Atherothrombosis, Inflammation, and Diabetes

Giuseppe G. L. Biondi-Zoccai, MD, Antonio Abbate, MD, Giovanna Liuzzo, MD, Luigi M. Biasucci, MD, FACC

Rome, Italy

Diabetes mellitus has been recognized as an independent major cardiovascular risk factor since the publication of the first large-scale epidemiologic investigations in the 1970s (1). There are over 100 million people worldwide with diabetes (5% to 8% of overall population), and this number is likely to increase significantly in the near future. According to data from clinical studies, most diabetics die of cardiovascular disease, and atherothrombosis accounts for about 8 to 10 of all diabetic deaths (2). Diabetes mellitus, in the absence of previous cardiovascular disease, may confer a risk of adverse cardiovascular events similar to that faced by nondiabetic individuals who have already had a previous myocardial infarction, thus representing a "coronary risk equivalent" and, therefore, diabetic patients should join secondary prevention programs, regardless of their cardiovascular history (3,4). Moreover, diabetic patients, constituting about 15% to 20% of patients presenting with acute coronary syndromes, are at considerably increased risk of excess morbidity and mortality, as compared with nondiabetic patients, as diabetics' risk of adverse events is twice that of nondiabetics, even in the most recent experimental or observational clinical studies.

Diabetes and related metabolic diseases, such as hyperinsulinemia, insulin resistance, and central obesity, are recognized as major contributors to cardiovascular morbidity and mortality. Interestingly, recent and compelling evidence has shown the significant and independent role of systemic and coronary inflammation in the initiation, progression, and precipitation of atherothrombosis superimposed on traditional risk factors (5). Given the increasing prevalence of diabetes and dysmetabolic syndrome, the diffuse atherosclerotic burden of diabetics, the possible synergistic effect of diabetes and inflammation in atherosclerosis, as well as the potential for a preventive and therapeutic benefit in the modulation of inflammation in diabetics, this review covers the basic epidemiologic and pathophysiologic aspects underlying these two disease processes (Fig. 1).

DIABETES MELLITUS, ATEROTHROMBOSIS, AND INFLAMMATION

The pathophysiologic process of atherosclerosis has been schematically summarized in several steps, each of them characterized by significant involvement of humoral and cellular inflammatory elements (5). Pathologic, angiographic, and other in vivo studies have shown that diabetes favors diffuse and accelerated progression of atherosclerosis (6). Diabetic plaques are also more commonly complicated and at greater risk of subsequent complications. Moreover, diabetics who die suddenly show an increased number of fissured atherosclerotic plaques, as compared with nondiabetics (7). In fact, the results of angioscopic examination show that diabetic patients with unstable angina have a higher incidence of plaque ulceration and intracoronary thrombus formation than nondiabetic patients, and diabetic plaques usually have a greater lipid core burden and a richer inflammatory component and are more commonly complicated by overlying thrombosis (8).

Genetic abnormalities. Several genetic abnormalities in glycemic metabolism have been associated with the development of diabetes, diabetic complications, or aspects of the dysmetabolic syndrome. All of these derangements could be also involved in atherogenesis. Transcription factors, such as peroxisome proliferator-activated receptor (PPAR)-alpha and -gamma, are now being intensively studied. Whereas PPAR-alpha primarily stimulates the beta-oxidative degradation of fatty acids, PPAR-gamma promotes lipid storage by regulating adipocyte differentiation. Peroxisome proliferator-activated receptor-alpha is the molecular target of lipid-lowering drugs such as fibrates, and PPAR-gamma interacts with insulin-sensitizer drugs such as thiazolidinediones, and they may significantly modulate atherogenicity and inflammation. In particular, mutations in the PPAR-gamma gene have recently been shown to be asso-

From the Institute of Cardiology, Catholic University, Rome, Italy.

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The role of transcription factors in homeostasis and disease is quite complex and still unclear, owing to the heterogenicity, cell specificity, and pleiotropy of their effects. However, substantial evidence has shown that PPARs often interact intracellularly with another transcription factor, nuclear factor-kappa-B (NF-kappa-B), whose activities are mainly proinflammatory and proliferative. Peroxisome proliferator-activated receptor-alpha–deficient mice show a prolonged systemic acute-phase response and a local innate and immune inflammatory reaction to common noxious stimuli, whereas PPAR-alpha

### Abbreviations and Acronyms

- AGEP = advanced glycation end products
- CRP = C-reactive protein
- HDL = high-density lipoprotein
- ICAM-1 = intercellular adhesion molecule-1
- I-kappa-B = NF-kappa-B–inhibiting protein
- IL = interleukin
- LDL = low-density lipoprotein
- NF-kappa-B = nuclear factor-kappa-B
- NO = nitric oxide
- PAI-1 = plasminogen activator inhibitor-1
- PPAR = peroxisome proliferator-activated receptor
- TNF-alpha = tumor necrosis factor-alpha
- VCAM-1 = vascular cell adhesion molecule-1

Figure 1. The pathogenetic mechanisms involved in the initiation of subclinical atherosclerosis and progression to atherosclerotic clinical events in diabetic patients include infection, inflammation, hyperglycemia, insulin resistance, dyslipidemia, and thrombosis. AGE = advanced glycation end products; CRP = C-reactive protein; HDL = high-density lipoprotein; HTN = hypertension; IL-6 = interleukin-6; LDL = low-density lipoprotein; PAI-1 = plasminogen activator inhibitor-1; SAA = serum amyloid A protein; TF = tissue factor; TG = triglycerides; tPA = tissue-type plasminogen activator.
activation appears to decrease vascular cell adhesion molecule-1 (VCAM-1) expression by activated endothelial cells, thereby switching off the proatherogenic-activated endothelium (10). Moreover, PPAR-alpha selectively downregulates human aortic smooth muscle cell secreting and proliferating activities (11). In particular, ligands of this transcription factor inhibit interleukin (IL)-1-induced production of IL-6 and prostaglandin and expression of cyclooxygenase-2 in endothelial cells. In hyperlipidemic patients, fenofibrate-dependent PPAR-alpha activation decreases levels of IL-6, fibrinogen, and C-reactive protein (CRP) (11). On the other hand, thiazolidinedione-activated PPARs inhibit both the proliferation and migration of vascular smooth muscle cells. In endothelial cells, the PPAR agonist troglitazone markedly attenuates tumor necrosis factor-alpha (TNF-alpha)–induced expression of VCAM-1 and intercellular adhesion molecule-1 (ICAM-1), whereas apolipoprotein E–deficient mice treated with troglitazone show reduced monocye/macrophage homing to atherosclerotic plaques (12). Finally, the combined activation of PPAR-alpha and -gamma appears to decrease CRP-mediated production of monocyte chemoattractant protein-1 by human endothelial cells, an effect similar to that achieved by simvastatin. Moreover, type 2 diabetic patients treated with troglitazone have increased levels of NF-kappa-B–inhibiting protein (I-kappa-B) and reduced NF-kappa-B activity, as well as systemic levels of reactive oxygen species, plasminogen activator inhibitor-1 (PAI-1), and CRP (13). Accordingly, the human promoter polymorphism 174 G/C of the IL-6 gene has been associated with an increased risk of cardiovascular disease and hypertension (14).

**Dyslipidemic abnormalities.** Diabetics usually show decreased high-density lipoprotein (HDL) cholesterol levels and increased low-density lipoprotein (LDL) cholesterol and triglyceride levels. Diabetes is most often associated with smaller, denser LDL that is more susceptible to oxidation and thus has greater atherogenicity (5). Even if diabetic patients with good glycemic control may have LDL levels similar to or slightly lower than those of nondiabetic individuals, fasting and postprandial levels of triglyceride-rich lipoproteins, especially very-low-density lipoprotein, are higher and those of HDL are lower than those of nondiabetics (15). In addition, glycation of LDL and other lipoproteins is quite common in diabetes, thus making the lipoproteins of diabetic patients more susceptible to oxidation and more atherogenic. Finally, several clinical studies have shown the benefit of lipid-lowering strategies in diabetics, even in cases of modestly increased or normal cholesterol levels, and these benefits are often greater than those found in nondiabetic patients (16).

**Oxidative stress.** Diabetics have decreased antioxidant activity and increased oxidative burden (17), and hyperglycemia increases the production of reactive oxygen species. These may shift the oxidant-antioxidant balance toward nonenzymatic oxidation of lipoproteins, thus contributing to atherogenic processes. It has also been hypothesized that reactive carbonyl groups may overwhelm the antioxidant mechanism of diabetic patients, leaving them unprotected to common oxidative stressors such as smoking.

**Advanced glycation end products (AGEP).** Diabetic hyperglycemia characteristically leads to nonenzymatic glycation of common macromolecules, as usually assessed through hemoglobin A1c (18). Nonenzymatic macromolecular glycation leads to the production of biomacromolecular aggregates known as AGEP. Glycated molecules as well as AGEP have been implicated in LDL modification, accumulation, and inflammatory activation. Oxidation of AGEP seems to play an important and perhaps primary role in initiating and amplifying lipid oxidation, thus contributing to the atherogenic profile of diabetics (19). Macromolecular glycated complexes increase arterial intimal noncellular growth and may involve proteins, nucleic acids, and lipid structures. The formation of complex and less degradable macromolecular aggregates and the impairment of scavenger cells cause quantitative imbalances in lipid levels and abnormal vascular clearance of proatherosclerotic lipids (20). Specific receptors for AGEP have been described, and their modulation has shown antiatherosclerotic effects in mice, whereas in normal rabbits, AGEP induces endothelial VCAM-1 and ICAM-1 expression and promotes atheroma formation (21).

**Prothrombotic and antifibrinolytic abnormalities.** Diabetes has several prothrombotic effects possibly involved in the initiation of atherosclerotic plaque, its progression, and complication. Diabetic rats show increased platelet adhesiveness and aggregability after common platelet-activating stimuli, such as adenosine and collagen (22). Platelet dysfunction is probably due in part to increased catabolism of arachidonic acid and increased production of thromboxanes. Diabetes is also associated with increased levels of fibrinogen, von Willebrand factor, factor VII, factor VIII, platelet factor 4, and PAI-1 levels. Peroxisome proliferator-activated receptor-alpha dysfunction or decreased activity seems to play a role in hyperfibrinogenemia (11), and fibrates may determine a reduction in fibrinogen levels through PPAR-alpha activation. Diabetic and hyperinsulinemic patients typically exhibit increased production of PAI-1 from visceral abdominal adipocytes. Finally, it is likely that elevated levels of tissue factor and PAI-1 and reduced levels of tissue-type plasminogen activator are associated with increased IL-6 production from adipocytes (according to the hypothesis of obesity as an inflammatory disease).

**Dysmetabolic syndrome.** The concept of dysmetabolic syndrome (metabolic syndrome X or insulin resistance syndrome) stands at the crossroads of hyperglycemia, overt type 2 diabetes mellitus, inflammation, and atherosclerosis (Fig. 2) (23). It is defined as a condition characterized at least in part by one or more of the following: glucose intolerance, relative hyperinsulinemia, peripheral insulin resistance, visceral or abdominal obesity, increased serum levels of inflammatory markers (such as C-reactive protein), and increased risk of cardiovascular disease.
triglyceride concentrations, hyperuricemia, microalbuminuria, hypertension, and endothelial dysfunction. All of these entities have in common the same final effect of hyperinsulinemia, low-grade inflammation, and metabolic dysfunction. However, diabetes and the dysmetabolic syndrome seem to have both an independent detrimental effect on cardiovascular outcomes, as shown by studies that adjusted for insulin levels, diabetic status, and glycemic control (24). Hyperinsulinemia is also an independent and significant risk factor for coronary heart disease development in apparently healthy subjects. The overwhelming prevalence of this syndrome has prompted the proposal of the so-called "thrifty gene hypothesis," according to which in relatively early times, the ability to store fat may have conferred survival advantage, thus enriching ethnic groups of genes facilitating fat storage without negative feedback control (25). Several pathophysiologic mechanisms have been proposed, including abnormalities in leptin gene expression, decreased activity of PPAR-alpha, and primary inflammatory or infective processes. Nonetheless, the concept of the dysmetabolic syndrome re-emphasizes the metabolic and inflammatory role of adipocytes. In fact, among the several cellular elements involved in vascular inflammation, adipocytes merit great interest, as dysfunction in lipid metabolism is characteristic of diabetes, and as adipocytes have recently been shown able to produce IL-6 and TNF-alpha (26).

Role of inflammation in development of diabetes mellitus. Although it is plausible to accept diabetes as a trigger for vascular inflammation, the converse is also true, as substantial evidence has shown that low-grade inflammation is an important pathogenetic determinant of type 2 diabetes. In the West of Scotland Coronary Prevention Study, increased CRP levels significantly predicted the risk of later developing type 2 diabetes, and this risk was independent of body mass index, fasting triglyceride or glucose levels, or statin use (27). Moreover, a high white blood cell count was an independent predictor of a worsening insulin action and the development of type 2 diabetes in Pima Indians (28), as well as in U.S. adult women (29). Increased levels of other markers of inflammation, such as sialic and orosomucoid acid, are also associated with the later occurrence of diabetes (30).

More recently, an abundance of clinical evidence has confirmed the pathogenetic role of inflammation in the onset of diabetes, showing that anti-inflammatory agents, such as statins (31), PPAR agonists (32), and other drugs, including angiotensin-converting enzyme inhibitors (33), may prevent or delay the onset of diabetes in high-risk subjects. Trials are currently underway to further validate this exciting hypothesis.

Role of nitric oxide (NO) in inflammation. Nitric oxide has several pleiotropic functions in the vascular milieu, beyond its well-known vasodilating capabilities. In vitro, NO selectively inhibits IL-1-induced VCAM-1 expression on endothelial cells. This inhibition is paralleled by reduced monocyte adhesion to endothelial monolayers. Nitric oxide also decreases the endothelial expression of other leukocyte adhesion molecules (E-selectin and, to a lesser extent, ICAM-1) and secretable cytokines (IL-6 and -8). Molecular assays indicate that NO represses VCAM-1 gene transcription, in part by inhibiting NF-kappa-B (34). The expression of macrophage colony-stimulating factor in endothelial cells, an important regulating factor of macrophage-derived foam cells in atherosclerotic lesions, as induced by oxidized LDL or TNF-alpha, is significantly downregulated by NO through inhibition of NF-kappa-B (35). The precise mechanisms of NO-mediated NF-kappa-B inhibition are probably the induction and stabilization of I-kappa-B-alpha.
Role of cytokines and infectious agents. Experimental observations have demonstrated the central mechanistic relevance of several cytokines and chemokines networks in atherosclerotic processes (5). In this regard, diabetes, insulin resistance, and glucose intolerance all seem to have important modulating effects, with global proatherosclerotic activity. At least partly as a consequence of hyperglycemia and imbalances in insulin homeostasis, diabetes commonly induces abnormalities in the expression and activity of various cytokines and vasoactive peptides, such as TNF-alpha, IL-6, angiotensin II, and endothelin-1 (36). Serum levels of TNF-alpha, IL-6, and several adhesion molecules are significant predictors of future cardiovascular events in healthy subjects as well as diabetics, and in diabetic patients, increased VCAM-1 levels are independently associated with cardiovascular mortality (37). Interleukin-1 and TNF-alpha are produced by inflammatory cells in the atherosclerotic plaque and induce the release of IL-6 from several cell types, including smooth muscle cells. Furthermore, circulating IL-6 stimulates the hypothalamic-pituitary-adrenal axis, activation of which is associated with central obesity, hypertension, and insulin resistance (38). Thus, it is intriguing that overweight and obese individuals have increased serum levels of CRP, IL-6, TNF-alpha, and leptin. Actually, it has been recently shown that IL-6 levels decrease in obese women after significant weight loss (39).

Hyperglycemic patients demonstrate a significant production of AGE products, which are specifically recognized by inflammatory cells in atherosclerotic plaque, and induce the release of inflammatory cytokines. These mediators stimulate endothelial and smooth muscle proliferation, endothelial activation, and collagen overproduction, thus contributing to plaque growth and progression. C-reactive protein, a nonspecific marker of inflammation that also has a direct inflammatory and proatherosclerotic plaque and induce the release of IL-6 from several cell types, including smooth muscle cells. Furthermore, circulating IL-6 stimulates the hypothalamic-pituitary-adrenal axis, activation of which is associated with central obesity, hypertension, and insulin resistance (38). Thus, it is intriguing that overweight and obese individuals have increased serum levels of CRP, IL-6, TNF-alpha, and leptin. Actually, it has been recently shown that IL-6 levels decrease in obese women after significant weight loss (39).

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looked at the association between these two conditions. Among 917 patients with acute coronary syndromes, diabetes and admission CRP levels were both independent predictors of long-term outcome (49). Other prospective studies considering CRP levels have confirmed the prognostic role of diabetic status (50,51). However, although the FReAgmin during InStability in Coronary artery disease (FRISC) cohort has shown that diabetes plays a prognostic role independent of CRP levels (49), several other smaller studies have shown that diabetes acts as a significant predictor on univariate but not multivariate analysis. Interestingly, in some series, the presence of diabetes was associated, independent of the prognosis, with elevated CRP levels (50,52), whereas in other studies, the two parameters appeared to be independent (53,54). After percutaneous coronary interventions, the baseline CRP level was shown to be a predictor of events (including target vessel revascularization), independent of diabetic status (a condition associated with a significantly increased risk of recurrence after revascularization procedures) (55–58).

Primary prevention studies among apparently healthy men (41) and women (59) have shown significant cardiac risk prediction by means of elevated CRP levels. In these data, no correlation between CRP levels and diabetes was described, and although diabetes was shown to be significantly associated with increased cardiac risk, CRP levels were still independent markers of prognosis. The MONI-toring trends and determinants in Cardiovascular disease (MONICA) Augsburg cohort study was the first study to evaluate the prospective value of CRP levels in primary prevention (60). Many baseline clinical variables, including a history of diabetes, were found to be associated with higher CRP levels, and during follow-up, CRP remained an independent predictor of adverse cardiovascular events. The independent prognostic role of CRP has also been reported in patients with peripheral artery disease (61).

In conclusion, these data appear to demonstrate that CRP levels predict cardiac risk independent of diabetic status. However, the fact that diabetics tend to have higher CRP levels and that, in some clinical studies, diabetes still remained a significant independent predictor suggests that inflammation and metabolic derangements may represent two different aspects of the same process and thus both could be considered as independent predictors of cardiac risk. To the best of our knowledge, no study to date has thoroughly looked at the prognostic value of CRP between diabetics and nondiabetics separately. Therefore, these conclusions need further confirmation. In addition, the relative role of cytokines (IL-6, TNF-alpha), oxidized LDL, fibrinogen, and other markers of risk prediction require further investigation.

Reprint requests and correspondence: Dr. Luigi M. Biasucci, Institute of Cardiology, Catholic University, Largo A. Gemelli 8, 00168 Rome, Italy. E-mail: lbiasucci@virgilio.it.

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