

Potential Mechanisms Promoting Restenosis in Diabetic Patients

DORON ARONSON, MD, ZACHARY BLOOMGARDEN, MD, ELLIOT J. RAYFIELD, MD

New York, New York

Diabetes is associated with greater restenosis rates after successful balloon angioplasty. The metabolic alterations that occur as a result of hyperglycemia or hyperinsulinemia can accelerate many of the pathophysiologic processes that lead to restenosis. Diabetes results in endothelial dysfunction and accelerated platelet deposition, which increase the propensity to thrombosis. Several growth factors known to promote the resten-

osis process are overexpressed in the presence of hyperglycemia. Advanced glycosylation promotes inflammatory cell recruitment and smooth muscle cell proliferation. Many of the potential mechanisms promoting restenosis in diabetic patients can be ameliorated by improved metabolic control.

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Restenosis limits the long-term beneficial effects of percutaneous transluminal coronary angioplasty and related procedures (1). The importance of diabetes as a consistent clinical predictor for restenosis after angioplasty has been demonstrated in several studies. The initial report from the National Heart, Lung, and Blood Institute Angioplasty Registry (2) indicated that the angiographic restenosis rate in diabetic patients was 47% compared with 32% in nondiabetic patients. Subsequent studies have reported restenosis rates of 49% to 71% among diabetic patients (3-9).

New angioplasty devices failed to make any major impact on the restenosis rate among diabetic patients. Diabetes is associated with a twofold increase in recurrent clinical events after directional coronary atherectomy (10). Likewise, diabetic patients have higher restenosis rates after stent placement (11) and after excimer laser angioplasty (12).

Restenosis is viewed as an intraluminal growth process after an arterial injury (see below), and risk factors for restenosis are likely to be risk factors for this growth process. In contrast to diabetes, other traditional risk factors for atherosclerosis, such as male gender, hypertension, hypercholesterolemia and continued smoking after angioplasty, have been reported as risk factors only inconsistently (1,3,7,8). In attempting to predict restenosis from clinical variables, Weintraub et al. (3) concluded that "the problem is that each of the clinical variables is a correlate without a clear relation to the underlying pathophysiological process." The intent of this review is to examine some of the putative mechanisms involved in the restenosis process in light of pathophysiologic events currently known to occur in the diabetic state.

Pathophysiologic Mechanisms of Restenosis in Relation to Diabetes

The complex pathophysiologic events leading to restenosis after balloon injury involve endothelial denudation; platelet deposition with mural thrombosis; vascular smooth muscle cell proliferation and migration; and extracellular matrix synthesis (13-17). Intimal smooth muscle cell hyperplasia is the predominant histologic finding in restenotic lesions (18). A fundamental element in the development of intimal hyperplasia is the transformation of smooth muscle cells from a contractile to a proliferative, secretory phenotype. The restenosis process is initiated when balloon inflation causes endothelial denudation and deep vessel injury. The exposure of subendothelial elements initiates platelet adhesion and activation. Activated platelets at the site of injury secrete growth factors that release smooth muscle cells from growth inhibition and induce their proliferation and subsequent migration from the media to the intima (15,17). Smooth muscle cell proliferation continues beyond the phase of platelet deposition (14). Activated smooth muscle cells themselves secrete growth factors that can act on surrounding cells and help to sustain proliferation and migration (19). The proliferative response becomes quiescent several months after intervention.

A second fundamental element of restenosis is the production and secretion of extracellular matrix by smooth muscle cells that have migrated into the injured intimal zone (17). The neointima is generally hypocellular, composed predominantly of extracellular matrix material that forms the bulk of neointimal growth (13,16).

Although vessel recoil may also contribute to loss of lumen, the markedly increased restenosis rate seen in diabetic patients after stent placement (11) underscores the role of enhanced smooth muscle cell proliferation as the major mechanism for restenosis in these patients, because coronary artery stenting provides a rigid endovascular lattice that prevents both elastic recoil and vascular spasm at the treated site (11).

From the Division of Endocrinology and Metabolism, Department of Medicine, Mount Sinai Medical Center, New York, New York 10029.

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Address for correspondence: Dr. Doron Aronson, Metabolism Section, Joslin Diabetes Center, One Joslin Place, Boston, Massachusetts 02215.

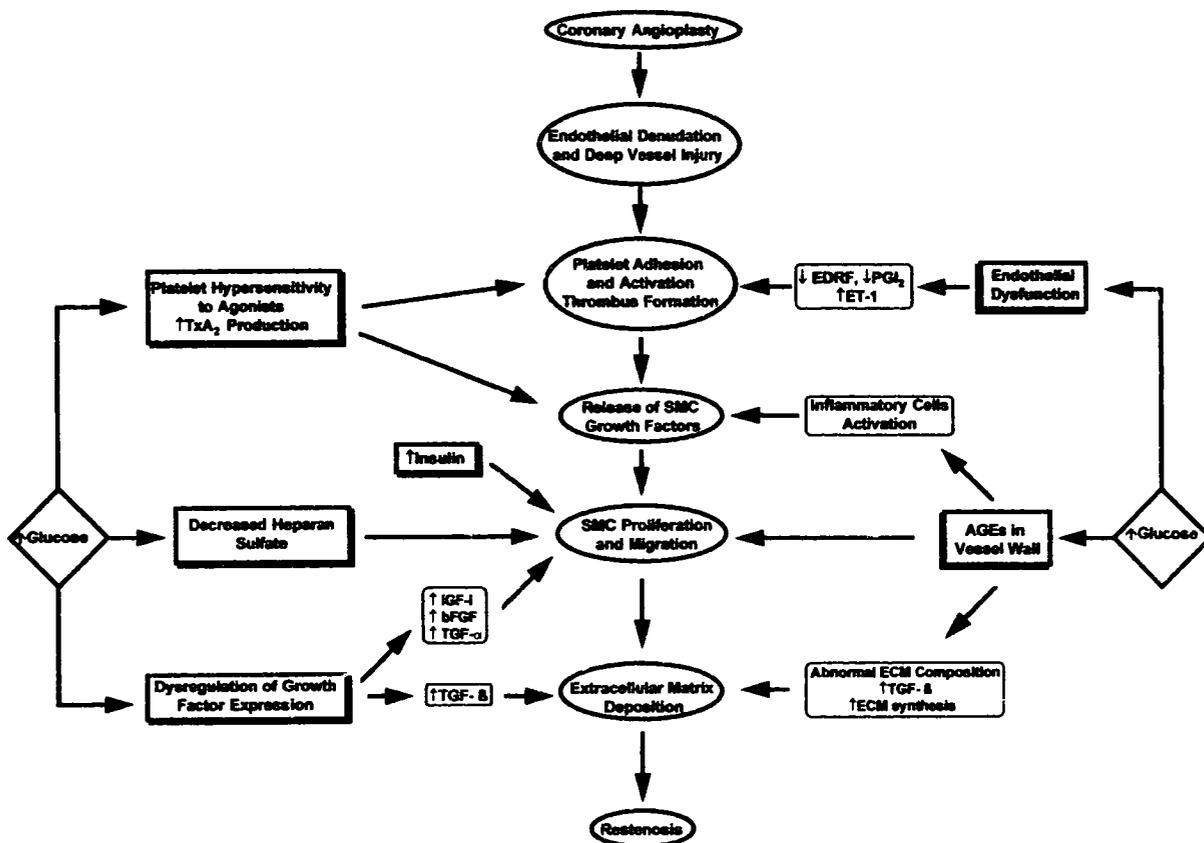


Figure 1. Proposed model for accelerated vascular lesion formation in diabetes. AGEs = advanced glycosylation end products; bFGF = basic fibroblast growth factor; ECM = extracellular matrix. EDRF = endothelium-derived relaxing factor; ET-1 = endothelin-1; IGF-I = insulin-like growth factor-1; PGI₂ = prostacyclin; TGF-α = transforming growth factor-alpha; TGF-β = transforming growth factor-beta; TxA₂ = thromboxane A₂.

In the diabetic state, several mechanisms combine to promote the various processes that constitute the biology of the restenosis process (Fig. 1). In the following section, these mechanisms are discussed.

Endothelial Dysfunction

The removal of the endothelial lining by balloon injury and the endothelial dysfunction that persists during endothelial regeneration both contribute to the restenosis process by promoting thrombosis, vasoconstriction and smooth muscle cell growth. The amount of endothelial denudation appears to correlate with the degree of later neointimal thickness (20). Furthermore, endothelial layer regeneration after vascular injury is critical in determining the eventual lesion formation (15,21). Indeed, intimal hyperplasia is greater in areas where endothelial regeneration occurred last (14).

The integrity and function of the endothelial cell layer are profoundly altered in diabetic animals and humans (Table

1). The best-characterized endothelial abnormality is impaired endothelium-dependent relaxation (22-24). Impaired endothelium-dependent relaxation can be induced by short exposure (several hours) to high glucose concentration (22,25) and occurs in different vascular beds including the coronary arteries (26).

Diabetes can impair endothelium-dependent relaxation by at least two mechanisms: 1) Advanced glycosylation end products (see later) have been shown to rapidly inactivate endothelium-derived relaxing factor in vitro through a direct

Table 1. Effects of Endothelial Dysfunction in Diabetes Mellitus on Restenosis

Diabetes-Induced Endothelial Dysfunction	Possible Promoting Effects on Restenosis
Inactivation of EDRF	Vasoconstriction; loss of antiproliferative activity of EDRF; increased platelet adhesion and aggregation
Reduced PGI ₂ synthesis	Vasoconstriction; increased platelet aggregation
Increased ET-1 production	Vasoconstriction; increased SMC proliferation
Impaired endothelial regeneration	Prolonged exposure of SMC to blood-borne mitogens; prolonged interaction of platelet with vessel wall

EDRF = endothelium-derived relaxing factor; ET-1 = endothelin-1; PGI₂ = prostacyclin; SMC = smooth muscle cell.

chemical reaction (27,28). 2) Increased generation of oxygen free radicals, which occurs in diabetes (22,29), also inactivates endothelium-derived relaxing factor before it can exert its action on smooth muscle cells (25,30). Vasospasm at the dilated coronary artery segment occurs commonly and may contribute to the early reduction in gain and promote clot formation (31). However, the loss of endothelium-derived relaxing factor has a profound effect on the arterial wall beyond the tendency to vasospasm because it inhibits both platelet aggregation and smooth muscle cell proliferation (32-34).

Prostacyclin is another endothelial vasodilator that also inhibits platelet aggregation and adhesion to the endothelium (34). Diabetes-induced alterations in prostaglandin metabolism result in a reversible decrease in prostacyclin production by endothelial cells (35,36).

Endothelin-1 is a potent natural vasoconstrictor synthesized and secreted from endothelial cells; it is also a mitogen for smooth muscle cells (32). Plasma endothelin-1 concentrations are significantly increased in diabetic patients (37). Physiologic concentrations of insulin enhance the transcription rate and secretion of endothelin-1 mRNA in endothelial cells (38,39). Hence, the hyperinsulinemic state that characterizes type II diabetes may result in a continual stimulus for endothelin-1 production and secretion.

In addition to the impaired function of endothelial cells, diabetes affects their migration and proliferation. Human vascular endothelial cell replication in culture is inhibited in the presence of high glucose concentrations (40,41). In spontaneously diabetic rats, the regeneration of the endothelium after catheter deendothelialization is slower than control rats, resulting in prolonged interaction of platelets with the denuded vessel and, subsequently, in greater neointimal proliferation (42).

The impaired ability of endothelial cells to regenerate after balloon injury along with the functional alterations in local production of vasodilators, antiaggregants and antiproliferative molecules that occur in diabetes are likely to have a considerable effect on the restenosis process by decreasing reendothelialization, increasing the propensity to thrombosis and reducing the capacity of endothelial cells to produce the appropriate growth inhibitors for smooth muscle cells.

Platelet Aggregation and Thrombus Formation

The earliest finding after balloon dilation is platelet deposition along the denuded surface, often with subsequent thrombus formation. The importance of thrombus formation in the restenosis process is emphasized by the observation that severe thrombocytopenia induced by antiplatelet antibodies inhibits neointimal accumulation after vascular injury (43). Similarly, antibodies directed against the platelet IIb/IIIa receptor integrin have been shown to significantly decrease the clinical restenosis rate (44).

Platelets that adhere and aggregate at sites of endothelial disruption release several prothrombotic, vasoconstrictive and growth-promoting mediators (Table 2) that stimulate neointimal growth (15,45,46) and have sustained biological effects on myointimal hyperplasia long after platelet deposition and thrombosis have resolved (31).

In diabetic patients, elevated fractions of activated platelets circulate in the absence of clinically detectable vascular lesions (47). In addition, platelets from diabetic subjects exhibit an enhanced adhesiveness, and hyperaggregability in response to various agonists, including epinephrine, collagen, arachidonic acid and thrombin (48-50). Platelet extracts from diabetic patients have been shown to have significantly greater growth-promoting potential for smooth muscle cells in culture than those from control subjects. However, after intensive insulin therapy, the platelet growth activity of these patients significantly declined and became similar to control levels (51).

Thromboxane A_2 , a potent platelet activator and vasoconstrictor, is synthesized in platelets during platelet activation. Increased thromboxane A_2 concentrations at sites of endothelial injury facilitate platelet aggregation and contribute to the neointimal proliferative response (52). Enhanced activity of the platelet arachidonic acid pathway with increased thromboxane A_2 synthesis occurs in diabetic subjects (53). There is a significant correlation between thromboxane A_2 production and fasting plasma glucose or hemoglobin A_{1C} , and normal thromboxane A_2 production can be restored by strict glycemic control (49,54).

The abnormal platelet function in diabetes is reflected in platelet interaction with deendothelialized vessels after balloon injury. Platelet accumulation on the injured aorta of diabetic rats is prolonged compared with control rats (42), resulting in a thicker intima with a greater number of layers of smooth muscle cells in diabetic rats (42).

Other abnormalities in the coagulation system in diabetic patients that enhance platelet aggregation and thrombus formation include elevated fibrinogen levels (55,56), increased Factor VII levels in response to hyperglycemia (56,57) and a decrease in the biological activity of antithrombin III as a result of nonenzymatic glycosylation of the lysine residue that binds it to its natural cofactor, heparin (56). In addition, a characteristic feature of insulin resistance and hyperinsulinemia is reduced plasma fibrinolytic activity caused by increased plasminogen activator inhibitor I activity (58,59). In vitro studies (60) have shown that proinsulin, a precursor of insulin, augments plasminogen activator inhibitor I gene expression in hepatocytes, resulting in an increased synthesis by the liver, which is the primary source of this molecule in plasma.

In summary, alterations in hemostatic mechanisms in the diabetic state can lead to further recruitment of platelets and mural thrombus formation through hypersensitivity of platelets to agonists at sites of vessel injury, decreased effectiveness of the inhibited thrombolytic system and elevated clotting factor levels.

Table 2. Growth Factors Involved in Pathogenesis of Restenosis*

Growth Factor and Source	Potential Role in Restenosis	Effect of Diabetes
PDGF Platelet alpha-granules; SMCs; endothelium	"Competence factor" for SMCs	SMCs express more PDGF beta-receptor mRNA; AGE-monocyte interaction results in PDGF release
IGF-1 SMCs; endothelium	"Progression factor" for PDGF-mediated mesenchymal cell growth; synergistic with PDGF	High insulin levels stimulate the IGF-1 receptor; insulin enhances IGF-1 expression
bFGF SMCs; endothelium	Powerful mitogenic activity for SMCs and ECs; anti-bFGF antibodies inhibit SMC DNA synthesis after balloon angioplasty	High glucose concentrations increase bFGF mRNA levels
TGF-beta† Platelet-alpha granules; endothelium; SMCs	Modulates extracellular matrix production; potent chemotactic factor for fibroblasts and monocytes	Overexpressed in hyperglycemia and in presence of AGEs
EDRF Endothelium	Loss of EDRF induces platelet aggregation, vasoconstriction and allows SMC proliferation	Inactivated by AGEs and oxygen free radicals that accumulate at accelerated rate in diabetes
ET Endothelium	Mitogen for SMCs	Enhanced secretion in hyperinsulinemic states
HS Endothelium; SMCs	Inhibitor of SMC proliferation	Decreased synthesis; increased turnover

*Data from references 13-17,32,45,62-67,76,77,79,81,82. †Has both growth-promoting and growth-inhibitory properties. AGE = advanced glycosylation end product; bFGF = basic fibroblast growth factor; EC = endothelial cell; EDRF = endothelium-derived relaxing factor; ET-1 = endothelin-1; HS = heparan sulfate; IGF-1 = insulin-like growth factor-1; PDGF = platelet-derived growth factor; SMC = smooth muscle cell; TGF-beta = transforming growth factor-beta.

Dysregulation of Growth Factor Expression

The metabolic alterations associated with diabetes can promote restenosis through unique alterations in local growth factor production and action, which in turn may induce proliferation of smooth muscle cells and extracellular matrix synthesis (Table 2). Alterations in growth factor expression have been implicated in the pathogenesis of proliferative diabetic retinopathy as well as in the mesangial cell proliferation and excessive matrix deposition that characterize diabetic nephropathy (61,62). Because many of these mitogenic mediators are overexpressed by vascular smooth muscle cells and endothelial cells in diabetic patients, it is reasonable to assume that they contribute to restenosis.

Platelet-Derived Growth Factor and Insulin-Like Growth Factor-1

Platelet-derived growth factor is a "competence factor" for smooth muscle cells in that it enables the cell to move from the quiescent (i.e., contractile) phenotype into the active phase of replication (63). Progression into actual replication requires a second class of growth factors, termed "progression factors,"

which trigger "competence" factor-primed cells to proliferate. One of the progression factors for platelet-derived growth factor-mediated cell growth is insulin-like growth factor-1 (64). Higher concentrations of insulin-like growth factor-1 have been demonstrated in smooth muscle cells of the synthetic phenotype from restenotic human coronary atherectomy plaques compared with smooth muscle cells of the contractile phenotype (65).

Smooth muscle cells from diabetic rats grow faster than normal smooth muscle cells in response to platelet-derived growth factor (66) and after balloon catheter injury (67). These cells express more platelet-derived growth factor-beta-receptor mRNA, which is typically expressed on the contractile phenotype of smooth muscle cells (66,67). Thus, vascular smooth muscle cells may be more sensitive to the growth stimulatory action of platelet-derived growth factor in the presence of diabetes.

Alterations in insulin-like growth factor-1 regulation that lead to increased expression and elevated tissue level of this growth factor have been implicated in the pathogenesis of renal hypertrophy in diabetic nephropathy (61,68). Similarly, insulin-like growth factor-1 may be involved in the pathogen-

esis of accelerated diabetic retinopathy (69), as patients with diabetic retinopathy have elevated insulin-like growth factor-1 levels in the vitreous humor (70). The role of insulin-like growth factor-1 in the development of macrovascular lesions is less clear.

Insulin as a Growth Factor for Vascular Smooth Muscle Cells

Physiologic concentrations of insulin are known to stimulate proliferation of cultured smooth muscle cells and fibroblasts (71,72). Although insulin itself is a poor mitogen (73), it can potentiate the expression of the more potent mitogen insulin-like growth factor-1 (74,75). Thus, in the presence of insulin resistance, where the metabolic action of insulin is ineffective, the proliferative response to hyperinsulinemia may still occur.

Transforming Growth Factor-Beta

Transforming growth factor-beta is the most important growth factor regulating the extracellular matrix production that characterizes intimal hyperplasia in its advanced stages (13,76,77). In the rat carotid artery injury model, administration of neutralizing antitransforming growth factor-beta antibodies reduces matrix accumulation in intimal lesions (78). Transforming growth factor-beta expression has been investigated in diabetes in relation to diabetic nephropathy because one of its dominant features is the expansion of extracellular matrix in the mesangial areas. In animal models of diabetes, the expression of transforming growth factor-beta in the kidney increases within a few days of the appearance of hyperglycemia (62,79), in association with active production of matrix components in the glomeruli (62). The increase in transforming growth factor-beta mRNA and protein in diabetic rats can be attenuated with insulin treatment (62). Recently, increased transforming growth factor-beta mRNA and protein were demonstrated in kidneys of patients with advanced diabetic nephropathy (62).

The expansion of the extracellular matrix in diabetic nephropathy bears many similarities to the extracellular matrix accumulation that results from arterial injury. In this context, it should be emphasized that mesangial cells act as specialized contractile smooth muscle cells that produce extracellular matrix components (80). Termination of extracellular matrix deposition is essential to avoid excessive neointimal growth after balloon catheter injury. Thus, failure to regulate or terminate transforming growth factor-beta expression in the presence of diabetes may potentially lead to excessive matrix deposition with greater encroachment on the arterial lumen.

Other Growth Factors

Hyperglycemia affects the expression of several other growth factors such as basic fibroblast growth factor and

transforming growth factor-alpha (81). Smooth muscle cells exposed to high glucose concentrations increase their basic fibroblast growth factor mRNA levels (81). Basic fibroblast growth factor is a potent mitogen for smooth muscle cells and may be involved in restenosis, because rats treated with blocking antibodies to basic fibroblast growth factor have decreased smooth muscle cell proliferation after balloon catheter injury (82). Transforming growth factor-alpha is known to be expressed in smooth muscle and endothelial cells (45,81). Thus, when overexpressed in the presence of hyperglycemia, it may promote restenosis.

Increased myointimal proliferation and matrix deposition in diabetic patients may be a consequence of altered production of local or circulating growth factors and/or modulations in the response to these factors. The cellular events that lead to altered regulation of growth factor expression in diabetes are largely unknown. Regulation of growth factor expression by glucose seems to occur at the transcriptional level and may be mediated by one of its intracellular metabolites (81). Regardless of the mechanism involved, the presence of diabetes can selectively alter gene expression in smooth muscle cells, leading to dysregulation of growth factors that are known mediators of the restenosis process.

Abnormal Extracellular Matrix Composition—Role of Heparan Sulfate

The extracellular matrix regulates cell migration, proliferation and matrix production by means of cytoskeletal signals and by posttranslational control of growth factors (83). An important factor in the release from growth inhibition following balloon injury is the loss of inhibitory control of the surrounding proteoglycan matrix. Matrix-associated heparan sulfate is a potent inhibitor of smooth muscle cell proliferation and plays an important role in the regulation of smooth muscle cell growth in injured arteries (13,17,84). After balloon injury, endothelial denudation, disruption of the proteoglycan matrix of the medial layers and activated platelet release of endoglycosidases that cleave heparan sulfate from the surface of smooth muscle cells all lead to the loss of the inhibitory effect of heparan sulfate. In addition, the removal of heparan sulfate renders cells receptive to growth factors and contributes to their phenotypic modulation (13,14,17).

Hyperglycemia induces an increase in selected matrix gene transcription that persists for weeks after restoration of normoglycemia *in vivo* (85). This results in an increased synthesis of extracellular matrix components such as collagen type IV, fibronectin and laminin. Abnormalities in the synthesis and metabolism of heparan sulfate have been repeatedly reported in association with both experimental and human diabetes (79,86,87). The density of heparan sulfate has been shown to be significantly reduced in the glomerular basement membrane of patients with diabetic nephropathy (79,86,87). Hyperglycemia causes a decrease in the *de novo* synthesis of heparan sulfate (88), probably because of decreased activity of the

enzyme *N*-deacetylase, which plays a key role in its biosynthesis (89). Interestingly, the administration of protamine as part of commonly used insulin preparations exacerbates smooth muscle cell proliferative lesions after balloon injury in rats and interferes with the growth inhibitory effects of heparin in culture and in vivo (90).

In summary, diminution of matrix-bound heparan sulfate can result in loss of tonic growth inhibition and facilitate smooth muscle cell proliferation. Hyperglycemia-induced augmentation in matrix component production may increase neointimal volume (Fig. 1).

Role of Advanced Glycosylation End Products in Restenosis

Advanced glycosylation end products are a heterogeneous class of nonenzymatically glycosylated adducts of proteins or lipids that accumulate in vascular tissues with aging and at an accelerated rate in diabetes. The degree of nonenzymatic glycosylation is determined mainly by the glucose concentration and time of exposure (91-94). Advanced glycosylation end products are abundant in most tissues and fluids and, through the induction of cytokines and growth-promoting mediators, may participate in tissue remodeling (91-94). Advanced glycosylation is implicated in the initiation and acceleration of multiple organ damage in diabetic patients, including macrovascular disease, nephropathy, and neuropathy (91-95), and may also promote the restenosis process.

Advanced glycosylation end products interact with vessel wall cellular components through a specific receptor system present on various cell types, including monocytes, endothelial cells and smooth muscle cells (94,96,97). Monocyte receptor-mediated interaction with advanced glycosylation end products results in the production of mediators such as tumor necrosis factor- α , platelet-derived growth factor and insulin-like growth factor-1 (98-100).

Monocytes and lymphocytes are known to migrate into the thrombus that forms in the balloon injury site. This cellular infiltrate may be an abundant source of growth factors and chemoreactant substances, facilitating and sustaining further cellular recruitment and smooth muscle cell growth (15-17). The presence of advanced glycosylation end products in the exposed vessel wall would be expected to promote the migration of inflammatory cells into the lesion with the subsequent release of growth-promoting cytokines. Furthermore, specific binding of glycosylated proteins by cultured rat smooth muscle cells is associated with increased cellular proliferation (93,94) that is likely to be cytokine or growth factor mediated.

Finally, advanced glycosylation end products influence the expression of selected extracellular matrix genes (e.g., collagen type IV, laminin) as well as transforming growth factor- β (101). Advanced glycosylation also alters the functional properties of several important matrix constituents. Glycosylation of matrix components such as collagen IV, laminin and vitronectin will decrease binding of anionic heparan sulfate, leading to greater turnover of heparan sulfate (92,102). Gly-

cosylation of the extracellular matrix also alters the normal interactions of transmembrane integrin receptors with their specific matrix ligands. For example, modification of the cell-binding domains of type IV collagen causes decreased endothelial cell adhesion (92,102).

Summary. Advanced glycosylation end products can mediate inflammatory cell recruitment and activation, stimulation of smooth muscle cell proliferation and abnormal matrix production, all of which can promote the restenosis process.

Conclusions

Diabetes influences various cellular events involved in the vessel response to injury (Fig. 1). The same mechanisms can potentially play a role in the intimal proliferation that causes luminal loss in the first years after bypass surgery with venous conduit (103).

An important question is whether the restenosis rate can be reduced in diabetic patients. Pharmacologic manipulations for the prevention of restenosis are based on insights into the pathophysiologic mechanisms of restenosis and include growth factor inhibitors (e.g., trapidil) (17), new antithrombotic agents (44), antiinflammatory agents and gene therapy (14,17). However, it may be possible to use simpler measures in diabetic patients. Many of the potential mechanisms promoting restenosis in diabetic patients are related to elevated glucose or insulin levels, or both. Moreover, the majority of these abnormalities are reversible on improved glycemic control. Therefore, we anticipate that rigorous glycemic control may reduce the restenosis rate in diabetic patients. However, some of the alteration induced by the metabolic abnormalities of the diabetic state may not be readily reversible. For example, hyperglycemia-induced gene transcription may persist for weeks after restoration of normoglycemia in vivo (85). This phenomenon has been termed hyperglycemic memory and may reflect irreversible intracellular and extracellular effects of hyperglycemia (85,102). In addition, normoglycemia is frequently achieved at the price of peripheral hyperinsulinemia. Clinical trials are needed to evaluate the impact of tight glycemic control on restenosis rates among diabetic patients.

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