Diabetes mellitus is increasing worldwide, resulting from the interaction of obesity, inflammation, and hyperglycemia. Activated immunity and cytokine production lead to insulin resistance and other components of the metabolic syndrome, establishing the link between diabetes and atherosclerosis. Hyperglycemia-induced endothelial dysfunction is mediated by increased oxidative stress, a promoter of adventitial inflammation and vasa vasorum neovascularization in experimental models of diabetic atherosclerosis. Recent studies have documented increased inflammation, neovascularization, and intraplaque hemorrhage in human diabetic atherosclerosis. This inflammatory microangiopathic process is independently associated with plaque rupture, leading to coronary thrombosis. Tissue factor, the most potent trigger of the coagulation cascade, is increased in diabetic patients with poor glycemic control. Circulating tissue factor microparticles are also associated with apoptosis of plaque macrophages, closing the link among inflammation, plaque rupture, and blood thrombogenicity. High-density lipoproteins, responsible for free cholesterol removal, are reduced in patients with insulin resistance and diabetes. High-density lipoprotein therapy leads to a significant decrease in plaque macrophages and increase in smooth-muscle cells. These beneficial effects may be responsible for coronary plaque stabilization in patients treated with recombinant Apolipoprotein A-I Milano/phospholipid complex. Finally, peroxisomal proliferator-activated receptors (PPARs) are now considered the nuclear transcriptional regulators of atherosclerosis. Three subfamilies, including PPAR-alpha, -delta, and -gamma, have been identified with crucial roles in lipid metabolism, plaque inflammation, expression of adhesion molecules and cytokines, and regulation of matrix metalloproteinases. Multiple experimental studies have documented plaque stabilization with PPAR-gamma agonists, a group of medications holding great promise in the treatment of diabetes atherosclerosis. (J Am Coll Cardiol 2004;44:2293–300) © 2004 by the American College of Cardiology Foundation.
Innate immunity and inflammation play a role in the development of insulin resistance and the development of diabetes mellitus. Since the hypothesis was proposed in 1997, at least 12 studies have shown that circulating markers of inflammation, acute-phase reactants, or interleukin-6 (the major cytokine mediator of the acute-phase response) are strong predictors of the development of type 2 diabetes (12). Thus, the pathophysiology of insulin resistance, the metabolic syndrome, and atherosclerosis may have a common proximal inflammatory basis (Fig. 3).

**EARLY STAGES OF DIABETIC ATHEROSCLEROSIS**

Five major molecular mechanisms have been implicated in hyperglycemia-induced endothelial damage: activation of protein kinase C isoforms via de novo synthesis of the lipid second messenger diacylglycerol, increased hexosamine pathway flux, increased advanced glycation end product (AGE) formation, increased polyol pathway flux, and activation of the proinflammatory nuclear transcription factor nuclear factor-kappa B (13,14). All of these mechanisms are independently associated with overproduction of superoxide by the mitochondrial electron transport chain (15). As a result, hyperglycemia-induced formation of reactive oxygen species (ROS) may lead to endothelial dysfunction, decreasing the bioavailability of nitric oxide and prostacyclin, increasing the synthesis of vasoconstrictor prostanoids and endothelin, and promoting atherosclerotic plaque formation (16). This attractive hypothesis needs further testing to completely elucidate the role of ROS in the pathogenesis of diabetic atherosclerosis.

Diabetic endothelial dysfunction is also expressed by increased vascular permeability related to hyperglycemia-induced ROS production occurring as early as two weeks after the onset of diabetes (17). Recently, endothelial dysfunction was documented by decreased flow-mediated brachial artery dilation in children with diabetes mellitus, leading to early atherosclerosis, as evidenced by increased carotid intimomedial thickness (18). Furthermore, diabetes-induced microvascular permeability is responsible for expression of the well-known endothelial mitogen vascular endothelial growth factor, the main promoter of angiogenesis and neovascularization responsible for diabetic microangiopathy (19,20).

**ADVANCED STAGES OF DIABETIC ATHEROSCLEROSIS**

Insulin resistance is associated with macrophage upregulation of CD36 protein with increased uptake of oxidized low-density lipoprotein in experimental obesity (21). Hyperglycemia increases macrophage matrix metalloproteinase (MMP) expression (22), which is up-regulated in human fibroblasts from patients with diabetes mellitus (23). In addition, MMP-2 is marker for microangiopathy in children with type 1 diabetes (24), suggesting a pivotal role for macrophages in the pathogenesis of diabetic atherosclerosis. Nevertheless, the absence of a reproducible animal model of atherosclerosis in type II diabetes mellitus may limit these observations.

Plaque composition may be different in patients with diabetes. Macrophage infiltration and thrombus formation are increased in advanced coronary plaques from diabetic patients with unstable angina (Fig. 4) (25). Recent autopsy studies have confirmed this observation (26).

New structural and functional features characterizing diabetic atherosclerosis have been recently identified, including adventitial inflammation and vasa vasorum neovas-
cularization leading to intraplaque hemorrhage, macrophage activation, and lipid core expansion evolving into high-risk atherosclerotic lesions. The mechanistic role of diabetic-induced oxidative stress in adventitial inflammation and neovascularization provides new pathogenic links between hyperglycemia and atherosclerosis.

**ADVENTITIAL INFLAMMATION**

Adventitial inflammation is frequently observed surrounding vasa vasorum in ruptured plaques from patients with unstable angina and acute myocardial infarction (27,28). To evaluate diabetes-induced oxidative stress and adventitial inflammation, Zhang et al. (29) documented increased cytokines (interleukin-6 and tumor necrosis factor-alpha), chemokines (macrophage chemotactic protein-1), and adhesive molecules in adventitial fibroblasts from streptozotocin-induced diabetic swine. Oxidative stress was responsible for this inflammatory response mediated by AGE-induced activation of the transcriptional factor nuclear factor-kappa B in coronary adventitial fibroblasts (29), providing a mechanistic link between diabetes, oxidative stress, and adventitial inflammation.

Adventitial inflammation can also extend into the tunica media, inducing atrophy and fibrosis. Increased content of macrophage-derived MMP-2 and -9 within the intimal-medial interface of advanced plaques has been described (30,31). This increased activity of MMP is associated with

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**Figure 3.** Several factors such as altered nutrition, inactivity, age, fetal metabolic programming, and genetic propensity are known activators of the innate immune system. Cytokine production leads to insulin resistance, type 2 diabetes, and other components of the metabolic syndrome, such as dyslipidemia. Activated innate immunity is a possible common antecedent of both type 2 diabetes and atherosclerosis. IL-6 = interleukin-6; TNFα = tumor necrosis factor-alpha. Modified with permission from Pickup (12).

**Figure 4.** (Left) Increased percent macrophage area in coronary tissue from patients with diabetes mellitus versus tissue from patients without diabetes. (Right) Photomicrographs of coronary atherectomy tissue immunostained with antihuman pan-macrophage antibody. Larger macrophage content is seen in coronary tissue from a patient with diabetes mellitus (A) than in coronary tissue from a patient without diabetes (B). Reprinted with permission from Moreno PR, et al. Circulation 2000;102:2180–4.
disruption of the internal elastic lamina, an independent predictor of plaque rupture (Fig. 5) (32).

Adventitial inflammation is also associated with vasa vasorum neovascularization leading to red blood cell extravasation, foam cell formation, and lipid core expansion (33,34).

**VASA VASORUM NEOVASCULARIZATION**

Neovascularization of the vessel wall is a consistent feature of atherosclerotic plaque development (35). Neovessels may serve as a port of entry to blood-derived cells, providing nutrients to aerobic cells like macrophages and smooth-muscle cells. Vasa vasorum neovascularization is present within the first two to four weeks of hypercholesterolemic diet, preceding endothelial dysfunction (36). Furthermore, neovessels are involved in plaque hemorrhage and the development of symptoms in cerebrovascular disease, and they may play a role in plaque rupture (37,38).

The amount of neovascularization correlates with the extent of inflammatory cells (39). We recently evaluated microvessel and inflammatory cell content in complex atherosclerosis. Macrophages, T-lymphocytes, and smooth-muscle cells were defined as CD68, CD3, and alpha actin-positive cells, respectively (Fig. 6).

Total and regional microvessel densities were increased in ruptured plaques when compared with non-ruptured plaques (Fig. 7). Furthermore, microvessel density was increased in lesions with inflammation, intraplaque hemorrhage, and in thin-cap fibroatheromas. Microvessels at the base of the plaque independently correlated with plaque rupture, suggesting a contributory role for microvessels in plaque instability (40,41).

Purushothaman et al. (42) recently studied microvessel and inflammatory cell content in 230 plaques from patients with diabetes mellitus. Ruptured plaques were excluded from this analysis. Microvessel content, macrophages/T-lymphocyte cells, and intraplaque hemorrhage were all increased in plaques from patients with diabetes mellitus. These differences in plaque composition may increase the risk of plaque rupture in diabetic atherosclerosis. However, more studies are needed to confirm these observations.

**CORONARY THROMBOSIS**

Diabetes is characterized by elevated concentrations of procoagulant factors, including fibrinogen, von Willebrand factor, and factor VII, with decreased concentration of antithrombotic factors including antithrombin III and protein C (43). Arterial thrombosis is mediated by tissue factor (TF), the most potent trigger of the coagulation cascade (44). Rauch et al. (45) reported that polymorphonuclear leukocytes might be involved in the transport of circulating TF to platelets by a CD15-dependent mechanism. Higher levels of plasma TF antigen (46) and circulating TF-positive microparticles with procoagulant activity have been de-
scribed in patients with acute coronary syndromes (47). Mallat and Tedgui (48) linked the apoptotic phenomena to atherothrombosis and to the production of TF. More recently, Hutter et al. (49) showed that apoptosis of plaque macrophages co-localize with TF expression, suggesting a potential pathogenic mechanism leading to increased plaque thrombogenicity.

Tissue factor is increased in patients with diabetes mellitus (50). Recently, Sambola et al. (51) identified significant reductions in TF activity and blood thrombogenicity in diabetic patients with improved glycemic control. These observations highlight the concept of high-risk blood in the pathophysiology of diabetic atherosclerosis leading to coronary thrombosis.

**NOVEL THERAPIES: IMPLICATIONS FOR PLAQUE REGRESSION**

Despite multiple advances, diabetes still remains a complex and challenging chronic condition to treat. A variety of therapeutic options, from lifestyle interventions and tight glycemic control to a large number of pharmacologic agents, are available to attenuate diabetic atherosclerosis. The Centers for Disease Control recently summarized current strategies for diabetes prevention by lifestyle intervention (52). Three large randomized trials definitively established that moderate weight loss through diet and physical activity reduces the incidence of type 2 diabetes in high-risk persons by 40% to 60% over three to four years (53–55). Furthermore, pharmacologic intervention with metformin (850 mg/day) reduced the incidence of diabetes by 31% (55). In addition, the use of ramipril reduced the incidence of newly diagnosed diabetes by 34% in the Heart Outcomes Prevention Evaluation (HOPE) trial (56).

For patients with established diabetes, control of blood pressure and the use of aspirin and angiotensin-converting enzyme inhibitors reduces cardiovascular events (57). Furthermore, aggressive lipid-lowering therapy is now a cornerstone in the treatment of diabetes mellitus, reducing cardiovascular events by 22% independent of baseline lipid levels (58).

In addition to established therapy, two novel alternatives are under active experimental and clinical investigation in patients with atherosclerosis and diabetes mellitus. These include HDL therapies and the peroxisomal proliferator-activated receptors (PPARs).

**HDL**

The HDLs are responsible for the removal of free cholesterol from the blood. Low plasma levels of HDL are associated with increased cardiovascular risk. Low levels of HDL are frequently seen in patients with insulin resistance, from the metabolic syndrome to overt diabetes mellitus. Badimon et al. (59,60) elegantly demonstrated the anti-atherogenic properties of HDL, reducing the number of fatty streaks and inducing disease regression in the rabbit experimental model. More recently, Rong et al. (61) evaluated the effects of HDL in advanced experimental athero-
sclerosis. Diseased thoracic aortic segments from hypercholesterolemic apolipoprotein E-deficient mice were transplanted in the abdominal aorta of apolipoprotein E-deficient mice not expressing (plasma HDL cholesterol approximately 26 mg/dl) or expressing (HDL approximately 64 mg/dl) a human apolipoprotein AI transgene. Mice with high plasma HDL showed significant changes in plaque composition, with macrophage area reductions >80% and smooth-muscle cell area increases >300%. These dramatic changes in plaque composition may explain the promising results in plaque regression documented by intravascular ultrasound in patients with coronary artery disease after five weeks of recombinant apolipoprotein A-I Milano/phospholipid complex (62).

Within the control of lipid modifying strategies, high-resolution magnetic resonance imaging can provide mechanistic information to understand plaque regression in humans. Corti et al. documented a decrease in plaque size in both carotid and aortic lesions without changes in luminal area after 12 months of aggressive lipid-lowering therapy (63). Longer follow-up showed a further decrease in plaque size along with a slight, but significant, increase in luminal area at 24 months (64). The same pattern for plaque regression was documented by Nissen et al. (65) using systematic intravascular ultrasound in patients with coronary artery disease, suggesting a major role for aggressive lipid-lowering therapy in plaque stabilization.

Figure 8. Direct and indirect effects of peroxisomal proliferator-activated receptors (PPARs). Peroxisomal proliferator-activated receptor activation by synthetic ligands induces metabolic changes that may limit inflammation and atherosclerosis indirectly. Alternatively, the expression of PPARs in most major vascular and inflammatory cells and PPAR regulation of relevant target genes in those cells raises the possibility that PPARs have a direct effect on inflammation and atherosclerosis. ABCA1 = ATP-binding cassette transporter 1; HDL = high-density lipoprotein; IFNγ = interferon gamma; MCP1 = monocyte chemoattractant protein-1; VCAM1 = vascular cell adhesion molecule-1. Reprinted with permission from Plutzky (67).

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs)

The PPARs are steroid hormone nuclear receptors that act as ligand-activated transcription factors controlling the expression of specific target genes that in turn regulate a variety of cellular functions (66). The PPARs are considered relevant nuclear transcriptional regulators of atherothrombosis (Fig. 8) (67). Three subfamilies have been described with different tissue distribution and effects: PPAR-alpha, -delta, and -gamma. The subfamily member PPAR-gamma plays a central role in adipogenesis and lipid metabolism and is highly expressed in endothelial cells, smooth-muscle cells, lymphocytes, and macrophages. The PPAR-gamma activators may reduce plaque inflammation, inhibit expression of adhesion molecules and cytokines, and reduce production of MMP (68).

Evidence indicates that PPAR-gamma activators can decrease thrombogenicity by reducing plasminogen activator inhibitor-1 and fibrinogen concentrations to improve fibrinolysis. In addition, PPAR-gamma activators may reduce production of endothelin-1, a potent vasoconstrictor and important atherogenic stimulus. Crucially, PPAR-gamma activators may reduce the lipid content of plaques by enhancing reverse cholesterol transport and by up-regulating the genes responsible for scavenger receptor class B type I human homologue, for adenosine triphosphate-
binding cassette transporter-1, and for apolipoprotein A1, thereby facilitating efflux of free cholesterol from the plaque and its transport to the liver. Using high-resolution magnetic resonance imaging, we recently observed plaque regression and features of plaque stabilization in the atherosclerotic rabbit model exposed to a new selective PPAR-gamma activator [69]. The combination of statin with PPAR-gamma activator was superior to either medication alone, with decreased macrophage content and matrix metalloproteinase activity and increased smooth-muscle cell/collagen content in atherosclerotic lesions. Considering the popularity of PPAR-gamma agonists in the treatment of diabetes, these medications hold promise in the continuing fight against atherosclerosis in patients with diabetes mellitus.

CONCLUSIONS

Diabetic atherosclerosis is an aggressive disease responsible for major health problems in modern society. The metabolic syndrome, an inflammatory disease affecting 47 million U.S. citizens, plays a pivotal role in atherosclerosis and requires major attention for adequate treatment. New understanding of the complex pathophysiology of atherosclerosis is consistent with a disease involving all three layers of the vessel wall, with rupture of the internal elastic lamina and active participation of adventitial vasa vasorum responsible for media and plaque neovascularization. This microangiopathic process is exaggerated in diabetes mellitus, associated with increased inflammation and intraplaque hemorrhage. Atherosclerotic microvessels are increased in the tunica media and at the base of the plaque in advanced diabetic plaques. In addition, microvessels are also increased in ruptured plaques from patients with diabetes mellitus. Coronary thrombosis is mediated by the interactions of systemic procoagulant factors, which are up-regulated in patients with diabetes mellitus. Tissue factor, the most potent trigger of the coagulation cascade, is increased in diabetics with poor glycemic control. Finally, novel therapies, including HDL and PPAR agonists, hold great promise for the future treatment of diabetic atherosclerosis.

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