosiglitazone) versus vehicle-fed apo E−/− mice. Macrophage infiltration into the expanding neointima was also reduced by this intervention. Note that these studies are in contradistinction to those of Levi et al with respect to the effect of rosiglitazone on hyperglycemia. Specifically, in Levi’s studies, apo E−/− mice were made frankly diabetic by relative deficiency of insulin using streptozotocin; in the Phillips studies, hyperglycemia was induced by feeding Western diet, thereby causing insulin resistance.

In other studies, Min et al administered troglitazone to Otsuka Long-Evans Tokushima fatty rats and found that smooth muscle cell proliferation was markedly reduced, in parallel with decreased neointimal expansion after carotid balloon injury. The role of dietary glycotoxins (AGEs) on neointimal expansion was directly tested in a apo E−/− mouse by Lin et al. Mice were randomized to high-AGE or low-AGE diet (the latter with 10-fold lower AGE content) and subjected to femoral artery injury. In parallel with decreased neointimal expansion in the low-AGE diet-fed mice, AGE content was reduced in the injured vessel wall.

The complex nature of murine/rodent models in dissection of the biologic response to arterial injury superimposed on chronic hyperglycemia was underscored by the studies of Stephenson et al. These investigators reported that db/db mice displayed markedly decreased neointimal expansion consequent to femoral artery endoluminal wire injury compared with wild-type controls. Four hours after acute arterial injury, medial smooth muscle death was reduced in db/db versus wild-type mice. However, smooth muscle cell proliferation did not appear to differ between db/db versus wild-type mice. These findings suggest important roles for leptin in modulation of stimulated smooth muscle cell properties. Oda et al reported that leptin stimulated phosphorylation and activation of MAP kinase and phosphatidylinositol 3-kinase activity in cultured smooth muscle cell; one consequence of which was increased smooth muscle cell proliferation and migration. Taken together, these considerations underscore the complexity of these model systems and suggest that preclinical testing of novel therapeutic targets in restenosis ultimately requires the use of species such as pigs, rabbits, or nonhuman primates before testing in human subjects.

**Conclusions**

The ultimate goal of our studies is to determine the potential role of RAGE blockade as a therapeutic strategy in the macrovascular complications of diabetes. Thus, the development of key models to test the role of the receptor in a time-specific and cell-specific manner has provided a tem-
plate for in-depth dissection of the role of RAGE in vascular pathology. The finding that blockade of RAGE affords benefit in mice bearing both insulin-deficient and insulin-resistant diabetes further supports the role of this receptor in hyperglycemic states. These considerations underscore the concept that the products of the downstream effects of high glucose and oxidant stress generate species that are signal transduction ligands of the receptor.

It is important to stress that RAGE-dependent mechanisms in vascular stimulation are not limited to diabetes. As the vasculature in euglycemic atherosclerotic plaques is also susceptible to oxidant stress, inflammation, and, thus, generation of AGEs, albeit to lesser degrees, it was logical to test the impact of RAGE blockade. Thus, it was not surprising that administration of sRAGE to euglycemic apo E−/− mice from ages 14 to 20 weeks attenuated atherosclerotic lesion area and complexity, without an impact on lipid levels. In conclusion, these findings underscore the usefulness of rodent, and particularly mouse, models in dissection of the mechanisms linking RAGE to accelerated atherosclerosis and restenosis, particularly in diabetes. Propelled by compelling data in animal models, we propose that testing of RAGE blockade in a multi-targeted approach in diabetes may lead to a successful reduction of the macrovascular complications of types 1 and 2 diabetes. Clinical trials to address these issues in human subjects are on the horizon.

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