Introduction

Adults with diabetes mellitus are much more likely to have cardiovascular disease than those without diabetes mellitus. Genetically engineered mouse models have started to provide important insight into the mechanisms whereby diabetes mellitus promotes atherosclerosis. Such models have demonstrated that diabetes mellitus promotes formation of atherosclerotic lesions, progression of lesions into advanced hemorrhaged lesions, and that it prevents lesion regression. The proatherosclerotic effects of diabetes mellitus are driven in part by the altered function of myeloid cells. The protein S100A9 and the receptor for advanced glycation end-products are important modulators of the effect of diabetes mellitus on myelopoiesis, which might promote monocyte accumulation in lesions. Furthermore, myeloid cell expression of the enzyme acyl-CoA synthetase 1 (ACSL1), which converts long-chain fatty acids into their acyl-CoA derivatives, has emerged as causal to diabetes mellitus-induced lesion initiation. The protective effects of myeloid ACSL1-deficiency in diabetic mice, but not in nondiabetic mice, indicate that myeloid cells are activated by diabetes mellitus through mechanisms that play minor roles in the absence of diabetes mellitus. The roles of reactive oxygen species and insulin resistance in diabetes mellitus–accelerated atherosclerosis are also discussed, primarily in relation to endothelial cells. Translational studies addressing whether the mechanisms identified in mouse models are equally important in humans with diabetes mellitus will be paramount. (Arterioscler Thromb Vasc Biol. 2014;34:705-714.)

Key Words: atherosclerosis ■ diabetes mellitus ■ endothelial cells ■ macrophages ■ mice

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link between diabetes mellitus and cardiovascular disease (CVD) was perceived. The realization that CVD is a major complication of diabetes mellitus emerged in the 20th century because more people with diabetes mellitus lived long enough to develop CVD. In the 21st century, the increase in diabetes mellitus in the United States and worldwide has been termed a pandemic, and diabetes mellitus–associated CVD is a major healthcare issue. Both type 1 diabetes mellitus and type 2 diabetes mellitus increase the risk of CVD and associated mortality, which are largely because of increased atherosclerosis. There are no gross differences in lesion morphology between lesions that develop in nondiabetic and diabetic settings. However, patients with diabetes mellitus often exhibit a more rapid progression of atherosclerosis and more widespread atherosclerosis. Human postmortem studies have revealed that elevated glycohemoglobin levels (28%), as a measure of suboptimal glycemic control, are associated with more extensive and more advanced atherosclerosis in the aorta and right coronary artery even in the absence of diabetic dyslipidemia in youths. Studies on coronary artery lesions obtained postmortem indicate that lesions from patients with either type 1 or type 2 diabetes mellitus have larger necrotic cores and a higher macrophage content as compared with lesions from subjects without diabetes mellitus. Interestingly, a significant increase in lesion macrophage area was only observed in diabetic subjets without dyslipidemia as compared with controls, and correlated strongly with glycohemoglobin levels but not with plasma lipid levels. These studies show that both type 1 and type 2 diabetes mellitus stimulate increased atherosclerosis in humans at least in part through mechanisms that are distinct from dyslipidemia.

Genetically engineered mice often serve as useful tools to study the mechanisms of complex diseases. However, whereas cardiovascular complications of diabetes mellitus have been recognized for centuries, mouse models of diabetes mellitus–accelerated atherosclerosis have not been available for long. The development, in the early 1990s, of apolipoprotein E–deficient (ApoE−/−) mice and low-density lipoprotein receptor–deficient (Ldlr−/−) mice as models of atherosclerosis enabled the later development of mouse models of diabetes mellitus–accelerated atherosclerosis. The ApoE−/− and Ldlr−/− mouse models have lipoprotein profiles that resemble those of humans better than those of wild-type mice. Another hurdle to overcome was the fact that extremely high (1000 mg/dL) plasma cholesterol levels mask the effects of hyperglycemia/diabetes mellitus on atherosclerosis. Thus, in the first study of the effect of diabetes mellitus in fat-fed Ldlr−/− mice, in which insulin production was reduced by the use of the β-cell toxin streptozotocin (STZ), diabetes mellitus had no effect on atherosclerosis. Instead, the first mouse model in which the adverse effect of diabetes mellitus on atherosclerosis was demonstrated was in a fat-fed BALB/c mouse model, in which plasma cholesterol levels were in the range of 400 mg/dL. In this model, aortic root atherosclerotic lesion size correlated with plasma glucose levels (Table). Without the more humanoid lipoprotein profiles created by knocking out Apoe or Ldlr, the lesions seen in the BALB/c mice were restricted to the aortic root. Other mouse models were later generated by using STZ to induce diabetes mellitus. In many of these models, however, diabetes mellitus resulted in markedly increased plasma lipid levels, which makes it difficult to distinguish the effects of hyperlipidemia from those of diabetes mellitus/hyperglycemia. The extent of dyslipidemia achieved in many fat-fed diabetic mouse models (1000–2000 mg/dL cholesterol) far overcomes the dyslipidemia observed even in the most dyslipidemic human subjects.

In the mid-2000s, we developed an Ldlr−/− mouse model of diabetes mellitus–accelerated atherosclerosis with cholesterol levels ≥400 mg/dL. This model (on the C57BL/6 background) expresses a lymphocytic choriomeningitis virus glycoprotein (GP) transgene under control of the insulin promoter. The GP protein is seen as self, and does not result in diabetes mellitus or atherosclerosis. Instead, the significance of the expressed GP transgene is that diabetes mellitus can be induced at will by a single virus injection, which causes T-cell–mediated destruction of the GP-expressing β-cells, similar to the autoimmune process leading to type 1 diabetes mellitus in humans. Diabetic Ldlr−/−;GP+ mice fed a semifluorified low-fat diet do not develop diabetes mellitus–induced hyperlipidemia but show accelerated lesion formation characterized by increased macrophage accumulation in the brachiocephalic artery and aorta, as compared with nondiabetic controls. Increased formation of atherosclerotic lesions in the setting of diabetes mellitus, without increased plasma lipid levels in diabetic mice, was also later observed in STZ-diabetic Ldlr−/− mice, STZ-diabetic chow-fed Apoe−/− mice, and more recently in a model in which Akita mice (which have a mutation in the Ins2 gene, rendering insulin nonfunctional) were crossed with mice carrying the human ApoE4 and LDLR genes (Table). Together, these different mouse models consistently show that diabetes mellitus accelerates formation of atherosclerotic lesions by promoting macrophage accumulation in susceptible arteries (Figure).

Mouse models have also provided insight into the effects of diabetes mellitus on progression of lesions of atherosclerosis. The virally induced Ldlr−/−;GP+ model was used to investigate the effects of diabetes mellitus on preexisting lesions that had been allowed to form during a 4-month period. These studies demonstrated that diabetes mellitus does not increase the size of advanced lesions but rather results in increased intraplaque hemorrhage in macrophage-rich areas in these lesions. The presence of intraplaque hemorrhage indicates that the lesion is more severe. Intraplaque hemorrhage has also been postulated to contribute directly to lesion progression and lesion cholesterol accumulation by the high levels of cholesterol in erythrocyte membranes. These studies demonstrate that diabetes mellitus not only stimulates lesion initiation, but that it has additional direct detrimental effects on advanced lesions (Figure).
Recently, studies have demonstrated that diabetes mellitus also hinders regression of lesions of atherosclerosis (Table). In these studies, STZ-injected mice and Akita mice were used as diabetes mellitus models. The Akita mice served as recipients of aortic transplants from \( Ldlr^{-/-} \) mice.30,31 These regression studies also demonstrated that the detrimental effects of diabetes mellitus on lesion regression are attributable to hyperglycemia, and that increased monocyte recruitment into the regressing lesions is an important contributor to the reduced regression in diabetic mice (Figure).31

Thus, mouse models have now been generated to investigate the effects of diabetes mellitus on the major stages of atherosclerotic lesions. The results emerging from these models are consistent: diabetes mellitus promotes atherosclerosis and hinders regression in large part by promoting monocyte accumulation in the artery wall. Moreover, these models seem to mimic the proatherosclerotic processes in humans with diabetes mellitus–accelerated atherosclerosis.

We are now at the exciting stage at which these mouse models have started to provide important insight into the cellular and molecular mechanisms responsible for the adverse effects of diabetes mellitus on atherosclerotic lesions of different maturity. The recent findings are discussed below. In addition, we have identified 4 questions that need to be addressed to make significant progress in our understanding of the mechanisms of diabetes mellitus–accelerated atherosclerosis during the next few years, and to identify new treatment strategies and targets.

Table. Mouse Models of Diabetes Mellitus–Accelerated Atherosclerosis and Hampered Regression

<table>
<thead>
<tr>
<th>Model</th>
<th>Diabetes Mellitus</th>
<th>Lesion Phenotype in Diabetic Mice</th>
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<tbody>
<tr>
<td>Lesion initiation</td>
<td>Fat-fed BALB/c</td>
<td>STZ</td>
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<td></td>
<td>Chow-fed ApoE (^{-/-})</td>
<td>STZ</td>
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<td></td>
<td>Low fat–fed ( Ldlr^{-/-} )</td>
<td>Viral (transgene)</td>
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<td>CCA-fed ( Ldlr^{-/-} )</td>
<td>STZ</td>
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<td></td>
<td>Chow-fed hApoE4</td>
<td>Akita</td>
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<tr>
<td>Lesion progression</td>
<td>Low fat–fed ( Ldlr^{-/-} )</td>
<td>Viral (transgene)</td>
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<td>Chow-fed ( Ldlr^{-/-} ) Reversa</td>
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<td></td>
<td>Chow-fed ( Ldlr^{-/-} )</td>
<td>STZ</td>
</tr>
<tr>
<td>Fat-fed ( Ldlr^{-/-} ) donors aortic transplant into Akita mice</td>
<td>Akita</td>
<td>Increased aortic root lesions</td>
</tr>
</tbody>
</table>

\( ApoE^{-/-} \) indicates apolipoprotein E–deficient mice; BCA, brachiocephalic artery; CCA, cholesterol and cholic acid–containing diet; hApoE4, human apolipoprotein E4; \( Ldlr^{-/-} \), low-density lipoprotein receptor–deficient mice; and STZ, streptozotocin.

Endothelial Cells

Endothelial cells play an important role in promoting atherosclerosis by expressing adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1), which allow monocytes to roll and adhere to the artery wall and then enter the subendothelial space. These endothelial adhesion molecules act by binding their monocyte counterparts (eg, very late antigen-4, also known as \( \alpha 4 \beta 1 \) integrin, binds VCAM-1).32 Endothelial cells also produce potent chemokines, such as chemokine (C-C motif) ligand 2 (CCL2 or monocyte chemoattractant protein-1), which further attract monocytes.32 Both type 1 diabetes mellitus and type 2 diabetes mellitus are associated with endothelial dysfunction, measured as reduced endothelium-dependent vasodilation.33–35 Endothelial expression of adhesion molecules and chemokines is more difficult to study in humans. However, mouse studies have demonstrated that endothelial cells express elevated levels of adhesion molecules, including VCAM-1, after diabetes mellitus induction by STZ,36 and that cytokine and chemokine (eg, IL1b and CXCL2) mRNA levels are elevated, as compared with nondiabetic mice.37 Consistent with these findings are studies in which nondiabetic mice were exposed to severe hyperglycemia (20 mmol/L glucose) through glucose clamps for 6 h. Mice exposed to hyperglycemia exhibited activating epigenetic changes in the promoter of nuclear factor \( \kappa B \) (NF-\( \kappa B \)) in aortic endothelial cells, which promoted increased VCAM-1 and CCL2 expression.38 Similar findings have been obtained in isolated human endothelial cells exposed to elevated glucose in vitro.39

It is therefore likely that diabetes mellitus, at least in part through hyperglycemia, causes changes in endothelial cells leading to increased monocyte recruitment and the downstream effects of diabetes mellitus on lesion initiation, progression, and regression.

Myeloid Cells

Diabetes mellitus has significant effects on myeloid cells (neutrophils, monocytes, and macrophages) in mouse models, and...
myeloid cells are now known to be key players in mediating the detrimental effects of diabetes mellitus on atherosclerotic lesions. Thus, it has been shown that peritoneal macrophages from STZ-diabetic mice, mice with virally induced diabetes mellitus, and obese hyperglycemic db/db (leptin receptor–deficient) mice express higher levels of cytokines and chemokines (tumor necrosis factor-α, interleukin-1β, CCL2) than do macrophages from nondiabetic mice.39,40 Lesion macrophages from regressing lesions in diabetic mice also exhibit elevated cytokine and chemokine expression (eg, TNFα, IL1β, IL6, CCL2), as compared with macrophages in regressing lesions from nondiabetic mice.41 A similar increase in cytokine expression has been demonstrated in several studies on monocytes from patients with type 1 diabetes mellitus and type 2 diabetes mellitus.42–45 Thus, human and mouse studies are in agreement that both type 1 and type 2 diabetes mellitus can induce a more inflammatory monocyte/macrophage phenotype. This inflammatory phenotype has been attributed to an increase in toll-like receptor 2 (TLR2) and TLR4 signaling.44,45 In a recent study, treatment of diabetic mice with a TLR4 antagonist resulted in both reduced atherosclerosis and reduced cytokine expression in macrophages without affecting atherosclerosis in nondiabetic mice.47 Thus, the TLR4 pathway might be activated by diabetes mellitus, and activation of TLR4 downstream events might contribute to the increased monocyte/macrophage inflammatory phenotype and diabetes mellitus–accelerated formation of early lesions. It is not yet known whether the effects of TLR4 antagonism are attributable to a direct effect on myeloid cells or whether other cell types respond to TLR4 activation and release factors that in turn act to promote myeloid cell activation in diabetic mice.

Furthermore, links have been made between diabetes mellitus, inflammation, and activation of the transcription factor NF-κB in myeloid cells. NF-κB mediates transcription of genes involved in innate immunity and inflammation, and it acts downstream of TLR4 and many other receptors. NF-κB seems to be activated to a greater extent under basal conditions in monocytes from type 1 diabetic subjects as compared with nondiabetic controls.43,49,50 Furthermore, monocytes from type 1 diabetic subjects with high glycated hemoglobin, hemoglobin A1c levels (>10%) exhibit higher NF-κB activity than monocytes of patients with lower glycated hemoglobin, hemoglobin A1c.51 This effect might be a result of elevated glucose levels because elevated glucose can lead to activation of NF-κB in human monocytes.51

In recent studies, we have demonstrated that the enzyme acyl-CoA synthetase 1 (ACSL1) is expressed at higher levels in myeloid cells in mice with diabetes mellitus induced by STZ or virus.48 ACSL1 is also markedly induced by the TLR4 ligand lipopolysaccharide in isolated macrophages, suggesting that ACSL1 might be a downstream mediator of TLR4 signaling in macrophages.52 ACSL1 acts to convert free fatty acids intracellularly into their acyl-CoA derivatives, a process

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that seems to be required for inflammatory macrophages to replenish membrane phospholipids after LPS stimulation.60,62 Interestingly, when ACSL1 expression is knocked down specifically in myeloid cells, the inflammatory effect of diabetes mellitus on monocytes and macrophages is lost, and diabetic mice are also protected from formation of lesions of atherosclerosis.62 Together, these studies suggest that activation of myeloid cells by diabetes mellitus, through induction of ACSL1, is required for the inflammatory myeloid cell phenotypes and diabetes mellitus–accelerated lesion initiation.

Another effect of diabetes mellitus on myeloid cells was recently uncovered in mouse models, the ability of diabetes mellitus to promote myelopoiesis.31 In both STZ-diabetic mice and diabetic Akita mice, increased numbers of neutrophils and monocytes (primarily the Ly6Chi monocyte population) were observed, as compared with nondiabetic controls. The effect of diabetes mellitus was mediated by the damage-associated molecular pattern protein S100A9, which causes proliferation of myeloid progenitor cells in the bone marrow.31 Interestingly, myeloid differentiation primary response 88, an adapter protein that mediates some of the downstream effects of TLR4, as well as effects of other TLRs, was not required for diabetes mellitus–induced myelopoiesis.31 Ly6Chi monocytes are recruited from bone marrow and spleen after inflammatory insult and are known to infiltrate lesions of atherosclerosis.53,54 Neutrophils are also present in atherosclerotic lesions, although their numbers are low compared with that of monocytes/macrophages. Leukocytosis has long been known to associate with cardiovascular disease,55 and this association holds true also in patients with type 1 diabetes mellitus,31 but a causative effect has not yet been established. It is conceivable that the inflammatory milieu created by the diabetic environment is responsible for the myelopoiesis. The role of myelopoiesis in diabetes mellitus–accelerated atherosclerosis is an exciting area that needs further studies.

Finally, proliferation of macrophages in lesions of atherosclerosis has recently been shown to contribute importantly to lesion macrophage accumulation in fat-fed mice,63 consistent with earlier studies demonstrating that the majority of lesion cells undergoing cell cycle traverse in fat-fed nonhuman primates are macrophages.64 In this context, it is interesting that lesion macrophage proliferation was increased in fat-fed and severely dyslipidemic diabetic mice, but not in diabetic mice fed a low-fat diet, although these mice were clearly hyperglycemic.24 Hyperglycemia is therefore unlikely to stimulate macrophage accumulation in lesions of atherosclerosis by promoting macrophage proliferation, or at least seems to be insufficient to induce lesion macrophage proliferation.

Together, the results of many studies indicate that myeloid cells are activated by diabetes mellitus, and that this activation is likely to contribute to the effects of diabetes mellitus on several different stages of atherosclerosis.

Smooth Muscle Cells

Are SMCs in lesions of atherosclerosis directly affected by the diabetic environment, or do they respond as a result of endothelial cell activation and monocyte recruitment into the artery wall? This question is still largely unanswered. SMC proliferation and accumulation were increased in lesions of atherosclerosis from diabetic mice and swine, but these lesions were also of a more advanced stage than lesions in nondiabetic animals, making it difficult to know whether the increased proliferation was attributable to a direct effect of diabetes mellitus on SMCs or to indirect mechanisms.22,58 Recent data obtained using an atherosclerotic Ldlr−/− mouse model of insulin resistance suggest that osteochondrogenic differentiation of SMCs results in increased vascular calcification in that model of insulin resistance.59 Vascular calcification is increased in patients with diabetes mellitus, especially type 2 diabetes mellitus, but the mechanisms regulating vascular calcification are complex, and cell types other than SMCs seem to play major roles. Other studies demonstrate that hyperglycemia causes changes in aortic gene expression, including in genes involved in inflammation and calcification, in a nonatherosclerotic mouse model.60 Another approach to evaluate the role of increased glucose metabolism in SMCs is to overexpress the glucose transporter-1 (GLUT1). Targeted overexpression of GLUT1 in SMCs leads to increased vascular inflammation, as assessed by an increased number of neutrophils and increased CCL2 levels in a balloon injury model in nonatherosclerotic mice.61 There were no differences in SMC numbers or medial thickness in uninjured mouse overexpressing GLUT1 in SMCs, demonstrating that increased glucose metabolism in SMCs is not sufficient to induce SMC proliferation.61 Thus, the evidence for diabetes mellitus having major proatherosclerotic effects on SMCs is not as strong as for endothelial cells and myeloid cells.

Second Question: Does Glucose Directly Affect Vascular Cells and Does This Promote Atherosclerosis?

Hyperglycemia seems to be required for the adverse effects of diabetes mellitus on atherosclerosis, at least in mouse models. This dependency on hyperglycemia was recently demonstrated in atherosclerosis regression models in which plasma glucose levels were reduced using a sodium glucose cotransporter 2 inhibitor, which results in increased renal secretion of glucose and normalization of hyperglycemia in diabetic mice.31 The inhibitor not only prevented the effect of diabetes mellitus on myelopoiesis but also prevented the adverse effects of diabetes mellitus on atherosclerosis regression and the inflammatory phenotype of lesion macrophages in regressing lesions.31 These studies support the concept that hyperglycemia is required for at least some of the effects of diabetes mellitus on lesions of atherosclerosis in mice but do not address which cell type is affected by hyperglycemia, although the neutrophil was implicated as the culprit responder to hyperglycemia.31 Studies like the SMC-targeted GLUT1 overexpression study described above,61 in which GLUT1 is overexpressed in a cell-type specific manner, have the potential to provide insight into the question of how glucose affects different cells in vivo. The SMC-specific GLUT1 overexpression study demonstrated that increased glucose uptake in SMCs drives neutrophils to invade the artery wall after injury.61 Thus, SMCs could possibly play a role in mediating the effect of hyperglycemia on atherosclerosis, as could neutrophils, other lesion cells, or cell types that affect the lesions indirectly.
A multitude of studies have investigated the effects of elevated glucose levels and advanced glycation end-products (AGEs) on cultured vascular cells or immune cells. AGEs activate the receptor for AGEs (RAGE), which also binds several other ligands implicated in the vascular complications of diabetes mellitus, including S100 proteins. For example, elegant mouse studies have demonstrated that hyperglycemia is required for S100A9 mediated effects on myelopoiesis, and that the effects of S100A9 are mediated through RAGE. Many in vitro and in vivo studies have demonstrated that RAGE mediates proatherosclerotic effects, and that its actions go beyond the diabetic condition. Glucose-induced production of reactive oxygen species (ROS) has also been the subject of intense studies, especially in endothelial cells. Studies in isolated endothelial cells have demonstrated glucose-induced changes, including increased ROS accumulation and epigenetic changes that are inhibitable by the overexpression of enzymes that act on ROS and methylglyoxal-modification of intracellular proteins, such as uncoupling protein 1, superoxide dismutase 2, or glyoxalase 1. No studies have been performed to date to evaluate the role of glucose-induced ROS production specifically in endothelial cells in diabetes mellitus–accelerated atherosclerosis, and such studies would be of great interest. A few studies have evaluated the effects of acute glucose administration on leukocyte adhesion to microvascular endothelial cells in vivo by using intravital microscopy. Such studies have revealed that intraperitoneal injection of glucose increases leukocyte rolling and adhesion to mesenteric microvessels in a dose-dependent fashion. A recent study found that elevated glucose increased leukocyte rolling and adhesion to microvessels of nondiabetic rats only when interleukin-1β was coadministered. Thus, it is possible that the inflammatory environment of diabetes mellitus enhances the effects of elevated glucose levels on myeloid cell adherence to the endothelium in vivo.

An intracellular pathway thought to show increased flux in the presence of elevated glucose is the aldose reductase/polyol pathway, in which intracellular glucose is converted to sorbitol by aldose reductase, and then further to fructose by sorbitol dehydrogenase. Aldose reductase pathway is generally thought of as one of the major mechanisms whereby increased glucose can affect cells. Expression of human aldose reductase at levels seen in humans selectively increased the effect of diabetes mellitus on atherosclerosis in Ldlr−/− mice. At least part of this effect was generated through aldose reductase expression in endothelial cells. Overexpression of aldose reductase in nondiabetic mice had no effect on atherosclerosis. These studies suggest that overexpression of aldose reductase has a selective role in atherosclerosis in the setting of diabetes mellitus. However, an aldose reductase inhibitor or aldose reductase-deletion selectively increased early and intermediate atherosclerosis in both nondiabetic and hyperlipidemic diabetic Apoe−/− mice, suggesting that basal levels of aldose reductase confer a protective effect on early and intermediate atherosclerosis in both nondiabetic and diabetic mice.

Finally, although the data implicate hyperglycemia in the proatherosclerotic effects of diabetes mellitus in mice, data from human studies are much less convincing. Intensive blood glucose lowering has long-term cardioprotective effects in young patients with type 1 diabetes mellitus, but cardiovascular benefits of reducing blood glucose or using intensive insulin therapy in people with type 2 diabetes mellitus are inconsistent. Therefore, if hyperglycemia acts to promote atherosclerosis in humans, it is likely to act in concert with other factors, especially in subjects with type 2 diabetes mellitus.

Third Question: Does Selective Insulin Resistance in Vascular Cells Promote Atherosclerosis?

Type 2 diabetes mellitus, and sometimes also type 1 diabetes mellitus, is associated with insulin resistance, among several other risk factors of cardiovascular complications. There has been a recent interest in the role of insulin resistance, per se, in relation to diabetes mellitus–accelerated atherosclerosis. For example, fat-fed Apoe−/− mice deficient in insulin receptor substrate 2 (IRS2) exhibit insulin resistance and increased atherosclerosis without changes in plasma lipids, and mice with reduced levels of insulin receptors and IRS1 also have increased atherosclerosis. Furthermore, recent studies demonstrate that insulin resistance may have direct effects at the level of the arterial wall, some of which are proatherogenic.

Studies in which the insulin receptor has systematically been deleted in different cell types involved in atherosclerosis have revealed that insulin signaling in endothelial cells is antiatherosclerotic. Lack of insulin receptors in endothelial cells results in increased atherosclerosis, increased leukocyte adherence to the endothelium, increased VCAM-1 expression, and an increased lesion complexity characterized by an increased number of SMCs. The antiatherosclerotic effect of insulin signaling in endothelial cells is likely to be mediated by Akt1 (one of the Akt isoforms expressed in endothelial cells). Thus, in addition to the effect of glucose on endothelial cells, a reduced ability of insulin to activate Akt is likely to contribute to increased atherosclerosis.

The role of insulin receptor signaling in myeloid cells is less clear. One study demonstrated that mice with insulin receptor–deficient or IRS2-deficient myeloid cells had reduced atherosclerosis. In another study, insulin receptor–deficient macrophages exhibited increased susceptibility to endoplasmic reticulum stress and apoptosis. Thus, lack of myeloid cell insulin receptors resulted in increased lesion necrotic core formation. The role of insulin receptor signaling in SMCs in lesions of atherosclerosis is unexplored.

Insulin resistance does not equally affect signaling pathways downstream of the insulin receptor, a phenomenon termed selective insulin resistance. Thus, in livers from insulin resistant mice, insulin failed to suppress glucose production, but its ability to stimulate fatty acid synthesis was retained. Both pathways were dependent on Akt activation, but mammalian target of rapamycin complex 1 selectively mediated the effects of insulin on hepatic lipogenesis and was spared from insulin resistance. It is unknown whether selective insulin resistance occurs in vascular cells. If it does, studies on insulin receptor–deficient cells should be complemented by studies in which specific branches of the insulin signaling...
pathway have been inhibited or deleted. This is an interesting area for future studies.

Fourth Question: Does Diabetic Atherosclerosis Have a Different Pathogenesis Versus Atherosclerosis in the Nondiabetic Setting?

Although the scientific community agrees that lesions of atherosclerosis from subjects and mice with diabetes mellitus have no distinguishing morphological features, a few mouse studies have suggested that the molecular mechanisms involved in diabetes mellitus–accelerated atherosclerosis might be in part different from those of atherosclerosis in the nondiabetic environment. For example, the higher expression of ACSL1 in myeloid cells from diabetic mice makes this pathway more important for lesion initiation in diabetic mice as compared with nondiabetic mice. Oxidative stress might also be more important in diabetes mellitus–accelerated atherosclerosis than in atherosclerosis in nondiabetic conditions. Thus, the NADPH oxidase Nox1 plays a preferentially more important role in atherosclerosis in diabetic hyperlipidemic Apoe−/− mice by generating superoxide and increasing monocyte adherence to the endothelium than it does in nondiabetic mice. Furthermore, lack of the antioxidant enzyme glutathione peroxidase-1 accelerates atherosclerosis only in diabetic mice, and not in nondiabetic mice, suggesting a greater importance of oxidative stress in diabetes mellitus–accelerated atherosclerosis. It is not yet known what cell type is mostly susceptible to the increased ROS, but the endothelial cell would be a likely target. Together, these studies provide clues that it might be possible to develop treatment strategies against CVD that are more efficient in patients with diabetes mellitus.

Future Research and Clinical Implications

Even with the lipid-lowering and blood pressure–lowering treatment strategies available today, patients with diabetes mellitus, and especially premenopausal women with diabetes mellitus, have a significantly higher risk of developing CVD. Therefore, additional treatment approaches and identification of new drug targets are urgently needed. In the last decade, research on diabetes mellitus–accelerated atherosclerosis has reached several important milestones, not least the development of mouse models that will allow sophisticated mechanistic studies in the years to come. Mechanism-based therapy that targets specific cell types and signaling pathways involved in diabetes mellitus–accelerated atherosclerosis may be useful in the treatment and prevention of diabetes mellitus–associated CVD, especially if the pathogenesis of atherosclerosis is indeed partly distinct in diabetes mellitus. For example, strategies to target specific inflammatory pathways might be particularly beneficial in the setting of diabetes mellitus. Two clinical trials are currently underway to investigate the roles of inflammatory pathways in CVD. Of these, the CIRT is relevant to patients with diabetes mellitus because it evaluates whether a low dose of methotrexate (commonly used in the treatment of rheumatoid arthritis) as compared with placebo will reduce major vascular events among postmyocardial infarction patients with diabetes mellitus or the metabolic syndrome. The outcome of this trial is very important and will help tailor additional mechanistic studies. Another interesting target is Nox1. Thus, although antioxidant therapy till date has yielded disappointing results, more targeted approaches to combat ROS formation in diabetes mellitus might prove to be more successful. For example, promising methods to target phagocytic cells through nanoparticles, which are ingested to deliver their cargo intracellularly in phagocytic cells, have now been developed.

In conclusion, mouse models have provided important mechanistic insight into how diabetes mellitus accelerates atherosclerosis and hinders atherosclerotic regression. We still have much to learn in the years to come. Specific targets for feasible therapeutic treatment strategies need to be discovered, and the translational impact of the mouse studies needs to be investigated. With a diabetes mellitus pandemic on our doorstep, there is no time to lose.

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Disclosures

None.

References


64. Yao D, Brownlee M. Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands. *Diabetes*. 2010;59:249–255.


Why did you choose the profession of scientific investigation?

Ever since I can remember, I have been fascinated by nature and the processes that make it work and evolve. My upbringing by a father who was a biology and geography teacher and my ever-curious mother, who went on the get a PhD after her daughters had grown up, played a big part in building my curiosity and excitement for scientific investigation. I vividly remember hunting fossils near where I grew up in Sweden or eagerly looking through the collections at the biology department of the high school where my father taught. As a young girl, I would bring small dead animals I found outside into my room for dissection. I just knew that I had to become a scientist. Studying biology and then human physiology at university reinforced my commitment.

How have mentors contributed to your professional development?

My PhD mentor in Sweden, Hans Arnqvist, is a diabetologist and physician-scientist. I developed my interest in diabetes mellitus and its vascular complications during my time in his laboratory. I remember his beeper going off during a laboratory meeting to alert him that one of his patients, a young woman in her 30s, had experienced a myocardial infarction; such events contributed to my decision to study the mechanisms of diabetes mellitus–accelerated atherosclerosis.

My postdoc mentor, Russell Ross, taught me a lot about science, including how to take each research project one or several steps farther, how to always aim higher, and how to never lose sight of the bigger picture. His contribution to my development as a scientist was profound.

During my transition to a faculty position at the University of Washington, Ed Krebs and Joe Beavo further expanded my knowledge of signal transduction, showed me how to write successful grant applications, and served as great role models for me. I would not be where I am today without these mentors, and I am grateful to have had an opportunity to work with them.

What wisdom do you impart on new investigators?

First and foremost, I think you have to love what you do. If your heart is not in it and if you do not feel blessed to get into the laboratory every morning (and to continue to think about it late at night), it is going to be difficult to succeed in science and be excited about it in the long term. Keen observation skills are important (a failed experiment often tells you as much as a successful one), and so are resilience and determination (many of the questions we ask are difficult, and finding an answer might take many months or even years). A quote by Albert Szent-Györgyi summarizes a strategy that I think often leads to success: “Think boldly, don’t be afraid of making mistakes, don’t miss small details, keep your eyes open, and be modest in everything except your aims.”

By carefully choosing your mentors and collaborators you will find your niche in science. In addition, this will build a network of scientists who will continue to support you through your career. For example, after studying with a clinician diabetologist, a brilliant atherosclerosis expert, and prominent scientists in the area of signal transduction, I was in a good position, I think, to establish my laboratory in the area of mechanisms of diabetes mellitus–accelerated atherosclerosis.

Finally, I really hope young scientists today will be able to look beyond the difficult funding climate and enjoy the thrill of making new discoveries and the excitement of the scientific process.

Which direction do you envisage your science taking?

The main focus of my research is on understanding the cellular and molecular mechanisms of diabetes mellitus–accelerated atherosclerosis using genetically engineered mouse models. We are now at the exciting stage when we can start to dissect these mechanisms by using cell type–selective overexpression or deletion of some of the key players. I am also interested in taking our research into more of a translational area. For example, we have started collaborations to investigate polymorphisms in some of the genes we have identified in mouse models in humans with diabetes mellitus and cardiovascular disease. We are also studying human myeloid cells to verify that the diabetes mellitus–induced pathways we identify in mouse models operate also in human subjects with diabetes mellitus.

In addition, I am eager to take more global approaches to our research questions, including applying metabolomic and proteomic approaches. The realization that molecules and signal transduction pathways do not act in isolation, but are part of large cellular networks, opens up many exciting possibilities for interdisciplinary collaboration, including collaboration between basic scientists and clinicians and new areas of investigation.

What are your nonscientific activities?

I love to spend time with my husband and our 2 sons, 15 and 10 years old. They also keep me grounded when things are hectic in the laboratory. I find peace in nature and I get inspired and delighted by a new or rare species of bird that I spot, the fantastic air acrobatics of the Anna’s hummingbirds in our yard, the majestic bold eagles that often circle above, the orca whales in Puget Sound, and the cherry trees blooming on the UW campus every spring. Nature fills me with awe, both outside the laboratory and in the laboratory.

What are your favorite foods and are they heart healthy?

Most of my favorite foods are heart healthy. I love sushi, for example. We are lucky to have a lot of excellent sushi and Pacific Rim fusion restaurants in Seattle. I do not often eat desserts, not because I diet, but because I do not like them much!
Karin E. Bornfeldt

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