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Forum Review

Involvement of Oxidative Stress in the Pathogenesis of Diabetes

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ABSTRACT

Pancreatic β-cell failure is the common characteristic of type 1 and type 2 diabetes. Type 1 diabetes is induced by pancreatic β-cell destruction, which is mediated by an autoimmune mechanism and consequent inflammatory process. Various inflammatory cytokines and oxidative stress produced by islet-infiltrating immune cells have been proposed to play an important role in mediating the destruction of β cells. The JNK pathway is also activated by such cytokines and oxidative stress and is involved in β-cell destruction. Type 2 diabetes is the most prevalent and serious metabolic disease affecting people all over the world. Pancreatic β-cell dysfunction and insulin resistance are the hallmark of type 2 diabetes. Once hyperglycemia becomes apparent, β-cell function gradually deteriorates, and insulin resistance is aggravated. This process is called "glucose toxicity." Under such conditions, oxidative stress is provoked, and the JNK pathway is activated, which is likely involved in pancreatic β-cell dysfunction and insulin resistance. In addition, oxidative stress and activation of the JNK pathway are involved in the progression of atherosclerosis, which is often observed under diabetic conditions. Taken together, it is likely that oxidative stress and subsequent activation of the JNK pathway are involved in the pathogenesis of type 1 and type 2 diabetes. *Antioxid. Redox Signal.* 9, 355–366.

INTRODUCTION

Failure of pancreatic β cells is the common characteristic of type 1 and type 2 diabetes. Type 1 diabetes mellitus is induced by destruction of pancreatic β cells, which is mediated by an autoimmune mechanism and the consequent inflammatory process. Various inflammatory cytokines and oxidative stress are produced during this process, which has been proposed to play an important role in mediating β-cell destruction. Type 2 diabetes is the most prevalent and serious metabolic disease, and β-cell dysfunction and insulin resistance are the hallmark of type 2 diabetes. Under diabetic conditions, chronic hyperglycemia gradually deteriorates β-cell function and aggravates insulin resistance. This process is called "glucose toxicity." Oxidative stress is provoked during this process and is likely involved in β-cell dysfunction and insulin resistance. Here we show that oxidative stress and subsequent activation of the JNK pathway are involved in the pathogenesis of both type 1 and type 2 diabetes.

OXIDATIVE STRESS AND β-CELL DESTRUCTION IN TYPE 1 DIABETES

Type 1 diabetes is mediated by an autoimmune mechanism or inflammatory process that is characterized by destruction of pancreatic β cells (26, 84, 105). The initial event in an immune response is the uptake of antigen by antigen-presenting cells (APCs) such as macrophages, and the cooperation of $CD4^+$ and $CD8^+$ T cells is crucial for islet infiltration and destruction of β cells (25, 134). $CD4^+$ Th1/Th2 balance is also critical for the development and resolution of immune responses. $CD4^+$ Th1 T-cells produce interleukin 2 (IL-2) and interferon γ (IFN- γ), which leads to activation of $CD8^+$ T

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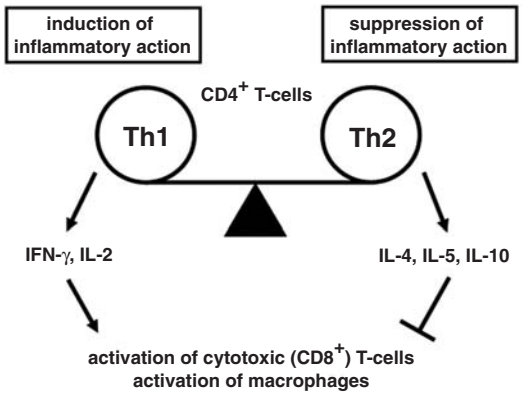

FIG. 1. Effects of Th1/Th2 balance of CD4+ T cells on the activation of CD8+ T cells and macrophages.

cells and macrophages. In contrast, CD4+ Th2 T cells secrete IL-4, IL-5, and IL-10, which leads to suppression of inflammatory action (Fig. 1). Activation of CD8+ T cells responds to class I molecules in β cells themselves, which is a critical step in the process of β-cell destruction (Fig. 2). Activated macrophages secrete various inflammatory cytokines and reactive oxygen species (ROS), which are also involved in the process of β-cell destruction (see Fig. 2).

Various inflammatory cytokines and ROS have been proposed to play an important role in β-cell destruction. Activated macrophages produce inflammatory cytokines, such as IL-1β and tumor necrosis factor α (TNF-α), and ROS, such as superoxide anion, hydrogen peroxide, and nitric oxide (NO) (see Fig. 2). Among various inflammatory cytokines, IL-1β has been proposed to play an important role in mediating β-cell destruction (4, 6, 18, 19, 20, 28, 57, 120, 122). The deleterious effects of IL-1β have been proposed to involve generation of NO and inhibition of mitochondrial function. Indeed, IL-1β–induced production of nitrite and iron–nitrosyl complex in β cells was confirmed using electron paramagnetic resonance (18).

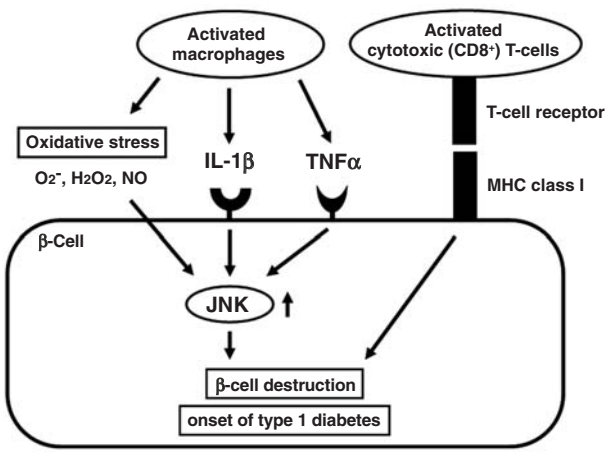

FIG. 2. Involvement of oxidative stress and the JNK pathway in pancreatic β-cell destruction in type 1 diabetes.

NO seems to be an important inducer of necrosis and perhaps even apoptosis, especially in rodent islets. Because pancreatic β cells contain very low levels of antioxidant enzymes, they may be more susceptible to the toxic actions of cytokines (27, 28). Overexpression of antioxidant enzymes protected in several insulin-producing tumor cell lines against the toxic effects of both oxidative stress and inflammatory cytokines (10, 17, 27, 44, 47, 74, 75, 78, 80, 127). For example, overexpression of the mitochondrial form of superoxide dismutase (Mn-SOD) protected β cells against oxidative stress and cytokines (10, 17, 44, 80). Targeted overexpression of the cytosolic SOD (Cu/Zn-SOD) in β cells protected mice from autoimmune and low-dose streptozotocin-induced diabetes (74, 75). Furthermore, it was shown that a SOD mimetic with longer half-life was effective to protect mice against the onset of type 1 diabetes (102). These data suggest that oxidative stress is involved in β-cell destruction and the onset of type 1 diabetes.

THE JNK PATHWAY AND β-CELL DESTRUCTION IN TYPE 1 DIABETES

Studies on insulin-secreting cells and primary β cells have revealed that IL-1β is a potent activator of the JNK pathway (76, 83, 133). More-convincing results have been obtained on the role of JNK in controlling IL-1β–mediated apoptosis. Transfection experiments and the use of cell-permeable peptide inhibitors demonstrated that inhibiting the JNK pathway confers protection against apoptosis induced by IL-1β in insulin-secreting cells (3, 12, 13). Given the fact that TNF-α and IFN-γ strongly potentiate the cytotoxic effects of IL-1β on β cells, it is of note that these two cytokines synergistically augment IL-1β–induced signaling via the JNK pathway in rat islets (5). This observation may provide at least a partial explanation at the signaling level for the synergistic toxic effects of cytokines on β cells.

It has been reported recently that JNK2 plays an important role in type 1 diabetes that is caused by autoimmune destruction of β cells (53). Studies of nonobese diabetic mice demonstrated that disruption of the JNK2 protein kinase decreased destructive insulitis and reduced disease progression to diabetes. CD4+ T cells from JNK2-deficient nonobese diabetic mice produced less IFN-γ but significantly increased amounts of IL-4 and IL-5, indicating polarization toward the Th2 phenotype. This role of JNK2 to control the Th1/Th2 balance of the immune response represents a mechanism of protection against autoimmune diabetes. These results indicate that JNK2 protein kinase plays an important role in the onset of type 1 diabetes.

OXIDATIVE STRESS AND β-CELL DYSFUNCTION IN TYPE 2 DIABETES

It has been suggested that although exposure of β cells to a high glucose concentration for relatively short periods stimulates insulin gene expression, prolonged exposure has adverse effects on various β-cell functions. Chronic hyper-

glycemia is a cause of impairment of insulin biosynthesis and secretion; once hyperglycemia becomes apparent, β-cell function gradually deteriorates. This process is called "β-cell glucose toxicity," which is often observed under diabetic conditions (54, 93, 103, 104, 115, 131). In the diabetic state, hyperglycemia *per se* and subsequent production of oxidative stress decrease insulin gene expression and secretion (9, 21, 31, 35, 39, 49, 54, 56, 58, 59, 63, 82, 85, 108, 109, 123, 124). It was previously shown that the loss of insulin gene expression is accompanied by decreased expression and/or DNA-binding activities of transcription factors pancreatic and duodenal homeobox-1 (PDX-1) (49, 58, 82, 85, 93, 104, 123) and RIPE3b1-binding protein (which was recently identified as MafA) (39, 104, 115, 131). After long-term exposure to a high glucose concentration, expression and/or DNA-binding activities of these two transcription factors are reduced. It is noted here that PDX-1, also known as IDX-1/STF-1/IPF1, plays a crucial role in pancreas development, β-cell differentiation, and induction of surrogate β cells (2, 32, 45, 55, 79, 89, 90, 91, 94, 97, 99). MafA is a recently isolated β-cell–specific transcription factor that functions as a potent activator of insulin gene transcription (62, 66, 86, 87, 100, 139).

Under diabetic conditions, oxidative stress is provoked (9, 21, 49) and involved in the β-cell glucose toxicity found in diabetes (31, 35, 39, 54, 56, 58, 59, 63, 82, 85, 108, 109, 123, 124). β Cells express GLUT2, a high-Km glucose transporter, and thereby display highly efficient glucose uptake when exposed to a high glucose concentration. Indeed, it was shown that expression of oxidative stress markers 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 4-hydroxy-2,3-nonenal (4-HNE) were increased in islets under diabetic conditions (35, 49). In addition, β cells are rather vulnerable to oxidative stress because of the relatively low expression of antioxidant enzymes such as catalase and glutathione peroxidase (15). Thus, it is likely that oxidative stress is involved in β-cell deterioration found in diabetes. Several sources of ROS exist in cells, such as the nonenzymatic glycosylation reaction (85), and the electron-transport chain in mitochondria (112) (Fig. 3).

It was shown that when β-cell–derived cell lines or rat isolated islets were exposed to oxidative stress, insulin gene promoter activity and mRNA expression were suppressed (56, 58, 59, 85, 123). In addition, when β-cell–derived cell lines or rat isolated islets were exposed to oxidative stress, binding of PDX-1 to the insulin gene promoter was markedly reduced. Furthermore, it was shown that the decrease of insulin gene expression after prolonged exposure to a high glucose concentration was prevented by treatment with antioxidants (39, 58, 123, 124). Reduction of expression and/or DNA-binding activities of PDX-1 and MafA by long-term exposure to high glucose was also prevented by an antioxidant treatment. These results suggest that chronic hyperglycemia suppresses insulin biosynthesis and secretion by provoking oxidative stress, accompanied by reduction of expression and/or DNA-binding activities of two important pancreatic transcription factors, PDX-1 and MafA. Therefore, it is likely that the alteration of such transcription factors explains, at least in part, the suppression of insulin biosynthesis and secretion, and thus are involved in β-cell glucose toxicity.

Next, to evaluate the potential usefulness of antioxidants in treatment for type 2 diabetes, obese diabetic C57BL/KsJ-db/db mice were treated with antioxidants (*N*-acetyl-L-cysteine plus vitamin C and E) (58). The antioxidant treatment retained glucose-stimulated insulin secretion and moderately ameliorated glucose tolerance. β-Cell mass was significantly larger in the mice treated with the antioxidants. Insulin content and insulin mRNA levels were also preserved by the antioxidant treatment. Furthermore, PDX-1 expression was more clearly visible in the nuclei of islet cells after the antioxidant treatment (58). Similar effects were observed with Zucker diabetic fatty rats, another model animal for type 2 diabetes (123). Taken together, these data indicate that antioxidant treatment can protect β cells against glucose toxicity. In addition, as a step to clinical trial of antioxidant for type 2 diabetes, we examined the possible antidiabetic effects of probucol, an antioxidant widely used as an anti-hyperlipidemic agent, on preservation of β-cell function in diabetic C57BL/KsJ-db/db mice (35). Immunostaining for oxidative stress markers such as 4-hydroxy-2-nonenal (HNE)-modified proteins and heme oxygenase-1 revealed that probucol treatment decreased ROS in β cells of diabetic mice. Probucol treatment also preserved β-cell mass, insulin content, and glucose-stimulated insulin secretion, leading to improvement of glucose tolerance (35). These data suggest potential usefulness of antioxidants for diabetes and provide further support for the implication of oxidative stress in β-cell glucose toxicity found in diabetes.

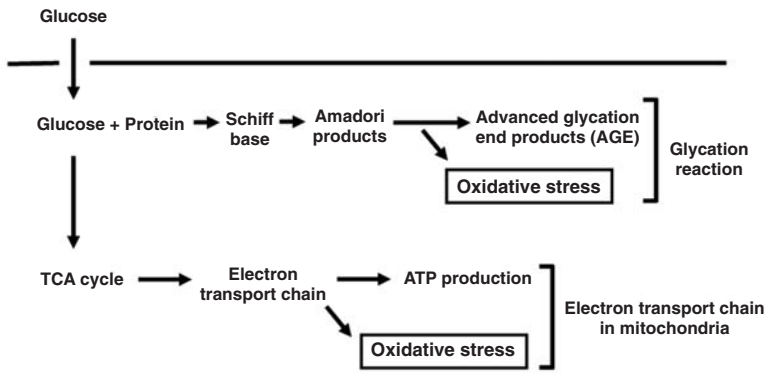

FIG. 3. Increase of oxidative stress under diabetic conditions.

It is noted here that lipotoxicity as well as glucose toxicity is involved in the deterioration of β-cell function found in type 2 diabetes (104, 116, 117). Indeed, it has been shown that deranged lipid metabolism in β cells, most typically represented by accumulation of intracellular triglycerides, causes β-cell damage through induction of iNOS and excess nitric oxide (NO) generation in Zucker diabetic fatty rats (116). Because serum free fatty acid levels and islet triglyceride content were decreased by probucol treatment, decrease of lipotoxicity might have also contributed to the preservation of β-cell function and amelioration of glucose tolerance after probucol treatment (35).

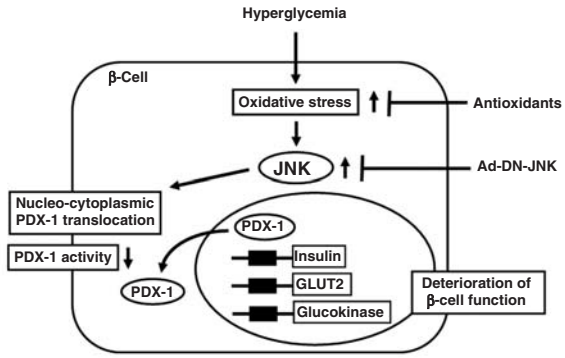

FIG. 4. Involvement of oxidative stress and the JNK pathway in pancreatic β-cell glucose toxicity found in type 2 diabetes.

THE JNK PATHWAY AND β-CELL DYSFUNCTION IN TYPE 2 DIABETES

It has been suggested that activation of the c-Jun N-terminal kinase (JNK) pathway is involved in pancreatic β-cell dysfunction found in diabetes. It was reported that activation of the JNK pathway is involved in reduction of insulin gene expression by oxidative stress and that suppression of the JNK pathway can protect β cells from oxidative stress (60). When isolated rat islets were exposed to oxidative stress, the JNK pathway was activated, preceding the decrease of insulin gene expression. Adenoviral overexpression of dominant-negative type JNK1 (DN-JNK) protected insulin gene expression and secretion from oxidative stress. Moreover, wild-type JNK1 (WT-JNK) overexpression suppressed both insulin gene expression and secretion (60). These results were correlated with change in the binding of the important transcription factor PDX-1 to the insulin promoter. Adenoviral overexpression of DN-JNK preserved PDX-1 DNA-binding activity in the face of oxidative stress, whereas WT-JNK overexpression decreased PDX-1 DNA binding activity (60). Thus, it is likely that JNK-mediated suppression of PDX-1 DNA binding activity accounts for some of the suppression of insulin gene transcription and of β-cell function, which fits with the phenomenon that PDX-1 DNA-binding activity is decreased in association with reduction of insulin gene transcription after prolonged exposure to a high glucose concentration. Taken together, it is likely that activation of the JNK pathway leads to decreased PDX-1 activity and consequent suppression of insulin gene transcription found in the diabetic state (Fig. 4).

As a potential mechanism for JNK-mediated PDX-1 inactivation, it was recently reported that PDX-1 is translocated from the nuclei to the cytoplasm in response to oxidative stress. When oxidative stress was charged on β-cell–derived HIT cells, both intrinsically expressed PDX-1 and exogenously introduced green fluorescent protein (GFP)-tagged PDX-1 moved from the nuclei to the cytoplasm (77). Addition of DN-JNK inhibited the oxidative stress–induced PDX-1 translocation, suggesting an essential role of JNK in mediating the phenomenon. Whereas the nuclear localization signal (NLS) in PDX-1 was not affected by oxidative stress, leptomycin B, a specific inhibitor of the classic, leucine-rich nuclear export signal (NES), inhibited nucleocytoplasmic translocation of PDX-1 induced by oxidative stress. Indeed, we identified an NES at position 82–94 of the mouse PDX-1 protein (77). Taken together, it is likely that oxidative stress induces nucleocytoplasmic translocation of PDX-1 through activation of the JNK pathway, which leads to reduction of its DNA-binding activity and suppression of insulin biosynthesis (see Fig. 4).

Furthermore, we recently showed that the forkhead transcription factor Foxo1 plays a role as a mediator between the JNK pathway and PDX-1 (78). In β-cell–derived cell line HIT-T15, Foxo1 changed its intracellular localization from the cytoplasm to the nucleus under oxidative stress conditions. In contrast to Foxo1, the nuclear expression of PDX-1 was decreased, and its cytoplasmic distribution was increased by oxidative stress. The overexpression of JNK also induced the nuclear localization of Foxo1, but in contrast, suppression of the JNK pathway reduced the oxidative stress–induced nuclear localization of Foxo1, suggesting an involvement of the JNK pathway in Foxo1 translocation (78). In addition, oxidative stress or activation of the JNK pathway decreased Akt phosphorylation in HIT cells, leading to the decreased phosphorylation of Foxo1 after nuclear localization. Furthermore, adenoviral Foxo1 overexpression reduced the nuclear expression of PDX-1, whereas repression of Foxo1 by Foxo1-specific small interfering RNA retained the nuclear expression of PDX-1 under oxidative stress conditions (78). Taken together, oxidative stress and subsequent activation of the JNK pathway induce nuclear translocation of Foxo1 through the modification of the insulin signaling in b cells, which leads to the nucleocytoplasmic translocation of PDX-1 and reduction of its DNA-binding activity (Fig. 5). Furthermore, it has been shown very recently that the protein kinase MST1 is activated by oxidative stress, which leads to facilitation of Foxo1 translocation from cytoplasm to nuclei (77). Therefore, it is also possible that oxidative stress triggers Foxo1 translocation from cytoplasm to nuclei, independent of Akt activity or Akt-mediated phosphorylation status of Foxo1.

In addition, the significance of JNK in the development of diabetes comes from the result of a genetic analysis in humans. Although islet-brain-1 (IB1) was known to regulate the JNK pathway (11, 24), it was reported that a missense mutation within the IB1-encoding MAPKIP1 gene (S59N) is associated with a late-onset type 2 diabetes (130). Thus, it is

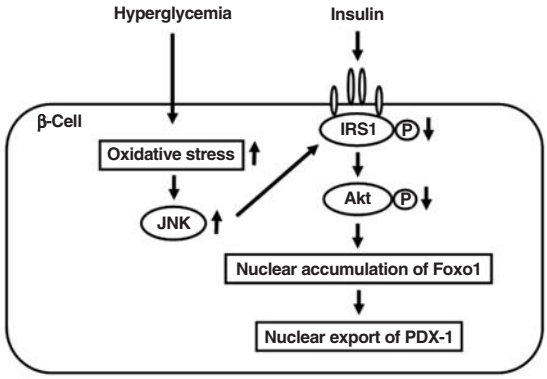

FIG. 5. Involvement of Foxo1 and insulin signaling in pancreatic β-cell dysfunction found in type 2 diabetes.

likely that activation of the JNK pathway is involved in deterioration of β-cell function found in type 2 diabetes.

OXIDATIVE STRESS AND INSULIN RESISTANCE IN TYPE 2 DIABETES

The hallmark of type 2 diabetes is insulin resistance as well as pancreatic β-cell dysfunction. Under diabetic conditions, various insulin target tissues such as liver, muscle, and fat become resistant to insulin. The pathophysiology of insulin resistance involves a complex network of insulin signaling pathways. After insulin binds to insulin receptor on the cell surface, insulin receptor and its substrates are phosphorylated, which leads to activation of various insulin signaling pathways. It has been shown that oxidative stress is involved in progression of insulin resistance as well as pancreatic β-cell dysfunction (30). It was previously reported that oxidative stress disrupted insulin-induced cellular redistribution of insulin receptor substrate-1 (IRS-1) and phosphatidylinositol 3-kinase (PI 3-K) and thus impaired insulin-induced GLUT4 translocation in the 3T3-L1 adipocyte (111, 128). It was also reported that treatment with antioxidants (*N*-acetyl-L-cysteine and taurine) prevented hyperglycemia-induced insulin resistance *in vivo* (38). Furthermore, in patients with type 2 diabetes, both short- and long-term administration of α-lipoic acid, an antioxidant, improved insulin resistance, as measured by both the euglycemic-hyperinsulinemic clamp and the Bergman minimal model (52, 72). These data indicate that oxidative stress is involved in the progression of insulin resistance.

THE JNK PATHWAY AND INSULIN RESISTANCE IN TYPE 2 DIABETES

In diabetic patients, hyperglycemia increased oxidative stress, which leads to activation of the JNK pathway. In addition, under diabetic conditions, various inflammatory cytokines and intracellular lipid accumulation also lead to activation of the JNK pathway. It has been suggested that activation of the JNK pathway is involved in insulin resistance as well as the pancreatic β-cell dysfunction found in diabetes (46, 132). It was reported that the JNK pathway is abnormally activated in the liver, muscle, and adipose tissue in obese type 2 diabetic mice and that insulin resistance in obese type 2 diabetic mice is substantially reduced in mice homozygous for a targeted mutation in the JNK1 gene (JNK-KO mice) (43). When the JNK-KO mice were placed on a high-fat/high-caloric diet, obese wild-type mice developed mild hyperglycemia compared with lean wild-type mice. In contrast, blood glucose levels in obese JNK-KO mice was significantly lower compared with those in obese wild-type mice. Intraperitoneal insulin tolerance tests showed that the hypoglycemic response to insulin in obese wild-type mice was lower compared with that in obese JNK-KO mice. Intraperitoneal glucose tolerance tests revealed a higher degree of hyperglycemia in obese wild-type mice than in obese JNK-KO mice. These results indicate that the JNK-KO mice are protected from the development of dietary obesity-induced insulin resistance.

Furthermore, targeted mutations in JNK were introduced in genetically obese mice (ob/ob) (43). Blood glucose levels in ob/ob-JNK-KO mice were lower compared with those in ob/ob wild-type mice, and the ob/ob wild-type mice displayed a severe and progressive hyperinsulinemia. Thus, JNK deficiency can provide partial resistance against obesity, hyperglycemia, and hyperinsulinemia in both genetic and dietary models of diabetes. Such improvement of insulin resistance and glucose tolerance might be accounted for by reduced body-weight gain by JNK deficiency in mice. Taken together, obese type 2 diabetes is associated with activation of the JNK pathway, and the absence of JNK results in substantial protection from obesity-induced insulin resistance. These results strongly suggest that JNK plays a crucial role in progression of insulin resistance found in type 2 diabetes (Fig. 6). It is noted here that, of the three isozymes of JNK (JNK1, JNK2, and JNK3), only JNK1 has been shown to be implicated in the development of insulin resistance (43).

It was also reported that overexpression of dominant-negative (DN) type JNK1 (Ad-DN-JNK) in the liver of obese diabetic C57BL/KsJ-db/db mice dramatically improved insulin resistance and markedly decreased blood glucose levels (43). In an intraperitoneal insulin tolerance test, the hypoglycemic response to insulin was larger in Ad-DN-JNK–treated db/db mice. Furthermore, in the euglycemic

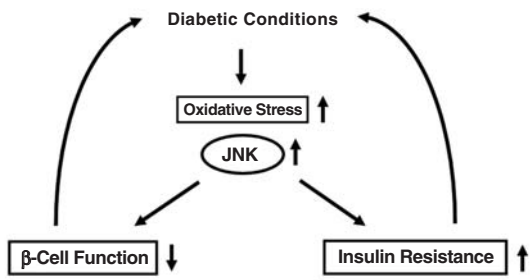

FIG. 6. Involvement of oxidative stress and the JNK pathway in the pathogenesis of type 2 diabetes.

hyperinsulinemic clamp test, the glucose infusion rate (GIR) in Ad-DN-JNK– treated mice was higher than that in Ad-GFP–treated mice, indicating that suppression of the JNK pathway in the liver reduces insulin resistance and thus ameliorates glucose tolerance in the db/db mice. Furthermore, hepatic glucose production (HGP) was significantly lower in Ad-DN-JNK–treated mice. In contrast, no difference was found in the glucose disappearance rate (Rd) between these two groups (96). These results indicate that reduction of insulin resistance and amelioration of glucose tolerance by DN-JNK overexpression are mainly due to suppression of hepatic glucose production. It has been reported that serine phosphorylation of insulin receptor substrate-1 (IRS-1) inhibits insulin-stimulated tyrosine phosphorylation of IRS-1, leading to an increase in insulin resistance (1). IRS-1 serine 307 phosphorylation was markedly decreased in Ad-DN-JNK–treated mice. An increase in IRS-1 tyrosine and Akt serine 473 phosphorylation was also observed in Ad-DN-JNK–treated mice (96). Therefore, an increase in IRS-1 serine phosphorylation may be closely associated with the development of insulin resistance induced by JNK overexpression. These results indicate that suppression of the JNK pathway enhances insulin signaling, which leads to amelioration of glucose tolerance. Taken together, these findings suggest that suppression of the JNK pathway in the liver exerts greatly beneficial effects on insulin-resistance status and glucose tolerance in both genetic and dietary models of diabetes.

Protein transduction domains (PTDs), such as the small PTD from the TAT protein of human immunodeficiency virus (HIV-1), the VP22 protein of Herpes simplex virus, and the third α-helix of the homeodomain of Antennapedia, a *Drosophila* transcription factor, are known to allow various proteins and peptides to be efficiently delivered into cells through the plasma membrane, and thus increasing interest has been expressed in their potential usefulness for the delivery of bioactive proteins and peptides into cells (1, 29, 34, 95, 98, 110, 114). It was recently reported that the cell-permeable JNK inhibitory peptide is effective for the treatment of diabetes. This peptide is derived from the JNK-binding domain of JNK-interacting protein-1 (JIP-1) and has been reported to function as a dominant inhibitor of the JNK pathway (13). To convert the minimal JNK-binding domain into a bioactive cell-permeable compound, a 20-amino-acid sequence derived from the JNK-binding domain of JIP-1 was covalently linked to a 10-amino-acid carrier peptide derived from the HIV-TAT sequence; then to monitor peptide delivery, this JNK inhibitory peptide was further conjugated with fluorescein isothiocyanate (FITC). When this peptide was injected intraperitoneally to C57BL/KsJ-db/db obese diabetic mice, the FITC-conjugated peptide showed fluorescence signals in insulin target organs (liver, fat, muscle) and in insulin-secreting tissue (pancreatic islets) (61). In insulin tolerance tests, reduction of blood glucose levels in response to injected insulin was much larger in JNK inhibitory peptide–treated mice (61). Furthermore, in the euglycemic hyperinsulinemic clamp test, the steady-state GIR in JNK inhibitory peptide–treated mice was significantly higher than that in untreated mice, indicating that JNK inhibitory peptide reduces insulin resistance in the db/db mice.

Endogenous hepatic glucose production (HGP) and glucose disappearance rate (Rd) in the JNK inhibitory peptide–treated mice also was evaluated. It is noted that Rd reflects glucose utilization in the peripheral tissues. HGP in JNK inhibitory peptide–treated mice was significantly lower than that in untreated mice. In addition, Rd in JNK inhibitory peptide–treated mice was significantly higher than that in untreated mice (61). These results indicate that JNK inhibitory peptide treatment reduces insulin resistance through decreasing HGP and increasing Rd. IRS-1 serine 307 phosphorylation was decreased in JNK inhibitory peptide–treated mice. An increase of IRS-1 tyrosine phosphorylation was observed in the peptide-treated mice. Concomitantly, glucose tolerance was also ameliorated in JNK inhibitory peptide–treated mice. In conclusion, suppression of the JNK pathway improves insulin resistance and ameliorates glucose intolerance, indicating that the JNK pathway plays a crucial role and could be a potential therapeutic target for diabetes (see Fig. 6).

OXIDATIVE STRESS AND ATHEROSCLEROSIS

Atherosclerosis is often observed in subjects with various metabolic diseases such as diabetes, hypertension, and hyperlipidemia. Among various risk factors, diabetes is thought to be one of the most important risk factors that facilitate the progression of atherosclerosis. Atherosclerosis is often observed as a macroangiopathy under diabetic conditions. We reported that increase of intima–media thickness (IMT) in the carotid artery, an index of the progression of atherosclerosis, is often observed in patients with diabetes (69, 135, 136) and that the progression of IMT is attenuated after treatment with various medicines for diabetes (65, 137). It is well known that hyperglycemia *per se* found under diabetic conditions facilitates the progression of atherosclerosis. Furthermore, hyperinsulinemia, which is often observed in subjects with insulin resistance, is also likely involved in the progression of atherosclerosis (Fig. 7).

Endothelial dysfunction is likely to be one of the earliest key events in atherosclerosis (14, 88). It has been thought that oxidative stress is involved in the deterioration of endothelial function, which is accompanied by inactivation of endothelial nitric oxide synthase (eNOS) and decrease of available nitric oxide (NO). Oxidative stress also induces expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), and receptors of oxidized lipid particles such as leptin-like oxidized LDL receptor (LOX-1). The expression of endothelial cell-surface receptors facilitates inflammatory cell recruitment and lipid deposition in the intimal layer. The subsequent ingestion of excess oxidized LDL particles by macrophages and monocytes results in inflammatory cytokine and growth factor release. Excessive ROS formation in diabetes further facilitates endothelial dysfunction, accompanied by inactivation of eNOS and decrease of NO (15). Indeed, it was reported that exposure of endothelial cells to a high glucose concentration inhibited eNOS expression and diminished NO bioavailability (16).

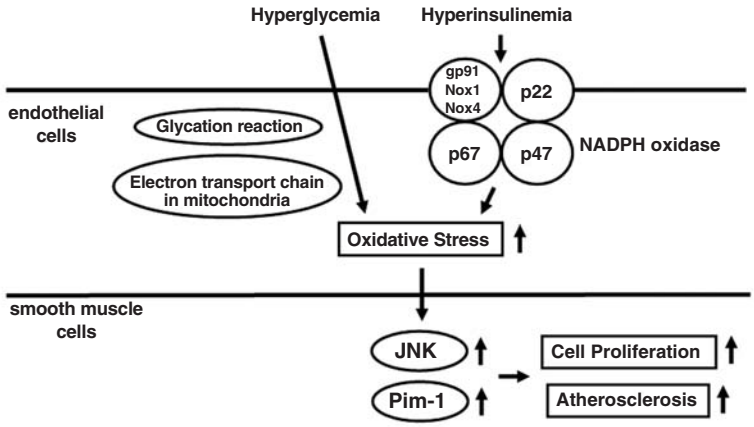

FIG. 7. Involvement of oxidative stress and the JNK pathway in the progression of atherosclerosis.

Proliferation of vascular smooth muscle cells (VSMCs) is also a key step in the development of atherosclerosis. It has been suggested that reactive oxygen species (ROS) and various growth factors are involved in the VSMC proliferation and the development of atherosclerosis (81). Induction of ROS is often observed *in vivo* under certain conditions such as diabetes, hypertension, hypercholesterolemia, obesity, and smoking, all of which are risk factors of atherosclerosis. Clinical mass studies have also provided support for the significance of ROS in the development of atherosclerosis (118, 126). Induction of ROS causes an increase in peroxidized lipids and thus enhances the foam-cell formation of macrophages and promotes migration of VSMCs. In addition, ROS directly stimulate DNA synthesis and accelerate VSMC proliferation, which plays a key role in the development of atherosclerosis. It has also been demonstrated that ROS regulate expression of various growth factors such as heparin-binding epidermal growth factor–like growth factor and insulin-like growth factor I and several growth-related proto-oncogenes such as c-Myc, c-Fos, and c-Jun (23, 106). Furthermore, using the suppression subtractive hybridization technique, we previously identified that ROS regulate expression of various other factors such as fibronectin, p105 co-activator, and ECA39 in VSMCs, all of which are likely involved in the progression of atherosclerosis (113). Taken together, it is likely that oxidative stress is involved in the VSMC proliferation and development of atherosclerosis through various pathways.

It has been shown that membrane-bound NADPH oxidase is the major source of ROS in the vasculature (40, 92) and that NADPH oxidase–derived ROS play a critical role in the development of atherosclerosis. NADPH oxidase is composed of the membrane-bound subunits gp91 phox (Nox2)/Nox1/Nox4, and p22 phox, and the catalytic site of the oxidase and cytosolic components p47 phox and p67 phox. In vascular cells, such as endothelial and smooth muscle cells, Nox 1 and Nox 4, rather than gp91 phox, are abundantly expressed. NADPH oxidase is activated by various factors such as angiotensin II, thrombin, platelet-derived growth factor, and tumor necrosis factor-α (22, 101, 121, 129). It has been reported that mice lacking p47 phox, which is an important component for NADPH oxidase, have lower levels of aortic ROS production compared with wild-type mice, and, when in a hypercholesterolemic apolipoprotein E-deficient [apoE (−/−)] background, had significantly fewer lesions in their descending aortas compared with apoE (−/−) mice (8). NADPH oxidase–derived ROS also play a crucial role in atherosclerosis in human. It has been reported that ROS production in atherosclerotic human coronary arteries is associated with NADPH oxidase subunit p22 phox (7) and recently that phagocytic NADPH oxidase overactivity is involved in oxidative stress and atherosclerosis in metabolic syndrome patients and that hyperinsulinemia may contribute to oxidative stress in metabolic syndrome patients through activation of NADPH oxidase (33). In addition, it was shown that high glucose stimulates ROS production through the activation of NADPH oxidase (42, 50) and that the p22 phox was significantly increased in rat and human diabetic arteries (37, 71). This activation of p22 phox may contribute to the acceleration of atherosclerosis in patients with diabetes. The role of p22 phox in the progression of atherosclerosis was also shown in type 2 diabetes patients. We previously reported that in type 2 diabetes subjects, the C242T polymorphism of the p22 phox gene, an essential component of NADPH oxidase in the vasculature, was closely associated with IMT of the carotid artery, an index of the progression of atherosclerosis (41). Average IMT in the diabetes patients with the CC genotype was significantly higher compared with those with the TC+TT genotypes, despite no difference in the risk factors. It is noted here that the presence of 242T allele is know to be associated with significantly reduced vascular NADPH oxidase activity (36). Furthermore, in stepwise multiple regression analysis, the p22 phox CC genotype was an independent risk factor for increased IMT in the diabetic subjects (41). These results show that the C242T mutation in the p22 phox gene is associated with progression of atherosclerosis in the diabetes patients and further strengthen the important role of NADPH oxidase in the progression of atherosclerosis.

Although various molecules and kinases are thought to be activated by oxidative stress in VSMCs, we recently reported that Pim-1, a proto-oncogene that encodes a serine/threonine kinase, is induced by oxidative stress and thus is likely involved in the progression of atherosclerosis (64). Pim-1 was substantially induced in neointimal VSMCs of balloon-injured rat carotid arteries, and *in vivo* infection with a dominant-negative Pim-1–expressing adenovirus (Ad-DN-Pim-1) markedly suppressed neointima formation

and cell-cycle progression in the balloon-injured arteries (64). In cultured VSMCs, ROS-stimulated cell-cycle progression and DNA synthesis were suppressed by DN-Pim-1 overexpression. Furthermore, Pim-1–producing cells were observed predominantly in the thickened intima of human thoracic aortas and coronary arteries (64). These findings suggest that oxidative stress and consequent induction of Pim-1 expression play a critical role in the progression of atherosclerosis (see Fig. 7).

THE JNK PATHWAY AND ATHEROSCLEROSIS

Although the JNK pathway is known to be activated by oxidative stress in VSMCs (138), oxidative stress and subsequent activation of the JNK pathway are likely involved in the progression of atherosclerosis. It is known that the JNK pathway is commonly activated by vascular remodeling–related molecules and plays a central role in the initiation of cellular responses, including cellular gene expression, growth, migration, or apoptosis. It was previously reported that the JNK pathway is activated in balloon-injured arteries (48, 70, 73). *In vivo* transfection of DN-JNK significantly suppressed activation of the JNK pathway and reduced VSMC proliferation in a balloon-injury model (51). Neointimal formation after balloon injury was also prevented by DN-JNK overexpression. Bromodeoxyuridine labeling index and total cell-counting analysis showed that DN-JNK remarkably suppressed VSMC proliferation in both the intima and the media after injury. In contrast, gene transfer of wild-type JNK (WT-JNK) significantly enhanced neointimal hyperplasia after balloon injury. Taken together, activation of the JNK pathway triggers VSMC proliferation, leading to neointimal formation, and the JNK pathway could be a new therapeutic target for atherosclerosis (see Fig. 7).

Furthermore, the role of JNK in atherosclerotic plaque formation *in vivo* was examined using atherosclerosis-prone apolipoprotein E knockout mice (ApoE (−/−) mice). Activation of the JNK pathway was closely correlated with the presence of clearly established plaques in ApoE (−/−) mice with a high-cholesterol diet. It was recently reported that atherosclerosis-prone ApoE (−/−) mice simultaneously lacking JNK2 [ApoE (−/−), JNK2 (−/−) mice], but not ApoE (−/−), JNK1 (−/−) mice, developed less atherosclerosis compared with ApoE (−/−) mice (107). Pharmacologic inhibition of the JNK activity also efficiently reduced plaque formation. Macrophages lacking JNK2 displayed suppressed foam cell formation caused by defective uptake and degradation of modified lipoproteins and showed increased amounts of the modified lipoprotein-binding and -internalizing scavenger receptor A (SR-A). Macrophage-restricted deletion of JNK2 was sufficient to decrease atherogenesis (107). These data suggest that JNK2-dependent phosphorylation of SR-A promotes uptake of lipids in macrophages, and thereby regulates foam cell formation. These data also further strengthen the significance of the JNK pathway in the progression of atherosclerosis (see Fig. 7).

CONCLUDING REMARKS

Oxidative stress and subsequent activation of the JNK pathway are involved in the pathogenesis of type 1 and type 2 diabetes. In the onset of type 1 diabetes, pancreatic β cells are relatively immediately destroyed by large amounts of ROS, various inflammatory cytokines, and activation of the stress signaling. Suppression of oxidative stress or the JNK pathway in β cells of type 1 diabetic mice suppresses the onset of type 1 diabetes. Oxidative stress is induced and the JNK pathway is activated under hyperglycemic conditions, which is possibly involved in deterioration of pancreatic β-cell function and insulin resistance found in type 2 diabetes. Suppression of oxidative stress or the JNK pathway in obese type 2 diabetic mice restored β-cell function and insulin sensitivity, leading to amelioration of glucose tolerance. In addition, oxidative stress and subsequent activation of the JNK pathway are involved in the progression of atherosclerosis, which is often observed as a macroangiopathy under diabetic conditions. Taken together, oxidative stress and subsequent activation of the JNK pathway are closely associated with the pathogenesis of diabetes.

ABBREVIATIONS

Ad, adenovirus; DN, dominant-negative; IRS-1, insulin receptor substrate 1; JNK, c-Jun N-terminal kinase; PDX-1, pancreatic and duodenal homeobox factor-1; ROS, reactive oxygen species; VSMCs, vascular smooth muscle cells; WT, wild type.

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