Review

Cytokine and Cytokine-Like Inflammation Markers, Endothelial Dysfunction, and Imbalanced Coagulation in Development of Diabetes and Its Complications

Ronald B. Goldberg

Division of Endocrinology, Diabetes, and Metabolism, Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, Florida 33101

Context: Recent developments indicate that pathophysiological mechanisms leading to β-cell damage, insulin resistance, and the vascular complications of diabetes include an activation of the inflammation cascade, endothelial dysfunction, and procoagulant imbalance. Their circulating biomarkers may therefore provide opportunities for early diagnosis and targets for novel treatments.

Evidence: Circulating biomarkers of these pathways such as TNFα, IL-6, C-reactive protein (CRP) (inflammation), vascular cellular adhesion molecule-1, interstitial cellular adhesion molecule-1, E-selectin, von Willebrand factor (endothelial dysfunction), plasminogen activator inhibitor-1, fibrinogen, P-selectin (procoagulant state), and adiponectin (antiinflammation) may be associated with development of both type 1 and type 2 diabetes and some studies, particularly in type 2 diabetes, have demonstrated that certain biomarkers may have independent predictive value. Similarly studies have shown that these biomarkers may be associated with development of diabetic nephropathy and retinopathy, and again, particularly in type 2 diabetes, with cardiovascular events as well. Finally, the comorbidites of diabetes, namely obesity, insulin resistance, hyperglycemia, hypertension and dyslipidemia collectively aggravate these processes while antihyperglycemic interventions tend to ameliorate them.

Conclusions: Increased CRP, IL-6, and TNFα, and especially interstitial cellular adhesion molecule-1, vascular cellular adhesion molecule-1, and E-selectin are associated with nephropathy, retinopathy, and cardiovascular disease in both type 1 and type 2 diabetes. Whereas further work is needed, it seems clear that these biomarkers are predictors of increasing morbidity in prediabetic and diabetic subjects and should be the focus of work testing their clinical utility to identify high-risk individuals as well as perhaps to target interventions. **(*J Clin Endocrinol Metab* 94: 3171–3182, 2009)**

Markers of well-accepted pathophysiologic pathways have been important in predicting development of diabetes and its complications as well as providing targets for therapy. Over recent years our understanding of the etiology of type 1 (T1D) and type 2 diabetes (T2D) and their vascular complications has widened considerably. Broadly speaking, the concept has arisen that an interplay between inflammatory and metabolic abnormalities leads to tissue damage in diabetes. In small and large vessels, the earliest indicator of these effects is endothelial dysfunction accompanied by the development of a prothrombotic state, whereas in islets and insulin-sensitive tissues, β-cell damage and impaired insulin signaling are the hallmarks. This has led to the identification of an expanded array of

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Abbreviations: AGE, Advanced glycation end product; CAC, coronary artery calcium; CHD, coronary heart disease; CIMT, carotid intimal medial thickness; CRP, C-reactive protein; CVD, cardiovascular disease; FFA, free fatty acid; HbA1c, glycosylated hemoglobin; ICAM, interstitial cellular adhesion molecule; IGT, impaired glucose tolerance; MCP, monocyte chemoattractant protein; NO, nitric oxide; PAI, plasminogen activator inhibitor; PPAR, peroxisomal proliferator-activated receptor; ROS, reactive oxygen species; s, soluble; T1D, type 1 diabetes; T2D, type 2 diabetes; tPA, tissue plasminogen activator; VCAM, vascular cellular adhesion molecule; vWf, von Willebrand factor.

circulating biomarkers of these processes that offer new opportunities for preemption, early diagnosis, and targeted therapy. This review focuses on developments in the identification of circulating cytokine and cytokine-like biomarkers of inflammation, endothelial dysfunction, and procoagulant imbalance and their interrelationships in the development of T1D and T2D and their vascular complications based on clinical studies.

Inflammatory processes in diabetes

The origins of heightened inflammatory activity in diabetes are diverse. In T1D, islet inflammation is thought to be a local phenomenon driven by a focal autoimmune attack on islet antigens. By contrast, in T2D, activation of inflammation results from systemic etiologic factors, such as central obesity and insulin resistance. The comorbidities of hypertension and dyslipidemia acting in concert with hyperglycemia on the background of the differing pathophysiologies and genetic substrates of T1D and T2D have been incriminated in the maintenance and exacerbation of the inflammatory reaction. Ultimately inflammatory mediators activate a series of receptors and transcription factors such as nuclear factor-κB, toll-like receptors, c-Jun amino terminal kinase, and the receptor for advanced glycation end products, which lead to β-cell dysfunction and apoptosis, impaired insulin signaling in insulin-sensitive tissues, systemic endothelial dysfunction, and altered vascular flow.

Activation of inflammation, endothelial dysfunction, and procoagulant pathways (Fig. 1)

An early feature of inflammation is the release of chemokines such as monocyte chemoattractant protein (MCP)-1, macrophage migration inhibition factor (MIF), and others from stressed tissues. In diabetes this has been most clearly demonstrated in vascular endothelium (1, 2) and adipose tissue (3). These factors increase expression of interstitial and vascular cellular adhesion molecules (ICAM-1, VCAM-1), and E-selectin (4) and attract monocytes and immunocytes that gain access to the stressed site. They undergo chemokine-induced proliferation and proinflammatory gene activation producing cytokines like TNFα, IL-1, IL-6, IL-18, interferon-γ (1, 5), and others. The underlying factors that stimulate expression of these genes include reactive oxygen species (ROS), oxidized lipids, reduced nitric oxide (NO), increased angiotensin II, free fatty acids (FFA) and advanced glycation end products (AGEs) (6, 7). Both endothelial cells and macrophages contribute to the generation of altered vasoreactivity and a procoagulant state through increased expression of plasminogen activator inhibitor (PAI)-1 and tissue factor and through platelet activation and acute phase reactions that increase levels of coagulation factors such as fibrinogen and factor VIII (1). Many of these molecules enter the circulation at levels that correlate with the degree of inflammatory activity. IL-6 appears to have a distinct messenger cytokine role, being the most important stimulator of C-reactive protein (CRP) production (8), a clinically relevant marker of inflammation (9).

Adipose tissue and adipokines

Adipose tissue contributes importantly to the inflammatory process in overweight subjects in both vascular and nonvascular tissues (10). Proinflammatory and procoagulant mediators released by stressed adipose cells have both local and systemic effects on metabolism and vascular function. Adipose tissue from obese individuals contains activated macrophages that together with adipocytes produce inflammatory adipokines such as MCP-1, MIF, TNFα, IL-6, procoagulant substances such as PAI-1, vasculoactive substances such as angiotensinogen and endothelin, and molecules that may contribute to insulin resistance such FFA, TNFα, and resistin (10, 11). Uniquely, adipocytes produce a collagen-like molecule, adiponectin, which enhances glucose uptake, NO production, and FFA oxidation (12); manifests antiinflammatory activity through inhibition of TNFα, cell adhesion molecules, and scavenger receptor expression (13); and has antiapoptotic activity in cultured β-cells (14). With increases in adiposity, at least partly due to suppression by TNFα, adiponectin levels fall in obese and diabetic individuals as well as subjects with coronary heart disease (CHD), suggesting that decreased levels of this adipokine may contribute to the metabolic and vascular abnormalities in T2D (15).

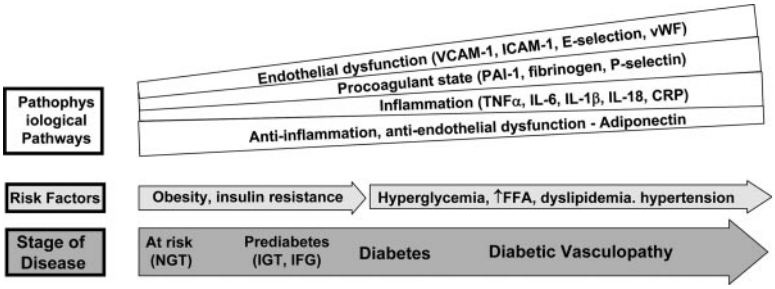

FIG. 1. Inflammation, endothelial dysfunction, and procoagulant state: conceptual diagram linking these pathophsyiological pathways to risk factors in the progression to diabetes and its complications. NGT, Normal glucose tolerance; IFG, impaired fasting glucose.

Biomarkers of diabetes development

T1D

The primary pathogenic process in T1D is mediated by cellular immune reactions, and islet autoantibodies are robust predictors of the risk of progression toward T1D. Activation of

autoimmunity enhances production of proinflammatory molecules, which may predict progression to diabetes. Thus, MIF, a proinflammatory chemokine, produced by beta cells may play a role in autoimmune insulitis, and although serum levels are lower in subjects with recent onset T1D, decreased levels are predictive of autoantibodies (16). Increased interferon-γ-inducible protein 10, a chemoattractant, and elevated IL-18, which up-regulates synthesis of interferon-γ from T cells, were noted in high-risk but not low-risk normoglycemic relatives of T1D (17, 18). Levels of the chemokines CCL3 and CCL4 (chemokine C-C motif ligand 3 and 4), which have been associated with experimental insulitis, were more likely to be elevated in high-risk antibody positive than antibody-negative relatives (19). In addition a higher frequency of high-sensitivity CRP levels greater than 0.5 mg/liter was noted in children at risk for T1D (20). Although preliminary, these findings suggest that proinflammatory pathways may be mediators of β cell damage, providing markers and targets for early intervention.

T2D

Proinflammatory, endothelial dysfunction, and procoagulant biomarkers in large prospective, observational studies of diabetes development. The concept that heightened inflammation is important in the pathogenesis of T2D (21) is supported by evidence that inflammation in islets, adipose tissue, liver, and muscle may provoke insulin resistance and β-cell dysfunction (5, 22, 23) and may therefore antedate the diagnosis of diabetes. The large observational studies that have tested whether biomarkers of these pathways have predictive value for diabetes development focused on a relatively few, well-characterized markers. Initially nonspecific indicators of inflammation such as white cell count and fibrinogen were found to be predictive of incident diabetes (24, 25). Subsequently PAI-1, CRP, and fibrinogen were shown to be independent predictors (Fig. 2), with PAI-1 being least confounded by direct measures of insulin sensitivity or adiposity (26). These observations are supported by several other prospective studies, in which tissue plasminogen activator (tPA) another marker of reduced fibrinolysis (27, 28) and von Willebrand factor (vWf), a marker of endothelial injury, were predictive (29). In addition, the change in PAI-1 levels over time was also found to be an independent predictor of incident diabetes (30). The basis for the strong relationship between PAI-1 and incident diabetes is intriguing particularly because it appears not to operate through insulin resistance or obesity; other relevant influences shown to increase PAI-1 levels include angiotensin II activity, adrenal steroids, PAI-1 gene polymorphisms, and the small baby syndrome (30).

There have been many studies demonstrating an association between CRP and/or IL-6 and incident diabetes,

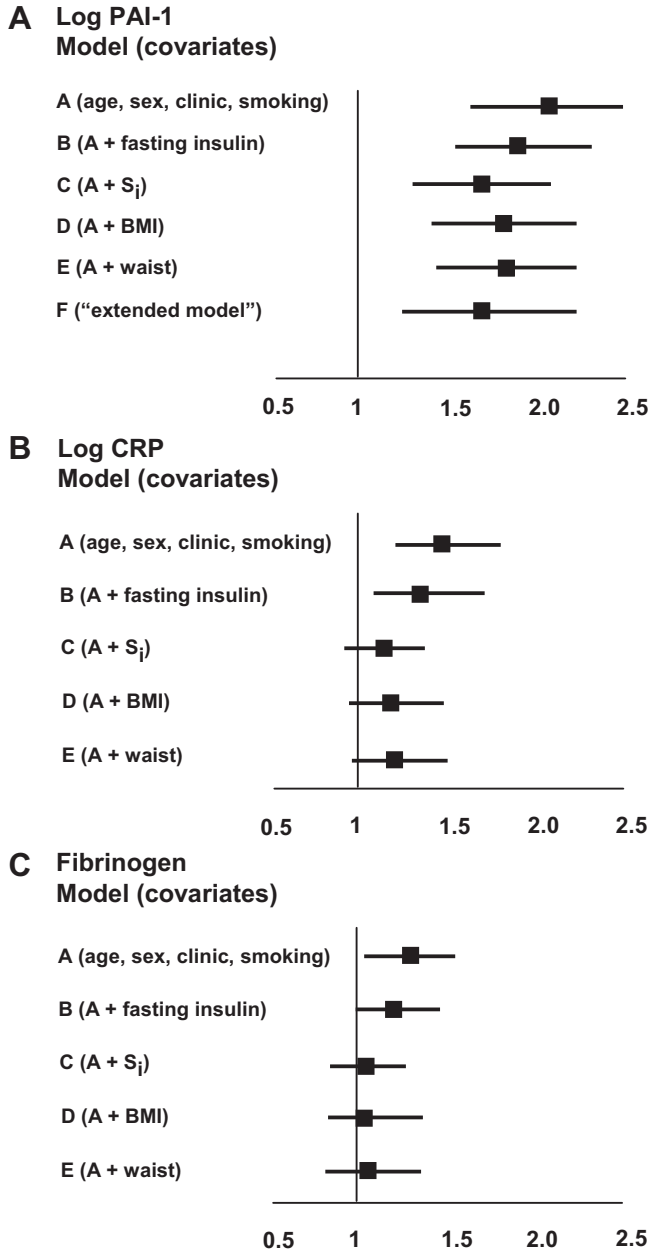

FIG. 2. Odds ratios (ORs) and 95% confidence intervals (CIs) for a 1 SD increase in levels of log PAI-1 (A), log CRP (B), and fibrinogen (C) in the prediction of development of type 2 diabetes. All three proteins were related to incident diabetes after adjusting for age, sex, smoking, and clinical center. When S_I (insulin sensitivity index) or body mass index (BMI) was included in the model, the relation was significantly attenuated for CRP and fibrinogen, whereas PAI-1 remained significantly related to incident diabetes (model F was an extended model that included as covariates age, sex, clinical center, smoking, ethnicity, S_I, BMI, family history of diabetes, and physical activity). $P = 0.0001$ for all, except model F ($P = 0.002$). [Reproduced with permission from A. Festa *et al.*: Diabetes 51:1131–1137. 2002 (26). ©The Endocrine Society.]

and several reported this to be independent of adiposity or insulin resistance (26, 27, 31). In addition, the early markers of inflammation, MCP-1, IL-8, and interferon-γ-inducible protein-10, were also found to predict diabetes, with MCP-1 independent of traditional risk factors (32).

Levels of MIF, which has been shown to impair insulin signalling, are increased in impaired glucose tolerance (IGT) and still further elevated in T2D (16). A metaanalysis also showed CRP to have predictive value for diabetes but not after adjustment for diabetes risk factors, waist to hip ratio, adiponectin, and γ-glutamyltransferase levels (31).

Although oxidative stress is thought to play an important role in activating inflammatory genes, clinical studies of markers of oxidative stress have not been consistently associated with development of diabetes (33–38). It is possible that oxidative stress markers do not adequately reflect the impact of increased ROS on β-cells or insulin signaling or that inflammatory, procoagulant, or endothelial dysfunction markers are more specific because they may be more proximate to the pathophysiology of hyperglycemia.

PAI-1 is less predictive of incident diabetes in subjects with IGT than in those with normal glucose tolerance (26), raising the possibility that PAI-1 loses its specificity as subjects progress to glucose intolerance. It is therefore possible that biomarkers may vary in predictive ability during different phases in the development of T2D. Thus, whereas subjects with T2D had elevated markers of both inflammation and endothelial dysfunction, those with IGT had only elevated inflammatory markers, suggesting that more metabolic disturbance is required before sufficient endothelial dysfunction manifests (39). However, markers of endothelial function, such as soluble (s) ICAM-1 and especially E-selectin (reflecting its specificity as a marker of endothelial dysfunction) were found to be independent predictors of diabetes in the Nurses Health Study (40). Biomarker studies in which timing of development of diabetes or its complications is known may shed further light on this question.

Adipokines and prediction of diabetes development

Several studies demonstrated that a falling adiponectin level is a strong predictor of incident diabetes independent of other risk factors (39–44) including, in one study, proinflammatory and endothelial dysfunction markers (39). Furthermore, increasing adiponectin levels induced by weight reduction was associated with a further reduction in risk (44). An exception to these findings comes from results in older adults in whom adiponectin was not an independent predictor for diabetes development when adjusted for PAI-1 (45), perhaps because adiponectin has been shown to suppress PAI-1 production in adipose tissue, indicating that they are functionally linked (46). Neither resistin nor retinol binding protein 4, adipokines associated with insulin resistance, has been shown to have value in predicting diabetes (47–49). Circulating adipocyte fatty acid binding protein, an adipokine that mediates intracellular fatty acid trafficking, was recently shown to be predictive of T2D in a Chinese population (50). Finally, although not an adipokine, levels of plasma fetuin A, an inhibitor of insulin signaling possibly linked to fatty liver, was positively associated with diabetes risk after adjustment for standard risk factors and CRP (51).

Implications for prevention of T2D

Overall, these findings strengthen the concept that heightened inflammation and impaired anti-inflammatory activity, endothelial dysfunction, and increased PAI-1 are part of the soil of T2D. Second, they present potential new targets for intervention. Thus, the use of the antiinflammatory agent salsalate has been shown to improve insulin resistance, increase adiponectin, reduce CRP, and lower glucose levels in at-risk obese nondiabetic and T2D subjects (52, 53). Another example relates to the efficacy of the adiponectin activators, the peroxisomal proliferator-activated receptor (PPAR)-γ agonists, which slow development of diabetes despite weight gain. Third, these new diabetes risk determinants may have clinical utility. In this respect PAI-1 appears to be one of the more powerful biomarker predictors for diabetes development; however, the sample requires special handling and the assay is not standardized. Adiponectin is more stable, but the assay has also not yet been standardized. On the other hand, CRP measurement procedures are well standardized. Because CRP appears to have predictive value for diabetes development, it constitutes a logical choice for further testing in high-risk situations. In addition, it may also be a novel index for of the efficacy of interventions for the prevention of diabetes.

Biomarkers associated with hyperglycemia, diabetes comorbidities, and antihyperglycemic agents

Interpretation of biomarker levels for prediction of diabetes and its complications is complicated by the presence of hyperglycemia, obesity, and other comorbidities as well as by antihyperglycemic agents that alter biomarker levels, presumably by influencing the activity of the underlying processes they reflect.

Hyperglycemia

Hyperglycemia has been shown to enhance oxidative stress; increase nuclear factor-κB, TNFα, IL-6, and adhesion molecules; impair NO-mediated vasodilatation; and have a procoagulant effect (54). In addition, CRP levels are higher in subjects with poor glycemic control (55, 56). Higher adiponectin levels are also associated with more favorable profiles of glycemia, lipids, and inflammatory markers in T2D (57). In both T1D and T2D subjects with a severely decompensated metabolic state, CRP, PAI-1, and ROS were significantly elevated and were lowered

after resolution of the hyperglycemia (58, 59). Whereas higher glycosylated hemoglobin (HbA1c) levels are associated with lower adiponectin values in T2D (60), adiponectin levels in T1D appear to be increased, and in one study, in proportion to HbA1c levels (61).

Obesity, insulin resistance, hypertension, dyslipidemia, and effects of lifestyle change

Normoglycemic obese individuals have elevated CRP, IL-6, and MCP-1 (3, 62); reduced adiponectin levels; increased PAI-1, coagulation factors (63), and markers of endothelial dysfunction; and an increase in ROS (64). The severity of many of these abnormalities is proportional to the degree of adiposity, but it is likely that the accompanying insulin resistance is a major contributor to these disturbances (65–67). In a study of T2D subjects, direct measures of insulin resistance correlated with levels of vWf, vasodilator responses, and values of PAI-1, tPA, IL-6, CRP, and TNFα, independent of obesity and hyperglycemia (68). These markers may also be elevated in normal-weight subjects with cardiometabolic abnormalities (69), and the presence of dyslipidemia and hypertension increases the frequency and severity of these biomarker abnormalities (70, 71). Importantly, weight reduction decreases levels of CRP, IL-6, and TNFα, (72) and lowers sICAM-1, sVCAM-1, and P-selectin (73). It is unclear whether the exercise-associated falls in CRP and IL-6 levels are independent of accompanying reductions in adiposity (74). A Mediterranean diet has been shown to improve markers of inflammation and endothelial dysfunction (75), whereas a eucaloric low-fat, high-carbohydrate diet has unfavorable effects on inflammatory markers with reduction in adiponectin levels (76, 77), an effect that is prevented if weight loss is achieved (78). Similarly, a low-fat diet accompanied by weight loss in subjects with IGT was associated with a significant reduction of CRP and fibrinogen levels (79) and PAI-1 (80).

Antihyperglycemic agents

Metformin lowered CRP levels in subjects with IGT, T2D, or polycystic ovarian syndrome, but this could be explained by weight and HbA1c reduction (79, 81). It had no effect on PAI-1 levels in one study (81), whereas in another TNFα, PAI-1, tPA, vWF, and adhesion markers were reduced compared with repaglinide treatment, which was not associated with any improvements (82). In subjects with IGT, metformin reduced markers of endothelial dysfunction independently of changes in weight or insulin sensitivity but not PAI-1, TNFα, or CRP (83). Thus, metform is at most only mildly anti-inflammatory and profibrinolyic but may have more significant effects on endothelial function, equivalent to that seen with the thiazolidinediones (84). However, the thiazolidinediones have also been shown to have powerful antiinflammatory as well as other vasculoprotective effects, reducing CRP, PAI-1, TNFα, and IL-6 levels and increasing adiponectin concentrations despite weight gain (85). These effects have been shown to occur within a few days of instituting treatment, before glucose levels are reduced (86). Inflammatory markers as well as PAI-1 are lowered by insulin treatment in decompensated diabetes (58), and a constant insulin infusion achieving euglycemia significantly lowers elevated inflammatory, ROS, and procoagulant markers in acutely stressed subjects with hyperglycemia (65). However, there is little evidence that improving glycemic control with basal or intensive insulin therapy in nonstressed subjects reduces markers of inflammation with T1D or T2D, although there have been reports of modestly beneficial effects on PAI-1 and endothelial function markers (59, 87, 88).

Whereas these findings raise optimism that clinical benefits may accrue from the apparently advantageous changes in markers of inflammation, endothelial function, and coagulation susceptibility induced by lifestyle change and antihyperglycemic treatments, there have been few long-term studies of either the durability of these effects or their importance for the development of diabetes or its complications, and these are sorely needed.

Biomarkers and diabetic microangiopathy

The classical pathophysiological determinants of diabetic microangiopathy, namely hyperglycemia, and hypertension are accompanied by heightened inflammatory activity, endothelial dysfunction, and disturbed coagulation (89–91), and these processes may constitute final common pathways to the vascular complications of diabetes. They may also underlie the association between obesity and insulin resistance with diabetic microangiopathy (92–94). Recent clinical studies are beginning to support the concept that elevated inflammatory factors and markers of endothelial dysfunction are associated with or predictive of microangiopathy.

Nephropathy

There is considerable experimental evidence that proinflammatory and adhesion molecules are important in the development of diabetic nephropathy (95). Cross-sectional studies in both T1D and T2D have shown that biomarker levels including CRP and adhesion molecules are increased in subjects with nephropathy (96, 97) (Fig. 3). In the Diabetes Control and Complications Trial, baseline E-selectin and fibrinogen levels but not CRP independently predicted development of nephropathy in T1D (98). In another prospective study, a composite endothelial dysfunction biomarker score contributed to mainly by sVCAM-1 and PAI-1 was independently predictive of pro-

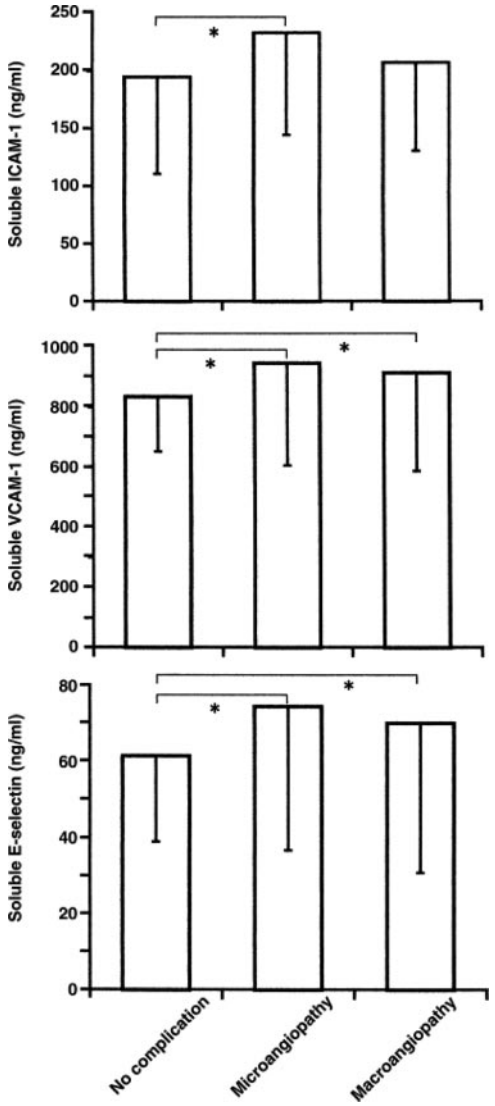

FIG. 3. Serum levels (mean ± SD) of sICAM-1, VCAM-1, and E-selectin in T2D subjects with no complications, a microangiopathy-dominant group, and a macroangiopathy-dominant group. *, $P < 0.05$. [Reproduced with permission from K. Matsumoto *et al.*: Diabet Med 19:822–826. 2002 (97). ©The Endocrine Society.]

gression of renal disease in T1D (99) over a 10-yr period of observation. Interestingly TGFβ, which is considered to be a key mediator of renal fibrosis, had no predictive effect in this study. A *post hoc* analysis of the Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria trial also demonstrated that a composite endothelial dysfunction but not an inflammation score was an independent predictor of progression of proteinuria to nephropathy (100). An additional finding is that IL-18, a proinflammatory cytokine involved in both innate and acquired immune responses, independently predicted conversion from normoalbuminuria to microalbuminuria in T2D (101). Other biomarkers such as AGEs, MCP-1, and PAI-1 have also been associated with diabetic nephropathy in small cross-sectional studies (102–104).

Several reports have shown adiponectin levels to be reduced in T2D diabetic subjects with nephropathy independent of confounders such as body weight, insulin resistance, or glycemic control (105, 106), and in one report levels were inversely associated with the degree of proteinuria (105). In contrast, a recent study found that total and high-molecular-weight adiponectin were increased in relation to nephropathy (107). Adiponectin levels are usually found to be increased in T1D subjects with nephropathy (108). No clear explanation for these rather significant disparities in findings is apparent other than the possibility that more advanced renal disease may impair adiponectin clearance, but this is unlikely to be the entire explanation. Recent studies indicated that the levels of leptin, an activator of the sympathetic nervous system that increases extracellular matrix, correlate positively with albuminuria and negatively with glomerular filtration rate (109).

Retinopathy

As for nephropathy, pathways of inflammation, endothelial dysfunction, and coagulation are thought to be important in the pathogenesis of diabetic retinopathy (110). In one clinical study of T2D, adhesion molecules were higher in subjects with retinopathy than those without (97), and in another population-based cohort, composite scores of both inflammatory and endothelial function markers were strongly associated with the presence of diabetic retinopathy (111). Similarly E-selectin values were found to be increased in a group with T1D and retinopathy (112) but not in a group with T2D and retinopathy (113). Adiponectin levels were found to be decreased in proportion to the severity of diabetic retinopathy in T2D subjects (107), but total and high-molecular-weight adiponectin were increased in the advanced stages of retinopathy in another (114).

Neuropathy

The role of subclinical inflammation in the development of diabetic neuropathy is less clear. A small study in T1D found increased levels of TNFα in subjects with diabetic neuropathy (115) and a recent report found that CRP was independently associated with development of diabetic neuropathy (116). It was also shown that the level of circulating heat shock protein 27, which has a role in cytoprotection and cell motility and is overexpressed in dorsal root ganglia in experimental diabetes perhaps as a compensatory mechanism, was associated with distal sensorimotor neuropathy (117).

Overall, the findings constitute a clinical database supporting the concept that diabetic microangiopathy is accompanied by elevated levels of inflammatory and endo-

thelial dysfunction biomarkers in a manner that was frequently independent of hyperglycemia and hypertension. Although most of the data demonstrate associative relationships only, at least two prospective studies suggest that markers of endothelial dysfunction in particular are predictive of diabetic nephropathy. These data are also important in that they suggest that pathways of inflammation and endothelial dysfunction are common features to both development of diabetes as well as its vascular complications. A unifying hypothesis would posit that inflammation occurs early in the development of diabetes along with increased adiposity and insulin resistance, contributing to β-cell damage. Unabated proinflammatory activity together with metabolic deterioration and endothelial damage could then combine to produce a diffuse vasculopathy. A distinctive, clinically useful biomarker of microangiopathic risk does not clearly emerge from these studies, although markers of endothelial dysfunction such as sVCAM-1, E-selectin, or vWF or a composite inflammation/endothelium dysfunction score, *e.g.* CRP and sVCAM-1, would appear to have predictive value and could represent treatment targets for diabetic nephropathy and retinopathy. In this regard it is of considerable interest that PPARγ and PPARα agonists, which have antiinflammatory and vasculoprotective properties, also have beneficial effects on progression of nephropathy and retinopathy (85, 118).

Biomarkers and diabetic macrovascular disease

Markers of inflammation and endothelial dysfunction are predictive of diabetic macrovascular outcomes and mortality

T2D. It is accepted that standard cardiovascular disease (CVD) risk factors do not adequately explain the excess CVD in diabetes, and heightened inflammation, endothelial dysfunction, and coagulation imbalance have been shown to be important in atherogenesis. The Hoorn population-based study was the first to show that vWF, sVCAM-1, and CRP were predictors of mortality over a 5- to 7-yr period in T2D subjects (119, 120). Similar findings were obtained in another 9-yr observational study of T2D in which vWF, E-selectin, CRP, tPA, and fibrinogen were shown to be independent predictors of progression of microalbuminuria and mortality (121). After this, several studies showed strong relationships between CRP and CHD events (122, 123) in subjects with T2D. In the largest of these, a CRP value greater than 3 mg/liter was an independent predictor of CHD death over an 8-yr period of follow-up (123). In an extended 11.7-yr follow-up of the Hoorn population, CRP and sICAM-1 as indices of inflammation and vWF and sVCAM-1 as indices of endothelial dysfunction were independently associated with increased CVD mortality, together explaining about 43% of the excess CVD risk (93) (Fig. 4). Endothelial dysfunction appears to be a greater hazard for CVD in T2D compared with nondiabetic subjects, attesting to the greater specificity or severity of endothelial dysfunction in diabetes as a marker of CVD (39, 121). The relationship of adiponectin to CVD is complex, with both positive and negative associations being reported (124). Adiponectin values were shown to be inversely related with CHD events in the one study reported in T2D; this relationship appeared to be mediated by high-density lipoprotein levels, with which adiponectin is strongly associated (125). Elevated markers of inflammation and endothelial dysfunction as well as decreased adiponectin may result from increased oxidative stress, and one fairly large cross-sectional study of T2D subjects demonstrated an inverse association between a measure of plasma antioxidant status and CHD, independent of traditional risk factors (126).

T1D. CRP, IL-6, and TNFα were independently associated with CVD events as well as microangiopathy in T1D (127, 128); sVCAM-1 and E-selectin also correlated with CVD events, but this effect was dependent on the presence of microalbuminuria. There is as yet no information on whether the elevated adiponectin levels found in T1D are related to atherosclerosis risk.

Biomarkers and subclinical atherosclerosis

Given the poor prognosis of established CVD in diabetes, there is interest in identifying new markers of subclinical atherosclerosis such as coronary artery calcium (CAC) and carotid intimal medial thickness (CIMT) to facilitate earlier diagnosis and prevention of CVD. There may be limitations to this approach because there are studies that show, for example, that CIMT is not a useful predictor of CHD in T1D (129). With that caveat, CRP levels were found to correlate with CIMT in a group of

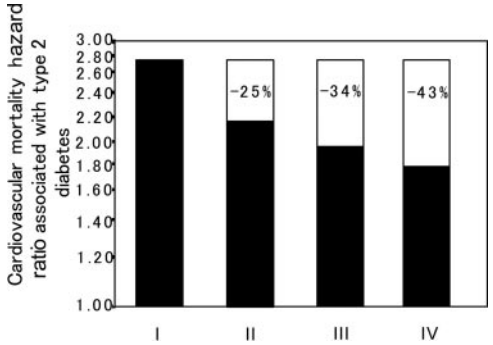

FIG. 4. Hazard ratio of cardiovascular mortality associated with T2D after adjustment for stratification variables (I), low-grade inflammation (II), endothelial dysfunction (III), and both low-grade inflammation and endothelial dysfunction (IV), showing percent reduction in mortality in each case. [Reproduced with permission from J. de Jager *et al.*: Arterioscler Thromb Vasc Biol;26:1086–1093. 2006 (39). ©The Endocrine Society.]

young T1D subjects (130) but not in older T2D individuals (131), and in another study of T2D, levels of IL-6 were associated with the amount of CAC (132). A marker of enhanced innate immunity, CD40 ligand, was also found to correlate with CIMT in subjects with T1D (133). Leptin levels were shown to associate with CAC in T2D, independently of body weight measures or other risk factors, pointing to a possible proatherogenic role for leptin (134). Recently in T1D subjects, cystatin C levels were also found to predict the extent of CAC (135) and asymmetrical dimethylarginine, a natural inhibitor of NO synthesis that is elevated by hyperglycemia and renal failure, was shown to be associated with risk of CVD events and decline of renal function (136). Lastly, in a group of T2D subjects, the circulating AGE pentosidine was shown to correlate with CIMT and aortic pulse wave velocity, a marker of arterial stiffness that is increased in diabetes (137).

Overall, these data demonstrate that markers of inflammation, particularly CRP, and endothelial dysfunction, have independent predictive value for clinical and subclinical CVD beyond traditional risk factors in diabetes. Whereas CRP appears to be the most robust inflammatory marker for potential clinical use, a clear advantage for any one biomarker of endothelial dysfunction does not emerge. vWF requires special handling of samples and therefore would not be suitable for clinical use. A composite score for sICAM-1, sVCAM-1, and E-selectin might be the most effective way to assess the risk predictive value of markers of endothelial dysfunction but would certainly add to expense, and these assays have not been standardized. Thus, CRP is the single biomarker with both predictive ability for CVD in diabetes and assay versatility in this setting; based on these features, CRP has been proposed for risk assessment of CHD in the at-risk general population. The recent demonstration that an elevated CRP level helps to identify apparently healthy individuals who will benefit from statin therapy, even though their low-density lipoprotein cholesterol levels were acceptable, raises the possibility that CRP could function as a marker for more aggressive atheropreventive strategies in younger, uncomplicated subjects with diabetes, in whom the benefit of aggressive statin therapy is not established.

Conclusion

Based on these findings, particularly the data from prospective studies, it seems unavoidable to conclude that biomarkers of inflammation, endothelial dysfunction, and coagulation can help to identify subjects at risk for developing diabetes, diabetic microangiopathy, and CVD. Many of the interventions used to treat diabetes can be shown to ameliorate these disturbances, although it remain to be proven that this will slow or delay disease. The challenge ahead is to more precisely characterize the clinical diagnostic and therapeutic value of the most promising of these biomarkers, namely CRP. The expanding impact of diabetes on health calls for novel approaches such as this.

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Address all correspondence and requests for reprints to: Ronald B. Goldberg, M.D., Professor of Medicine, 1450 NW 10th Avenue, Miami, Florida 33101. E-mail: rgoldberg@med.miami.edu.

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