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Diabetes is a proinflammatory state: a translational perspective

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Abstract

The diabetic state confers an increased propensity to accelerated atherogenesis. Inflammation is pivotal in atherosclerosis; in addition to the established risk factors, inflammation appears to play a pivotal role in diabetes and its complications. Evidence for increased inflammation includes: increased levels of plasma C-reactive protein, the prototypic marker of inflammation; increased levels of plasminogen-activator inhibitor; increased monocyte superoxide and proinflammatory cytokine release (IL-1, IL-6 and TNF-α); increased monocyte adhesion to endothelium; increased NF-κB activity; and increased Toll-like receptor 2 and 4 expression and activity in diabetes. Thus, it appears that both Type 1 and Type 2 diabetes are proinflammatory states and that these could contribute to increased diabetic vasculopathies.

Keywords

atherosclerosis; C-reactive protein; diabetes; inflammation; Toll-like receptor

Diabetes affects more than 194 million people worldwide and more than 16 million in the USA [1,201]. The prevalence of diabetes is increasing, with the lifetime risk for Americans born in 2000 estimated at 38.5% for women and 32.8% for men [2].

Type 2 diabetes mellitus (T2DM) is the sixth leading cause of death in the USA [3]. Type 1 diabetes mellitus (T1DM) also confers an increased propensity to micro- and macro-vascular complications. Diabetic complications include microvascular complications, such as retinopathy, neuropathy and nephropathy, and macrovascular complications, including cardiovascular, peripheral vascular disease and cerebrovascular diseases. Cardiovascular disease risk is two- to four-times greater in individuals with T2DM relative to individuals

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without diabetes [4–9]. It is referred to as a cardiovascular risk equivalent since the risk of dying from coronary heart disease for T2DM patients is comparable to that for individuals without diabetes who have had a myocardial infarction (MI) [4–9]. Approximately two-thirds of individuals with diabetes die from heart disease or stroke [4–10]. However, established risk factors, such as dyslipidemia, hypertension and smoking, cannot explain this increased prevalence of macrovascular disease in diabetes [4]. Thus, the diabetic state itself is an independent risk factor for premature atherosclerosis [8].

Potential mechanisms that could mediate the premature atherosclerosis in diabetes are shown in **Box 1**. Thus, there are various mediating mechanisms, such as dyslipidemia, an increased procoagulant state, the metabolic syndrome, microalbuminuria, glycation of proteins leading to advanced glycation end products (AGEs), oxidative stress and inflammation that could culminate in the increased propensity to vascular complications in diabetes. In addition, it has been suggested recently that traditional and insulin resistance-related metabolic risk markers should be considered to better evaluate cardiovascular and T2DM risk [11]. In this article, we will focus on evidence for increased inflammation in diabetes and some of the mediating mechanisms.

Inflammation & atherosclerosis

Much evidence supports a pivotal role for inflammation in all phases of atherosclerosis, from the initiation of the fatty streak to the culmination in acute coronary syndromes [12–14]. The earliest event in atherogenesis appears to be endothelial cell dysfunction. Various noxious insults including obesity, hypertension, diabetes, smoking and dyslipidemia, can result in endothelial cell dysfunction, which manifests primarily as deficiency of nitric oxide and prostacyclin and an increase in endothelin-1, angiotensin II, plasminogen activator inhibitor (PAI)-1, cellular adhesion molecules and cytokines/chemokines. Following endothelial cell dysfunction, mononuclear cells, such as monocytes and T lymphocytes, tether and roll along the endothelium, initially loosely and thereafter adhere firmly to the endothelium and then transmigrate into the subendothelial space. The rolling and tethering of leukocytes on the endothelium is orchestrated by adhesion molecules such as selectins (E-selectin, P-selectin), cell-adhesion molecules (ICAM-1 and VCAM-1) and integrins.

Chemotaxis and entry of monocytes into the subendothelial space is promoted by chemokines such as monocyte chemoattractant protein (MCP)-1, IL-8 and fractalkine. Thereafter, macrophage colony-stimulating factor promotes the differentiation of monocytes into macrophages. Macrophages incorporate lipids from oxidized low-density lipoprotein via the scavenger receptor pathway (CD36, scavenger receptor-A) becoming foam cells, the hallmark of the early fatty streak lesion. Following the fatty streak lesion, smooth muscle cells migrate into the intima, proliferate and form the fibrous cap. It is currently believed that lipid-laden macrophages and smooth muscle cells, during the process of necrosis and apoptosis, release matrix metalloproteinases and cathepsins, which cause a rent in the endothelium. Since the macrophage is enriched in tissue factor, this is released from the macrophage and comes in to contact with the clotting cascade, resulting in thrombus formation and acute coronary syndromes (unstable angina and MI). Macrophages also interact with T cells and other cells via activation of the CD40–CD40 ligand (CD40L) pathway, which contributes to plaque vulnerability and destabilization. Various knockouts and transgenic experiments have underscored the importance of the various cytokines, chemokines and adhesion molecules in atherogenesis, emphasizing the pivotal role of inflammation in atherosclerosis [12–14].

Inflammation & diabetes

C-reactive protein

Inflammation plays a crucial role in atherosclerosis and is involved in many of the metabolic abnormalities associated with diabetes, the most important of them being insulin resistance. The prototypic marker of inflammation is C-reactive protein (CRP). Numerous studies, especially in normal individuals, have shown that CRP levels in the highest quantile predicts cardiovascular events [14,15]. There is also evidence supporting the suggestion that CRP levels are increased in diabetes. The earliest data underscoring the relationship between inflammation and diabetes were the demonstration by Pickup's group that both serum IL-6 and high-sensitivity (hs)CRP are elevated in diabetics [16]. Pickup *et al.* showed that T2DM subjects with more then two features of the metabolic syndrome in fact had more inflammation (increased serum CRP and serum IL-6 levels) compared with those with less than two features of the metabolic syndrome and matched controls [16]. This was confirmed by the present investigators in T2DM with and without macrovascular complications [17]. Ford *et al.* showed in the Third National Health and Nutrition Examination Survey (NHANES-III) population that individuals with diabetes or with impaired fasting glucose had increased levels of CRP compared with those with a normal fasting glucose level [18]. Also, compared with this group, participants with impaired fasting glucose, newly diagnosed diabetes and previously diagnosed diabetes had 0.99 (0.72–1.37), 1.84 (1.25–2.71), and 1.59 (1.25–2.01) odds of having an elevated CRP concentration after adjustment for age, sex, race or ethnicity, education and body mass index (BMI). Tan *et al.* also showed that T2DM patients had higher CRP ($p < 0.01$) than matched nondiabetic controls, and both endothelium-dependent and -independent vasodilation were impaired ($p < 0.01$) in these subjects relative to CRP concentrations [19]. In the Hoorn study, Yudkin's group demonstrated that in diabetics, levels of CRP and von Willebrand factor (vWF) were a significant predictor of cardiovascular mortality, as well as all-cause mortality, and that this risk was independent of the known conventional risk factors [20].

Several studies in different populations worldwide have consistently reported increased levels of CRP in diabetes and in metabolic syndrome. Furthermore, it has been proposed that hsCRP should be added as a clinical criterion for metabolic syndrome and that a hsCRP-modified coronary heart disease (CHD) risk score should be created, as reviewed by us previously [21]. Elevated CRP concentrations have not only been reported in diabetes, but also appear to predict T2DM. One study by the Atherosclerosis Risk in Communities (ARIC) Investigators showed that increased inflammatory markers, including white blood cell count, plasma fibrinogen and sialic acid levels, were associated with the risk of developing T2DM [22]. The Cardiovascular Health Study reported serum CRP concentrations were associated with the development of diabetes in the elderly [23]. In the Women's Health Study, elevated inflammatory markers, namely serum CRP and IL-6, were associated with the development of T2DM in healthy middle-aged women [24]. A supportive observation was made in the West of Scotland Coronary Prevention Study where CRP was shown to be an independent predictor of risk for the development of T2DM in middle-aged men [25]. These were similar findings to the MONICA Augsburg Cohort Study that reported low-grade inflammation being associated with increased T2DM risk in middle-aged men [26]. In the USA, Pradhan *et al.* investigated whether elevated plasma IL-6 and CRP levels were associated with the development of T2DM in over 27,000 healthy women [27]. In the 4-year follow-up period, 188 women developed T2DM. For these women, baseline IL-6 and CRP were higher than in controls. The relative risk of future T2DM in women, between the highest and lowest quartiles of these inflammatory markers, was 15.7 for CRP. These data thus support a role for inflammation in diabetogenesis. Furthermore, data collected from the Third National Health and Nutrition Examination Survey suggested a possible role of inflammation in insulin resistance and glucose intolerance. Over 2500 men and women were studied for associations

between plasma CRP, fasting insulin, glucose and glycated hemoglobin (HbA_{1C}). Elevated CRP was associated with higher insulin and HbA_{1C} levels in both sexes and with raised glucose in women [28]. Further confirmation of this `inflammatory' hypothesis has come from the Insulin Resistance Atherosclerosis Study (IRAS), where those individuals that converted to T2DM had higher baseline levels of inflammatory proteins, including plasma fibrinogen, CRP and PAI-1, than those that did not develop diabetes. The authors also concluded that chronic inflammation is a risk factor for the development of T2DM [29].

With regards to T1DM, we and others have convincingly shown increased levels of hsCRP in subjects with T1DM compared with age-, gender- and BMI-matched controls [30–33].

Schalkwijk *et al.* reported elevated levels of plasma CRP in T1DM patients without macrovascular disease compared with controls [31]. Furthermore, Schalkwijk *et al.* showed that CRP was higher in T1DM patients with microalbuminuria [31]. In the EURODIAB study, Schram *et al*. showed that T1DM cases with complications had significantly greater CRP levels than those without; however, they failed to compare subjects without T1DM [32]. In a Japanese population, CRP levels have been shown to be significantly elevated in T1DM compared with control and correlated with mean and maximum intima-media thickness [33].

Cytokines & chemokines

The monocyte–macrophage is crucially important and the most readily accessible cell in the artery wall. The importance of studying monocyte function and atherogenesis in T2DM is further underscored by the study of Moreno *et al.*, which showed that coronary tissue from diabetic subjects exhibits a larger content of lipid-rich atheroma and macrophage infiltration than tissue from nondiabetic subjects [34]. Furthermore, Burke *et al*. also demonstrated that lesions of T2DM subjects had larger mean necrotic cores and greater total and distal plaque load than nondiabetic subjects [35]. Necrotic core size correlated positively with diabetic status, independent of other risk factors. Intimal staining for macrophages, T cells and HLA-DR was also significantly greater in diabetic subjects, respectively. The association of increased macrophage infiltrate was independent of cholesterol levels and patient age. These studies demonstrate that in sudden coronary death, inflammation and necrotic core size play a greater role in the progression of atherosclerosis in diabetic subjects.

Reactive oxygen species (ROS), such as O_2^-, have been shown to be increased in monocytes and neutrophils of T2DM patients [36–42]. Studies from our laboratory show increased O_2^- levels in lipopolysaccharide-activated monocytes in T2DM patients with and without macrovascular complications and in diabetic neutrophils [41,42]. Furthermore, in support of a proinflammatory state in diabetes, we have convincingly shown that monocytic release of IL-1β and IL-6 is increased in T2DM [42]. It is important to note that IL-1β also plays a key role in the pathogenesis of T2DM. Recently, 70 patients were randomized to receive subcutaneous injection of human recombinant IL-1 receptor antagonist once daily or placebo. At 13 weeks, in the IL-1RA group there was significant decrease in HbA_{1C}, increased β-cell secretory function and a reduction in proinsulin-to-insulin ratio, an indicator of β-cell stress [43].

Desfaits *et al.* have observed a significant increase in the levels of lipopolysaccharide-stimulated TNF-α release from monocytes in T2DM [44]. An early event in atherogenesis is the binding of monocytes to endothelial cells and their transmigration into the intima [45]. *In vitro*, hyperglycemia increases binding of monocytes to human endothelial cells [46]. While increased adhesion of monocytes to endothelial cells has been reported in T2DM [47,48], this has been largely in patients with hypertriglyceridemia. We demonstrated convincingly that even patients matched with controls with regards to the lipid levels had increased adhesion of their monocytes to human aortic endothelial cells [42]. Soluble cell adhesion molecules are

shed from activated cells, such as endothelial cells. Increasing evidence supports the role of plasma levels of cell-adhesion molecules (ICAM, VCAM, E-selectin and P-selectin) as molecular markers of atherosclerosis [49–51]. T2DM confers elevated levels of soluble adhesion molecules, such as ICAM, VCAM and E-selectin [42,52–54], as shown by numerous investigators. Furthermore, increased levels of ICAM and VCAM have been reported in the atherosclerotic lesion of T2DM [55].

With regard to proinflammatory cytokines in T1DM, Kulseng *et al.* have reported increased mononuclear cell TNF secretion in T1DM [56]. In the first comprehensive study of T1DM in North America, we compared monocyte function and biomarkers of inflammation in T1DM subjects without macrovascular disease with that in matched control subjects (n = 52 per group) [40]. hsCRP, sICAM, soluble CD40L and nitrotyrosine levels were significantly elevated in T1DM subjects compared with control subjects. Monocyte superoxide anion release was significantly increased in the resting and activated state in T1DM compared with control subjects. Monocyte IL-6 levels were significantly elevated in T1DM subjects compared with control subjects in the resting state and after lipopolysaccharide activation. Monocyte IL-1β levels were increased in the activated monocytes in T1DM compared with control subjects [40]. In a subsequent study, we examined systemic and cellular biomarkers of inflammation in T1DM patients with microvascular complications (T1DM-MV patients) and T1DM patients without microvascular complications (T1DM patients) compared with matched control subjects and determined the microcirculatory abnormalities in the T1DM and T1DM-MV patients using computer-assisted intravital microscopy (CAIM). Severity index, as assessed by CAIM, was significantly increased in the T1DM and T1DM-MV patients compared with the control subjects [39]. There was a significant increase in CRP, nitrotyrosine, VCAM and monocyte superoxide anion release, and IL-1 release in T1DM-MV compared with T1DM patients. T1DM-MV patients had significantly increased CAIM severity index and microalbumin-to-creatinine ratio compared with T1DM patients. Furthermore, pp38MAPK, pp65 and pERK activity were significantly increased in monocytes from the T1DM and T1DM-MV patients compared with those from the control subjects, and pp38MAPK and pp65 activity was significantly increased in the T1DM-MV compared with the T1DM patients.

Nuclear factor-κB

Nuclear factor (NF)-κB plays a pivotal role in the regulation of several genes [57–59]. In nonstimulated cells, NF-κB exists in a latent dimer form in the cytoplasm being bound to IκB, an inhibitor protein. Several subunits of NF-κB (P50, P65, c-rel, P52, rel B) and IκB (α, β, γ, δ, ε and Bcl3) are present. The main form of activated NF-κB is P50/P65. Upon stimulation, IκB is phosphorylated, translocated to the nucleus and affects gene expression by binding to κB elements in their promoters. Compelling evidence for increased inflammation in diabetes can be found in the work from Hoffman *et al.*, where they show that diabetic patients with high HbA_{1C} have increased NF-κB p65 activity [60]. In addition, they demonstrated a significant correlation between NF-κB p65 and HbA_{1C} and significant increase in NF-κB activity in peripheral blood mononuclear cells of both T1DM and T2DM compared with controls. We have shown that monocytes from T1DM subjects with and without microvascular complications have significantly increased NF-κB activity compared with controls and that there is a significant increase in T1DM with complications compared with T1DM without complications [39]. This increase in NF-κB activity correlated with significant increases in monocyte cytokine release, thus providing definitive evidence for increased inflammation in T1DM.

Plasminogen activator inhibitor-1

Circulating levels of PAI-1 are a key regulator of fibrinolysis, and inhibit breakdown of fibrin by inhibiting tissue plasminogen activator (tPA). There is substantial experimental and

epidemiological evidence that PAI-1 contributes to the development of cardiovascular disease. PAI-1 excess has been identified in youthful survivors of acute MI and plasma PAI-1 activity is increased in MI survivors who develop recurrent MI [61]. A nested case–control study identified a strong association between elevated PAI-1 antigen and activity with increased independent risk of MI in middle-aged men and women [62]. Furthermore, transgenic mice that overexpress a stable form of human PAI-1 develop spontaneous intravascular coronary thrombosis and MI [63]. Several groups have reported increased PAI-1 localized to atherosclerotic plaques and this is exaggerated in diabetics [64]. Furthermore, PAI-Tg has been shown to accelerate the development of atherosclerosis in ApoE$^{-/-}$ mice [65], while PAI-deficient mice crossed to an ApoE$^{-/-}$ background appear to be protected against the development of atherosclerosis [66]. Furthermore, high concentrations of free fatty acids, very low-density lipoprotein, triglycerides and CRP (which are all characteristic of diabetes), augment PAI-1 expression. All these data point to the crucial role for PAI-1 in atherothrombosis. Decreased fibrinolysis, primarily due to increased PAI-1 activity, has been demonstrated in patients with coronary artery disease [67]. Elevated PAI-1 is considered a strong risk factor for coronary artery disease and has been shown, in most reports to be elevated in Type 2 diabetes [68]. In the IRAS study, levels of PAI-1 were the strongest independent predictor of future diabetes and cardiovascular risk [69].

Toll-like receptors

Members of the Toll-like receptor (TLR) family play a critical role in the inflammatory components of atherosclerosis. TLRs are a family of pattern-recognition receptors that are important in the regulation of immune function and inflammation [70–75]. Their activation by various ligands triggers a signaling cascade leading to cytokine production and initiation of an adaptive immune response [70–75]. TLRs are upregulated in several inflammatory disorders. However, there is a paucity of data on TLRs in diabetes, a cardiovascular risk equivalent. Among the TLRs, TLR2 and TLR4 play an important role in atherosclerosis. TLR2 and 4 can recognize components of the bacterial cell wall, such as lipopolysaccharide and peptidoglycans and lipopeptides. The activation of these receptors on cells of the innate immune system leads to the production of cytokines and chemokines, and the upregulation of cell surface molecules. TLRs are expressed in multiple tissues; the predominant site of TLR expression is on cells of the innate immune system, especially monocytes.

Toll-like receptor 2 and 4 expression is upregulated in atherosclerotic plaque macrophages and in animal models of atherosclerosis [70–75]. TLR4 binds to the lipopolysaccharide (endotoxin) of the outer membrane of Gram-negative bacteria. TLR2 recognizes and signals bacterial lipoproteins, peptidoglycans and lipoteichoic acid from Gram-positive bacterial cell walls. Knockout of TLR4 is associated with reduction in lesion size, lipid content and macrophage infiltration in hypercholesterolemic apoE$^{-/-}$ mice [76]. In addition, TLR2/LDLR$^{-/-}$ and, in a recent paper, TLR2/ApoE$^{-/-}$ mice are protected from the development of atherosclerosis [77,78]. Furthermore, two groups have demonstrated that deficiency of myeloid differentiation factor 88 (MyD88), one of the downstream TLR intracellular signaling molecules, results in reduction in plaque size, lipid content, expression of proinflammatory genes, and systemic expression of proinflammatory cytokines, such as IL-1 and TNF [70–78]. There is a paucity of data examining the role of TLR2 and TLR4 in hyperglycemia/diabetes. TLR4 mRNA expression is induced in adipose tissue of *db*/*db* mice [79]. Mohammad *et al.* showed increased TLR4 expression in T1DM nonobese diabetic (NOD) mice and this correlated with increased NF-κB activation in response to the TLR4 ligand, lipopolysaccharide, resulting in increased proinflammatory cytokines [80]. In addition, Kim *et al.* demonstrated that TLR2$^{-/-}$ mice had a reduced incidence of diabetes compared with wild-type NOD mice by approximately 50% [81]. Song *et al.* reported increased TLR4 mRNA expression in differentiating adipose tissue of *db*/*db* mice [79]. Wen *et al.* have shown viral mimic-induced development of diabetes in

C57BL/6-rat insulin promoter-B7.1 mice that do not normally develop diabetes [82]. Further mechanistic studies suggested that diabetes was induced via direct recognition of this virus-like stimulus by pancreatic islets through TLR3 expression. Park *et al*. have shown significant differences in *TLR2* polymorphisms between T1DM and controls; however, they failed to examine TLR2 expression [83]. Creely *et al.* demonstrated increased TLR2 but not TLR4 expression in adipocytes of T2DM; however, they failed to examine any correlations with glycated hemoglobin or downstream readouts and their sample size was very small (n = 5) [84]. This may have explained the failure to observe an increase in TLR4 expression, in spite of an increased endotoxin level, the ligand for TLR4. We recently demonstrated for the first time that TLR2 and TLR4 surface expression and mRNA were significantly increased in T1DM monocytes compared with controls [85]. Downstream targets of TLR, NF-κB, MyD88, Toll/interleukin receptor domain-containing adaptor-inducing IFN-β (Trif) and phosphate interleukin receptor-associated kinase (IRAK), were significantly upregulated in T1DM. Finally, release of IL-1β and TNF-α were significantly increased in monocytes from T1DM compared with controls and correlated with TLR2 and TLR4 expression ($p < 0.005$). In addition, TLR2 and TLR4 expression were significantly correlated to HbA_{1C}, carboxymethyllysine and NF-κB ($p < 0.02$). Furthermore, we reported a significant correlation between TLR2 and TLR4 expression and glycemic control as well as AGE levels as denoted by increased carboxymethyllysine (CML), NF-κB, IL-1β and TNF-α release (a readout of TLR activation). Subsequently, we have also demonstrated increased levels of ligands for TLR2 and TLR4 such as heat-shock protein 60, Homeobox group protein-1 (HMGB1) and endotoxin in T1DM patients compared with matched controls, providing another line of evidence for TLR2 and TLR4 activity contributing to increased inflammation in T1DM [86]. Current studies in the laboratory are focused on examining TLR2 and TLR4 expression in T2DM subjects compared with matched controls.

CD40–CD40L

Increasing evidence shows that CD40–CD40L interaction plays a crucial role in the pathogenesis of atherosclerosis [87–89]. Disruption of CD40 signaling in hypercholesterolemic mice diminishes the formation and progression of atherosclerotic plaques. Furthermore, interference with CD40 ligation promotes changes in plaque composition associated in humans with less rupture-prone lesions, such as increased content of smooth muscle cells and fibrillar collagen, as well as decreased lipid and macrophage accumulation. CD40L can occur in soluble form in plasma (sCD40L). Patients with unstable angina have elevated levels of sCD40L compared with those with stable angina or healthy volunteers. Also, there exists a significant correlation between elevated levels of sCD40L and future cardiovascular events in apparently healthy, middle-aged women. Recently, Varo *et al.* demonstrated a significant ($p < 0.001$) association between plasma sCD40L and T1DM, as well as T2DM, independent of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, blood pressure, BMI, gender, CRP and soluble ICAM-1 [90]. Furthermore, in a pilot study, administration of troglitazone (12 weeks, 600 mg/day) to T2DM patients significantly diminished sCD40L plasma levels by 29% [91]. Lim *et al.* also showed similar results; diabetics with microangiopathy had increased levels of plasma sCD40L compared with those without microangiopathy and matched controls [92]. Jinchuan *et al.* demonstrated that CHD patients with diabetes had increased levels of increase of CD40 and CD40L co-expression on platelets as well as sCD40L compared with controls ($p < 0.01$) [93]. A positive correlation was found between serum AGE levels in patients with DM and CD40–CD40L system.

Molecular mechanisms

With regard to molecular mechanisms we first explored the effects of hyperglycemia on superoxide (O_2^-) anion release in monocytes. Incubating monocytes with low glucose (5.5

mmol/l) and high glucose (15 mmol/l), we showed that O_2^- anion release was increased with hyperglycemia and this was associated with increased protein kinase C (PKC) activity. Using specific antisense oligonucleotides, we showed that O_2^- anion release was driven by PKC-α and that ROS and O_2^- derived in the monocyte from NADPH oxidase and not from the mitochondrial respiratory chain. Using antisense oligonucleotides to PKC-α obliterated the increase ROS and O_2^- anion release under hyperglycemic conditions in monocytes, while antisense to PKC β-II had no effect [94].

We then examined the mechanism for increased monocytic IL-1β release under hyperglycemic conditions. Data from inhibitor and siRNA experiments indicate that IL-1β release under hyperglycemia is mediated by PKC-α, via phosphorylation of p38 MAPK and ERK1/2 leading to NF-κB activation, resulting in increased mRNA and protein for IL-1β [95]. At the same time, it appears that NADPH oxidase via p47phox activates NF-κB, resulting in increased IL-1β secretion [95]. We also demonstrated that IL-6 release from monocytes under hyperglycemia appears to be mediated via upregulation of PKC-α and -β, through p38 MAPK and NF-κB, resulting in increased mRNA and protein for IL-6 [96]. Shanmugham *et al*. showed that hyperglycemia also significantly induces MCP-1 expression [97]. Hyperglycemia-induced MCP-1 mRNA expression and monocyte adhesion were blocked by specific inhibitors of oxidant stress, PKC, ERK1/2 and p38 MAPKs. In addition, Srinivasan *et al.* have shown increased IL-8 and increased monocyte adhesion under hyperglycemic conditions [98]. Glucose-stimulated monocyte adhesion is primarily regulated through phosphorylation of p38 with subsequent activation of AP-1, leading to IL-8 production. Inhibition of the p38 pathway in diabetic *db*/*db* mice significantly reduced monocyte adhesion by 50%. Taken together, these data indicate that chronic elevated glucose in diabetes activates the p38 MAPK pathway to increase inflammatory IL-8 gene induction and monocyte/endothelial adhesion.

Recently, we have examined the molecular mechanisms for increased TLR2 and TLR4 expression under hyperglycemia. Knocking down both TLR2 and TLR4 in the cells resulted in a 76% decrease in high-glucose-induced NF-κB activity, suggesting an additive effect. Furthermore, PKC-α knockdown decreased TLR2 by 61%, whereas inhibition of PKC-δ decreased TLR4 under high glucose by 63%. siRNA to p47Phox in THP-1 cells abrogated high-glucose-induced TLR2 and TLR4 expression. Additional studies revealed that PKC-α, PKC-δ and p47Phox knockdown significantly abrogated high-glucose-induced NF-κB activation and inflammatory cytokine secretion. Collectively, these data suggest that high glucose induces TLR2 and TLR4 expression via PKC-α and -δ, respectively, by stimulating NADPH oxidase in human monocytes [99]. Furthermore, in order to understand the mechanisms for increased circulating and monocytic IP-10 in T1DM, we tested the effect of TLR2 and TLR4 siRNA on hyperglycemia-induced MCP-1 and IP-10 production, and demonstrated that downregulation of TLR2 and TLR4 abrogates hyperglycemia-induced IP-10 release and MCP-1 via NF-κB inhibition [100].

Role of the adipose tissue

There is now considerable evidence that abdominal adiposity appears to be an important contributor to the inflammatory state in diabetes [101–103]; however, this will not be discussed in detail in this review. In 194 female twins, Greenfield *et al.* performed an analysis controlling for genetic influences in monozygotic twins, and demonstrated that within-pair differences in CRP were associated with within-pair differences in total and central body fat, triglycerides, high-density lipoprotein and blood pressure [104]. These relationships are likely to contribute significantly to the prospective associations between CRP and T2DM and coronary events.

Furthermore, the visceral adipose tissue has been shown to be positively related to CRP, MCP-1, ICAM-1 and PAI-1 antigen [105]. These data suggest that adipose tissue distribution remains an important determinant of systemic inflammation in T2DM.

Conclusion

It is clear that diabetes is a proinflammatory state **(Box 2)**. This is evidenced by increased levels of hsCRP, TLR2, TLR4 and PAI-1, as well as soluble cell adhesion molecules, sCD40 and proinflammatory cytokines. Future studies are needed to answer the question of whether the diabetes begets inflammation or if a proinflammatory state precipitates the development of diabetic vasculopathies. Potential therapeutic strategies that could be used to target inflammation in diabetes include the statins, PPAR-γ agonists, metformin and insulin.

Expert commentary

The first studies related to inflammation and diabetes were mainly focused on studying patients with T2DM and demonstrated that there are several cellular disturbances that contribute to the proinflammatory state of diabetes. Recent studies have shown that in T1DM there are distinct similarities to T2DM and T1DM is also a proinflammatory state. Recent evidence for that also comes from *in vivo* data demonstrating increased TLR2 and TLR4 expression and activity in diabetes.

Five-year view

Type 1 and Type 2 diabetes mellitus are associated with increased inflammation as manifest by increased levels of hsCRP, monocyte proatherogenic activity, increased cytokines and increased adipose tissue inflammation. All of these could result in increased micro- and macro-vascular complications of diabetes. More importantly, there are several therapeutic strategies that could be employed to target the increased inflammation in diabetes and forestall vascular complications. Some of these therapeutic interventions include metformin, insulin, statins and PPAR-γ agonists, which have been shown to be anti-inflammatory. Over the next 5 years, more extensive and prospective clinical trials will be needed to answer the questions with regards to the potential beneficial effects of these interventions in diabetes, with different end points assessing different diabetic complications.

Box 1 Potential atherogenic mechanisms in diabetes

- Lipid and lipoprotein aberrations
- Procoagulant state
- Metabolic syndrome
- Microalbuminuria
- Glycation of proteins (advanced glycation end products)
- Oxidative stress
- Inflammation

Box 2 Evidence supporting increased inflammation in diabetes

- Increased levels of high-sensitivity C-reactive protein
- Increased levels of Toll-like receptor 2 and 4
- Increased levels of plasma and monocytic cytokines, IL-1, TNF, IL-6
- Increased levels of soluble cell-adhesion molecules
- Increased levels of plasminogen activator inhibitor-1

- Increased CD40/soluble CD40 ligand
- Decreased levels of adiponectin
- Increased activity of protein kinase C, MAPK, NADPH oxidase, nuclear factor-κB

Key issues

- Type 1 and Type 2 diabetes are associated with increased inflammation.
- Evidence for increased inflammation include increased levels of circulating biomarkers of inflammation such as high-sensitivity C-reactive protein and proinflammatory cytokines in diabetes.
- Diabetes is accompanied by increased cellular inflammation, as evidenced by increased monocytic superoxide, cytokines and adhesion to endothelium.
- Diabetes is also a procoagulant state as evidenced by increased levels of plasminogen activator inhibitor-1 and the CD40–CD40 ligand.
- Recent additional evidence for the proinflammatory state of diabetes is increased levels of the Toll-like receptor (TLR)2 and TLR4, and increased downstream signaling and adapter proteins in Type 1 diabetes.
- In addition, there is increased adipose tissue inflammation, increased macrophage and T-cell recruitment in the adipose and decreased levels of adiponectin in Type 2 diabetes.

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