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Molecular and Cellular Mechanisms by Which Diabetes Mellitus Promotes the Development of Atherosclerosis

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Abstract

Recent decades have witnessed a significant increase in the incidence of diabetes mellitus primarily driven by population trends toward weight gain and sedentary lifestyles. Diabetes mellitus is a leading cause of blindness, renal failure, lower limb amputation, and an independent risk factor for atherosclerotic cardiovascular disease (CVD) and stroke. In fact, individuals with diabetes have a two- to fourfold increased risk of myocardial infarction (MI) and stroke that accounts for over 65% of diabetic mortality. Despite a vast amount of research, the molecular and cellular mechanisms that predispose individuals with diabetes to the development and progression of atherosclerosis are not understood. This chapter summarizes the current state of our knowledge of these conditions as well as some of the animal models that are being used to further increase our understanding. In addition, pathways and mechanisms that may link diabetes, and hyperglycemia in particular, to the development and progression of atherosclerosis are discussed. The continued investigation of identified pathways, linking hyperglycemia and diabetes mellitus to atherosclerosis, and the discovery of novel mechanisms and targets will be important to the development of new and effective antiatherosclerotic therapies tailored to individuals with diabetes.

Keywords: accelerated atherosclerosis; animal models; diabetes mellitus; hyperglycemia; molecular mechanisms

Introduction

The last few decades have witnessed a dramatic, worldwide increase in the incidence of diabetes mellitus. Driven by changes in lifestyle and an escalating rate of obesity, the number of individuals with diabetes is expected to reach 300 million by the year 2025 [1, 2]. Therefore, the diabetes epidemic is, and will continue to be, a global health crisis. Complications associated with diabetes make it a leading cause of blindness, renal failure, and lower limb amputations as well as an important, independent risk factor for atherosclerotic cardiovascular disease (CVD). CVD accounts for over 65% of diabetic mortality. In fact, diabetes mellitus is “a coronary heart disease risk equivalent” meaning that individuals with diabetes have the same risk of myocardial

infarction (MI) as nondiabetic persons who have had a previous MI [3–5]. Despite a tremendous amount of research, the treatment and prevention of diabetes-associated CVD is currently limited by our lack of understanding of the molecular and cellular mechanisms by which diabetes mellitus promotes atherosclerosis. This chapter will focus upon our current understanding of this problem, the model systems that are being used to further increase our knowledge and the potential future directions of this research.

Diabetes Mellitus

Diabetes mellitus is currently defined as a condition with casual blood glucose values are ≥ 11.1 mmol/L, or fasting plasma glucose ≥ 7.0 mmol/L, or glucose levels ≥ 11.1 mmol/L 2 h after an oral glucose tolerance test [6]. Pathologically, the term diabetes mellitus describes a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia. These include conditions under which the body cannot produce insulin and/or cannot effectively respond to the insulin that is being produced. There are two major forms of diabetes; type 1 (T1D), formerly known as juvenile diabetes or insulin-dependent diabetes mellitus (IDDM) and type 2 (T2D), formerly known as adult onset or noninsulin-dependent diabetes mellitus (NIDDM). T1D and T2D are, in essence, two distinctly different diseases that present an overlapping array of signs and symptoms.

T1D is characterized by an absolute insulin deficiency that results from an autoimmune-mediated depletion of insulin secreting pancreatic β cells [7, 8]. Typically, T1D develops with a rapid onset in children, with a peak incidence at 14 years of age. T1D makes up approximately 10% of the total number of cases of diabetes mellitus. Recent breakthroughs in β cell transplantation may lead to an eventual cure for T1D. Until that time, the only treatment is the tight self-monitoring of plasma glucose and the administration of exogenous insulin according to carbohydrate intake.

T2D accounts for the remaining 90% of cases of diabetes mellitus. T2D is characterized by a relative insulin deficiency that arises from a combination of peripheral tissue insulin resistance and impaired β cell function [9, 10]. Tissues expressing the insulin responsive GLUT4 glucose transporter, including adipose, and skeletal muscle and liver are particularly relevant to insulin resistance. The onset of T2D is relatively gradual and, until recently, with rare exceptions, affected only adults. However with the dramatic increase in childhood obesity, there has been an alarming increase in the diagnosis of T2D in children. Depending on severity, T2D can be controlled by using a combination of lifestyle modifications (diet and exercise), medications that increase insulin sensitivity and exogenous insulin.

Despite their distinctly different etiologies, both T1D and T2D are characterized by chronic hyperglycemia. Furthermore, there is a strong correlation between hyperglycemia and both micro- and macrovascular disease [11–15].

The negative effects of elevated glucose levels on vascular function can include decreased proliferation of endothelial cells, the impairment of some parameters of vascular responsiveness, and increased endothelial programmed cell death [16–18]. It is well established that aggressive blood glucose lowering significantly decreases the incidence and severity of microvascular disease including retinopathy, renal failure, and peripheral nerve dysfunction [11, 12]. Recent evidence suggests that increased glycemic control also correlates with a reduction in macrovascular disease, however the relationship between glucose lowering and a decrease in CVD has been much more difficult to demonstrate [12, 19, 20]. Several explanations have been put forth to rationalize the inability to clinical trials to demonstrate a strong improvement in cardiovascular outcomes through glycemic control including the possibilities that the trials were underpowered, too short in duration or were too focused upon fasting glucose rather than postprandial glucose levels. Alternatively, these findings may indicate that the quality of glycemic control presently achievable is insufficient to be effective in protecting against macrovascular disease. Therefore, even short-term deviations in the control of blood glucose may promote vascular dysfunction.

The pathophysiology of T2D-associated CVD is further complicated by multiple risk factors, collectively known as the metabolic syndrome, that commonly accompany chronic hyperglycemia. The metabolic syndrome is clinically defined as a combination of abdominal obesity, insulin resistance (prediabetes), atherogenic dyslipidemia, and hypertension [21]. The metabolic syndrome is a major cause of morbidity and mortality with CVD being the primary clinical outcome [21]. Other complications can include respiratory difficulties, chronic skeletal muscle problems, gallbladder disease, infertility, hepatic steatosis, circulatory problems, and certain cancers [22, 23].

Therefore, while a role for hyperglycemia in the development and progression of atherosclerosis is supported by a great deal of basic research, the clinical role of elevated glucose levels in macrovascular disease is less clear. Furthermore, despite a great deal of research the mechanisms that may link high glucose concentrations to the molecular and cellular pathways of disease development are not fully understood. This review will focus on potential direct proatherogenic consequences of hyperglycemia.

Model Systems

A major challenge facing researchers, who study the role of diabetes mellitus in macrovascular disease, is the inherent resistance of most rodent strains to atherosclerotic plaque development. In commonly used rodent strains, hyperglycemia and diabetes alone are not sufficient to promote the development of lesions [24]. Even in strains that can develop vascular lesions, diabetes-accelerated atherosclerosis appears, in many cases, to depend upon hyperlipidemia, either resulting from a high fat diet or a genetic deficiency, as with the

ApoE-deficient or LDLR-deficient mouse [24, 25]. The general resistance of mice to atherogenesis may result, at least in part, from the observation that wild-type mice, in contrast with humans, have barely detectable low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels. The induction of hyperlipidemia, through dietary or genetic manipulation, “humanizes” the lipid profiles of mice by significantly elevating both VLDL and LDL [24–26].

Rodent models of diabetes mellitus and hyperglycemia can be generally categorized as; (i) models in which diabetes is chemically induced, (ii) strains that have been inbred and selected for the existence of chronic hyperglycemia, or (iii) genetically engineered mouse models of diabetes (reviewed in [27]). Each model will possess a number of advantages and disadvantages depending upon the particular study and the experimental design. In general, models of T1D are useful to study the effects of chronic hyperglycemia in isolation whereas T2D models may be more physiologically relevant, albeit more complicated by additional CVD risk factors such as obesity, dyslipidemia, hypertension, and perhaps hyperinsulinemia [27].

Commonly used, inbred models of spontaneous, chronic hyperglycemia, including the nonobese diabetic (NOD) mouse, the KK mouse, the Goto Kakizaki rat, and the bio-breeding (BB) rat, which are very useful in the study of hyperglycemia and many aspects of diabetes mellitus, including microvascular complications, are resistant to the development of atherosclerosis.

Many researchers chemically induce hyperglycemia in existing models of atherosclerosis such as the LDLR or ApoE knockout mouse models that spontaneously develop atherosclerosis [28]. The most commonly used animal models involve the administration of compounds, such as alloxan or streptozotocin (STZ), which are selectively toxic to pancreatic β cells. Alloxan (2,4,5,6-tetraoxypyrimidine;5,6-dioxuracil) can be administered parentally, intravenously intraperitoneally, or subcutaneously [29]. It is rapidly taken up not only by pancreatic β cells but also by hepatocytes. Beta cells are believed to be especially sensitive to the reactive oxygen species that are produced in the cyclical interconversion of alloxan to dialuric acid [30]. The effective range of diabetogenic dose is relatively narrow and great care must be taken to minimize the side effects of this compound [29]. STZ is usually administered as a single large dose that is directly toxic to β cells or by multiple low doses (e.g., five consecutive doses of 40 mg/kg body weight) that appear to induce β cell destruction by an immune-mediated process. STZ is a nitrosourea derivative that enters pancreatic β cell through the GLUT 2 transporter [31]. In low-to-moderate doses, STZ is selectively toxic to pancreatic β cells. STZ-induced diabetes accelerates atherosclerosis in ApoE-deficient mice and BALBc mice fed an atherogenic diet [24, 25]. An advantage of the alloxan and STZ-induced models of diabetes mellitus is that they permit the examination of the effects of hyperglycemia in the absence of other variables that are often associated with T2D including obesity and hyperinsulinemia. One complication of chemically induced

hyperglycemia is that, over time, dyslipidemia develops thereby making it difficult to differentiate whether accelerated lesion development is a result of elevated plasma glucose or lipid levels [25, 32, 33]. In fact, in at least some models, the STZ-associated dyslipidemia appears to be essential for the acceleration of atherosclerosis and STZ-induced hyperglycemia alone fails to accelerate lesion development [33–35].

Transgenic models of T2D, such as the ob/ob mouse have been crossed with atherosclerosis-prone strains including the LDLR knockout mouse. Not surprisingly, these double knockout mice exhibit severe hypertriglyceridemia, hypercholesterolemia, and accelerated atherosclerosis beyond either model individually [36]. Recently a transgenic model of T1D has been developed in which β cell death can be induced, at will, by viral infection in an LDLR-deficient (proatherogenic) genetic background [32]. The advantage of this model is that β cell death can be specifically induced at any time and the uninfected mice act as a normoglycemic control. Hyperglycemia, in the absence of dyslipidemia, is sufficient to accelerate atherosclerosis in this model [32].

Potential Mechanisms by Which Hyperglycemia Accelerates Atherosclerosis

Atherosclerosis is a disease of the walls of major muscular arteries that involves inflammation and the accumulation of lipids (reviewed in [37, 38]). It is believed to originate as a response to insult or injury to the endothelial layer. The damaged endothelial cells increase the expression of adhesion proteins (β 2 integrin, PCAM-1, VCAM, ICAM, P-, and E-selectins) and chemotactic factors (monocyte chemotactic protein, MCP-1) factors that actively recruit monocytes and T lymphocytes to the vessel wall. Increased endothelium permeability allows the infiltration of monocytes and T lymphocytes as well as of lipoprotein particles, especially LDL, into the intima. The activated monocytes differentiate into macrophages expressing scavenger receptors (SR-A, CD36) that recognize oxidized, and otherwise modified, LDL particles. Scavenging macrophages become engorged with LDL-derived cholesterol and take on the appearance of a foam cell. Early lesions consist almost solely of foam cells and are known as fatty streaks. As lesion development progresses, activated macrophages/foam cells and T lymphocytes further simulate the inflammatory process through the secretion of matrix metalloproteases (MMPs) and cytokines (IFN- γ) that stimulate vascular smooth muscle cell (VSMC) growth and migration into the intima. VSMCs produce and secrete extracellular matrix that becomes a fibrous cap that covers the lesion. Foam cell death leaves a growing mass of extracellular debris, known as the necrotic core that includes lipids and many procoagulant proteins (tissue factor). The action of MMPs on the components of the fibrous cap can result in cap thinning and plaque instability. The majority of MIs and strokes

are thought to occur when plaques rupture and the blood comes into contact with the necrotic core, forming a thrombus or clot [37, 38].

In diabetic patients, the atherogenic process is thought to occur through an identical series of events. Atherosclerotic lesions isolated from diabetic patients are generally more advanced but otherwise indistinguishable from those isolated from nondiabetic patients [39, 40]. Individuals with diabetes have a two- to fourfold-increased risk of coronary heart disease and a fourfold-increased risk of mortality from heart disease [4]. The reasons for this are not clear. Diabetes/hyperglycemia may promote the development of CVD by increasing the incidence or extent of endothelial injury, by accelerating the development or growth of a plaque, and/or by decreasing plaque stability.

Several mechanisms by which hyperglycemia may promote atherosclerosis have been proposed. In general these involve the effects of elevated levels of intracellular glucose and the increased availability of glucose-derived molecules in cells of the vascular wall (Fig. 13.1).

The Polyol Pathway

The conversion of glucose to sorbitol occurs at a low rate under normoglycemic conditions due to the high K_m of aldose reductase for glucose. The flux through this pathway is significantly elevated under diabetic conditions as intracellular glucose concentrations rise [41]. High sorbitol concentration may be directly toxic to some cell types. In others, increased aldose reductase activity has been shown to lead the increased consumption of NADPH and

FIGURE 13.1. Streptozotocin (STZ)-induced hyperglycemia promotes accelerated atherosclerosis in ApoE-deficient mice. ApoE-deficient mice were given ten intraperitoneal injections of STZ (40 mg/kg body weight) or an equal volume of saline solution (control) before 8 weeks of age. Plasma glucose levels increase to approximately 20 to 25 mM in diabetic mice versus 7 to 9 mM in controls. Atherosclerotic lesions at the aortic sinus (indicated by arrows), examined at 15 weeks of age, are significantly larger and more advanced in the hyperglycemic mice than in nondiabetic mice or diabetic mice with a subcutaneous, slow release insulin pellet (diabetic + insulin).

depletion of GSH levels that can result in increased levels of reactive oxygen species (ROS) and subsequent cellular damage [42]. It has also been proposed that the conversion of sorbitol to 3-deoxyglucosone can feed into the production of advanced glycation endproducts (AGEs—see below).

Although the link between the polyol pathway and atherogenesis has been inferred, there is very little evidence to support a role in macrovascular disease. Studies focusing on the microvascular benefits of aldose reductase inhibition have, to date, produced mixed results [43–45]. Zenarestat, a potent aldose reductase inhibitor and potential treatment for diabetes-associated neuropathy, did reach phase III before the trial was suspended due to signs of renal toxicity in some patients [45, 46].

The Hexosamine Pathway

The shunting of excess intracellular glucose through the hexosamine pathway may also be relevant to the development of macrovascular complications of diabetes (Fig. 13.2). In a typical cell, under normoglycemic conditions, 1% to 3% of total glucose will be converted to glucosamine-6 phosphate by the enzyme glutamine:fructose-6 phosphate amidotransferase (GFAT) [47]. When intracellular glucose levels rise, flux through this pathway increases. In

FIGURE 13.2. A summary of the potential links between diabetes mellitus, hyperglycemia, and accelerated atherosclerosis.

addition, increased GFAT expression and activity have been reported in tissues from humans with diabetes [48]. Increased hexosamine pathway flux has been implicated in several diabetes-associated complications including; insulin resistance [47, 49], pancreatic β cell death [50], as well as atherosclerosis [51]. While it is not entirely clear how hexosamine pathway byproducts promote cellular dysfunction most research has focused on the modification of proteins by *O*-linked glycosylation. The enzyme *O*-*N*-acetylglucosamine transferase (OGT) catalyses the addition of *N*-acetylglucosamine to serine and threonine residues of a variety of proteins. It is known that *O*-linked glycosylation can act in competition with phosphorylation of the same serine and threonine residues. It is well established that elevated glucosamine concentrations drive the *O*-linked glycosylation of proteins including transcription factors [52], nuclear pore proteins [53], as well as signaling factors [54] potentially altering their function, stability, and/or activity. Glucosamine has been shown to desensitize insulin-stimulated glucose uptake in both adipocytes [47] and skeletal muscle cells [55], probably by inhibiting the translocation of the glucose transporter, GLUT4, to the cell surface [56]. In addition, increased hexosamine pathway activity can promote the transcription of proinflammatory and prothrombotic factors including TGF- α , TGF- β , and PAI-1 [57, 58]. Therefore, the hyperglycemia-induced *O*-GlcNAc modification of proteins may change gene expression patterns and alter the function of specific factors that contribute to a proatherogenic, prothrombophilic vascular environment. More studies are required to test this theory and to precisely determine the factors and downstream pathways that may be involved in the acceleration of vascular disease.

Endoplasmic Reticulum Stress Pathways

Recently we have begun to investigate an additional intracellular effect of glucosamine that has not previously been examined in the context of diabetes and atherosclerosis—its ability to promote the accumulation of unfolded proteins in the endoplasmic reticulum (ER), thereby leading to ER dysfunction and cell injury [59–62]. In eukaryotic cells, the ER provides a contained environment for the synthesis and modification of membrane proteins and proteins destined to be secreted. These co- and posttranslational modifications, including disulfide bond formation and N-linked glycosylation, play an important role in the subsequent folding and oligomeric assembly of proteins [63]. Misfolded polypeptides are retained in the ER and subsequently targeted for degradation [64, 65]. Agents or conditions that disrupt ER homeostasis by interfering with glycosylation, disulfide bond formation, ER Ca^{2+} stores, and/or the general overloading of the ER with proteins will cause the accumulation of unfolded or misfolded proteins in the ER, a condition defined as ER stress (reviewed in [66, 67]).

In mammals, conditions of ER stress lead to the activation of the unfolded protein response (UPR) through activation of three distinct, integral ER

membrane proteins designated PERK, Ire1, and ATF6 [67]. Together, these proteins signal (i) the general inhibition of protein expression, through the phosphorylation of the translation initiation factor eIF2 α [68], and (ii) the specific induction of ER-resident chaperone expression including GRP78, GRP94, PDI, and calreticulin, that function to assist in the folding of the accumulated polypeptides [69–71]. The ER stress response also induces the expression of the transcription factor, GADD153, which is known to play a role in ER stress-induced growth arrest and programmed cell death [72]. The ultimate fate of a cell is determined by the balance of protective (i.e., ER chaperone expression) and proapoptotic signals triggered by ER dysfunction. We have shown that glucosamine can induce ER stress in cells that are relevant to atherogenesis—including human macrophages, aortic SMCs, endothelial cells as well as hepatocytes (Ref. [62] and unpublished data).

In recent years additional cellular responses to ER stress have been identified (Fig. 13.3). Our lab and others have shown that ER stress can activate

FIGURE 13.3. Links between the hexosamine pathway and atherothrombosis. The rate limiting enzyme of the hexosamine pathway, glutamine:fructose-6 phosphate amidotransferase (GFAT) converts fructose-6 phosphate to glucosamine 6-phosphate (GlcN-6P). Glycosylation of serine and threonine residues of intracellular proteins is catalyzed by OGT, a reaction that is reversed by *O*-GlcNAcase. The *O*-linked glycosylation of transcription factors including SP-1 have been shown to increase expression levels of PAI-1, TGF- α , and TGF- β . Glucosamine and/or downstream metabolites of glucosamine can also promote endoplasmic reticulum (ER) stress leading to lipid accumulation and activation of intracellular inflammatory pathways through the activation of the transcription factors SREBP and NF- κ B, respectively.

and dysregulate the sterol regulatory element binding proteins (SREBPs), transcription factors that control lipid biosynthesis and uptake [62, 73, 74]. ER stress-inducing agents have been shown to promote the activation of NF- κ B, the transcription factor that is responsible for promoting the expression of genes involved in inflammatory processes [75]. Finally, ER stress has been shown to induce the activation of caspases and promote apoptosis of human aortic endothelial cells and hepatocytes [62, 76]. Together, the dysregulation of lipid metabolism, activation of inflammatory pathways, and the induction of cell-specific apoptosis represent the hallmark features of atherosclerosis. In addition to glucosamine, other independent cardiovascular risk factors including homocysteine [73] and obesity [77] as well as the accumulation of intracellular free cholesterol [78] have been shown to induce ER stress. Thus ER stress pathway may be a common mechanism of general atherogenesis.

The potential role of ER stress-induced cellular dysfunction in the development and/or progression of human disease make this pathway a plausible therapeutic target. Studies are underway to develop and test potential antiatherogenic interventions that target ER stress pathways in models of diabetes mellitus.

Protein Kinase C Activation

The ubiquitous protein kinase C (PKC) family of signaling factors are known to play an important role in vascular function and in the pathogenesis of micro- and macrovascular disease. Specifically, glucose-induced PKC activation has been implicated in decreased endothelial vasodilation [79] and increased production of ROS [80] that could contribute to endothelial dysfunction. PKC inhibition blocks endothelial expression and activity of a number of adhesion molecules, including ICAM-1, P-, and E-selectins, under conditions of elevated glucose concentration [81]. AngII- and oxLDL-induced VSMC proliferation is dependent upon PKC activity [82, 83]. PKC may also be involved in foam cell formation through its involvement in the induction of receptors for oxidized LDL macrophages [84]. Most PKC isoforms can be activated by diacylglycerol (DAG), an intermediary of glucose and fat metabolism, and conditions of diabetes and high glucose concentration have been shown to increase membrane-associated PKC activity via the DAG activation pathway in cultured VSMCs and in animal models [85]. In addition, it has been proposed the PKC may also be activated by AGE-RAGE signaling pathways (see below). Selective inhibitors of PKC have shown promise in blocking nephropathy and retinopathy in animal models of diabetes [86]; however, there is no direct evidence that these inhibitors affect atherogenesis. The ubiquitous nature of expression, the large number of PKC isoforms and the lack of isoform-specific inhibitors are complicating factors in determining the potential role of this kinase in atherogenesis.

Advanced Glycation Endproducts

AGEs are formed through a nonenzymatic process, known as the Maillard reaction, involving the reaction of the aldehyde groups of reducing sugars with the amino groups of proteins [87, 88] (Fig. 13.4). Because the reaction is nonspecific, requiring only a free amine, AGEs are a very heterogeneous group of compounds. The final step in the reaction, the conversion of Amadori products into cross-linked advanced glycation products, occurs very slowly in vivo, and therefore AGEs are predominantly composed of stable proteins with long half-lives such as collagen, laminin, and lens crystallins.

AGEs gradually accumulate during the natural aging process and have been implicated in characteristic changes associated with aging including the loss of collagen elasticity and flexibility, the formation of cataracts and β amyloid plaques [89]. Formation of AGEs has been noted during the heating of food however, in healthy individuals, AGEs are poorly absorbed by the gut and rapidly cleared by the kidney [90]. Individuals with impaired renal function, a common complication of diabetes, are especially prone to the accumulation of dietary AGEs and therefore exogenous sources of AGEs may play a significant role in this population [91, 92].

In vivo, the Maillard reaction rate is directly proportional to the prevailing glucose concentration resulting in the enhanced levels of AGEs and AGE

FIGURE 13.4. The formation of advanced glycation endproducts (AGE) by the Maillard reaction. AGEs are produced by a multistep reaction involving the sequential formation of Schiff bases, Amadori products, and finally a heterogeneous population of cross-linked advanced glycation endproducts. AGEs can interact with cellular receptors (RAGE) that activate intracellular inflammatory pathways and the production of ROS that may contribute to atherogenesis. Conditions of oxidative stress can also accelerate the formation of AGEs. The possible sites of post-Amadori-cross-link elimination by aminoguanidine and the cross-link breaker, ALT-711, are indicated.

intermediates in individuals with diabetes mellitus. This is clearly demonstrated by the routine clinical use of the Amadori-intermediate, glycated hemoglobin (HbA1c), as a diagnostic marker of chronic hyperglycemia.

The association of AGEs with chronic hyperglycemia has attracted a great deal of interest into the possible role of AGEs in diabetic complications. There are several potential ways that AGE-modified proteins could be damaging; the formation of AGEs may alter protein function [93], disrupt extracellular matrix [94, 95], and/or lead to the modification of lipoprotein particles thereby increasing their atherogenicity. However it now appears that the predominant vascular effect of AGEs occurs through their interaction with RAGE (receptor for AGE) found on macrophages, endothelial, and SMCs [96–98]. The AGE–RAGE interaction triggers a signal transduction cascade that results in the production of intracellular reactive oxidative species that can initiate an inflammatory response [99, 100]. The presence of ROS also feed back to accelerate the formation of AGEs.

There is a great deal of evidence supporting a role for AGEs in microvascular disease. AGEs accumulate in the kidney, retina, and peripheral nerve of diabetic rats [101]. Nephropathy can be induced in nondiabetic animals by the administration of AGEs [91, 92]. Neutralizing antibodies against RAGE also slow the progression of nephropathy in diabetic mice [102]. The administration of compounds that block AGE cross-linking (aminoguanidine and pyridoxamine) or interventions that break established cross-links (3-phenacyl-4,5-dimethylthiazolium chloride or ALT-711) have been shown to be protective against nephropathy and cardiovascular stiffness in diabetic models [93, 103].

There is a growing body of evidence supporting a role for AGEs in the development of atherosclerosis. AGEs have been localized to atherosclerotic plaques and RAGE is expressed by vascular endothelial cells, SMCs, and macrophages [104, 105]. Administration of both aminoguanidine and the AGE cross-link breaker, ALT-711, are associated with a significant decrease in plaque area and complexity in diabetic ApoE^{−/−} mice [106]. The strongest evidence in support of the AGE–RAGE pathway comes from experiments that focus upon interfering with receptor function. This has been accomplished using a soluble form of RAGE (sRAGE) that retains its ligand binding ability [107]. Daily intraperitoneal injections of sRAGE into STZ-induced diabetic mice showed dose-dependent attenuation of early lesion development in terms of lesion area at the aortic root and lesion complexity relative to controls. Importantly, plasma glucose and lipid levels were not affected by sRAGE treatment suggesting the effect was glycemia and lipid independent [107]. It has further been demonstrated that sRAGE can block the progression and even induce the regression of established lesions in diabetic mice [108]. In support of these findings, in vascular damage assays RAGE null mice show [109] decreased intimal/medial thickening. As of yet there have been no published reports to show the effects of the RAGE knockout genotype on plaque development in mouse models of diabetes-accelerated atherosclerosis.

Interventions that interfere with AGE formation and action have shown some beneficial effects at the clinical level [110]. ALT-711 (under the commercial name, Alagebrium) has demonstrated safety and efficacy in a phase II clinical trial in the treatment of systolic hypertension and heart failure, conditions associated with vascular stiffening likely resulting from collagen cross-linking [111].

Some controversy still remains regarding the role of AGEs in atherosclerosis, in part due to the ability of other ligands, including cytokines of the S100/calgranulin family, to interact with and activate RAGE [112, 113]. The role of AGE–RAGE is further complicated by the fact that virtually every large, well-controlled clinical trial has shown that antioxidant supplementation has no positive impact on cardiovascular risk in diabetic patients [114–117]. This paradox supports the possibility that other, AGE-independent, mechanisms and pathways may play an important role in the atherogenic process.

It is important to note that the above mechanisms are not mutually exclusive. Atherosclerosis is a complex disease that involves the interaction of several different cell types and many different molecular factors. Therefore, it is very possible that atherogenesis is regulated and affected by several independent and interdependent pathways that are altered under conditions of hyperglycemia. Furthermore, diabetes mellitus, especially T2D, is often associated with a cluster of conditions including hypertension, obesity, and dyslipidemia—each a CVD risk factor in its own right. Thus the interplay between this combination of risk factors in the context of a particular genetic background and modulated by a myriad of environmental influences will all contribute to the development and progression of the disease.

Conclusions

Because of the cardiovascular risks of diabetes and the increasing prevalence of T2D, it is essential that we further increase our knowledge of how and why diabetes mellitus and hyperglycemia promote CVD. Currently, and for the near future, the primary strategy for managing CVD in the diabetic population will be through the control of hyperglycemia and through the treatment of associated complications such as hypertension and dyslipidemia using established medications such as ACE inhibitors, statins, and fibrates.

The continued identification and investigation of existing and novel pathways linking hyperglycemia and diabetes mellitus to atherosclerosis is important to the development of new and effective antiatherosclerotic therapies that are tailored to individuals with diabetes. A great deal of research has been focused upon the role of hyperglycemia in the development and progression of atherosclerosis in cell culture and animal model systems. Several mechanisms have been identified that appear to link glucose to proatherogenic processes. The most promising of these; the polyol pathway,

PKC activation, the hexosamine pathway, and the AGE–RAGE interaction show potential and are actively being evaluated as targets for putative antiatherogenic therapies. Thus far, however, all interventions targeting the effects of hyperglycemia, including direct glucose lowering, appear to show greater effect in the treatment of microvascular complications than in the control of macrovascular disease. This is likely due, at least in part, to the complexities of atherosclerosis and current limitations of the animal models available to researchers who study the development and progression of atherosclerosis. Additional studies are obviously required to further improve our understanding of this important disease.

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