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Review Article

Autoimmune perspective of insulin-dependent diabetes mellitus: cytokines as therapeutic targets

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CONTENTS

Abstract

Insulin-dependent diabetes mellitus (IDDM) is believed to be an autoimmune disorder characterized by specific and progressive loss of pancreatic beta cells. Macrophages and dendritic cells are the first to invade the pancreas. These cells recognize beta cell autoantigens and then present to $CD4^+$ T helper (Th) cells. $CD4^+$ T cells are, in turn, documented to activate $CD8^+$ cytotoxic T cells and macrophages. The activated $CD8^+$ T cells and macrophages may serve as final effectors to destroy beta cells. Cytokines are important mediators secreted by these immune cells involved in beta cell destruction and thus may serve as attractive therapeutic target sites. Several cytokine-based therapies such as monoclonal antibodies directed against cytokines or their receptors, soluble cytokine receptors and cytokine receptor antagonists, which can block the production/action of proinflammatory cytokines (IL-1β, TNF-α, IFN-γ) may be investigated. Immunostimulatory agents directed at specific augmentation of Th2 subset (IL-4, IL-10) or Th3 subset (TGF-β) of $CD4^+$ Th cells may also offer an alternative approach.

Introduction

Insulin-dependent diabetes mellitus (IDDM) is believed to be an autoimmune disorder characterized by specific and progressive loss of pancreatic beta cells (1, 2). Several environmental and nutritional factors have been identified which may selectively damage beta cells (3) through the release of specific beta cell autoantigens such as insulin (4), glutamic acid decarboxylase (5), IA-2 (6) and ICA 69 (7). The excess release of insulin (8) or cytokines (9) from cells recruits macrophages to the islets (Fig. 1). These macrophages and dendritic cells recognize beta cell autoantigens and activate T helper (Th) cells. This process may induce cytokine transcription and consequently further add to the cytokine pool in the islets (10). This increase in cytokine pool activates mononuclear cells such as macrophages and cytotoxic lymphocytes, which may induce nitric oxide (NO)-dependent necrotic and apoptotic destruction of beta cells.

Environmental stresses such as viral infection (11), streptozotocin (STZ) treatment (12) and recent onset IDDM (13, 14) have been reported to increase the mRNA expression and release of interferon α (IFN-α) from beta cells which may consequently produce insulitis. Transgenic expression of IFN-α in nondiabetes-prone mice leads to development of diabetes mellitus (15). Recently, a patient receiving IFN-α treatment for chronic hepatitis was reported to develop fulminant type 1 diabetes (16). Moreover, systemic administration of poly ribonucleotide, poly [I:C], an inducer of IFN-α/β, is reported to accelerate the development of diabetes in BB rats (17) and to prevent diabetes in NOD mice (18). Therefore, IFN-α may be involved in the development of diabetes in humans and BB rats but it may prevent the development of diabetes mellitus in NOD mice.

Resident macrophage-derived cytokines: initiators of beta cell death

Electron microscopic and immunohistochemical analysis of islet lesions suggests that macrophages and

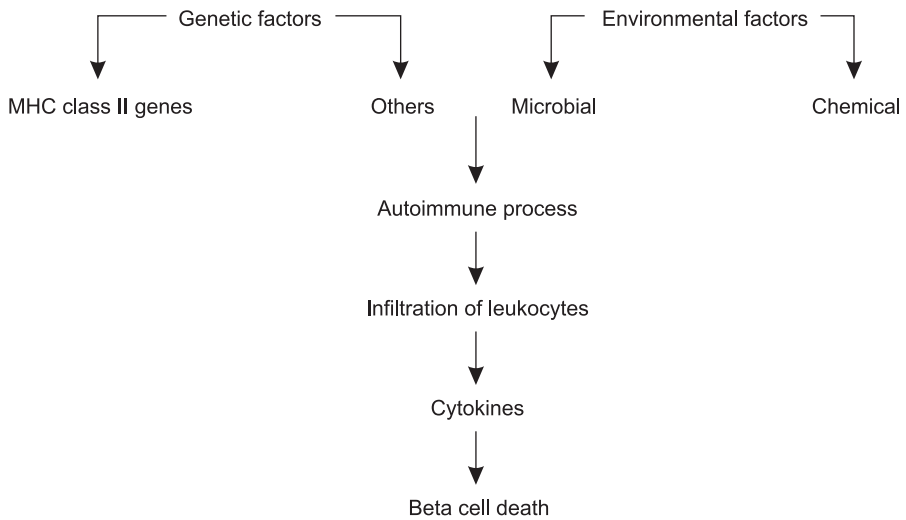

Fig. 1. Schematic representation of the pathogenesis of IDDM.

dendritic cells are the first to invade the pancreas during the early stages of insulitis in BB rats, NOD mice and low dose STZ-treated diabetic mice (19-22). Macrophages are highly cytotoxic to pancreatic islet cells *in vitro* (23). Moreover, oxygen free radical formation (24) and islet killing capacity of macrophages is noted to be augmented at the onset of diabetes in BB rats (25, 26). Transfer of peritoneal macrophages from diabetic NOD mice accelerates the onset of IDDM in prediabetic NOD mice (27). Administration of silica, a substance toxic to macrophages, to NOD mice and BB rats has been reported to prevent insulitis and diabetes (28, 29). Furthermore, spleen cells obtained from silica treated BB rats do not induce insulitis or diabetes in recipient neonatal BB rats (30). These observations strongly implicate the role of macrophages in pathogenesis of IDDM.

Interleukin (IL)-12 is the main cytokine secreted by these immune cells (31) and it may mediate their beta cell cytotoxic effect. Exogenous administration of IL-12 is reported to accelerate the onset IDDM in young female NOD mice (32). IL-12 mRNA expression in pancreatic islets correlates with beta cell destruction (33). Moreover, a homodimeric IL-12 p40 subunit which acts as a antagonist to IL-12, is reported to suppress diabetes development in NOD mice (34). In addition, transgenic NOD mice over expressing the IL-12 p40 homodimer in islets, are markedly protected against IDDM (35). Transgenic NOD mice with a disrupted IL-12 gene have been demonstrated to be less prone to cyclophosphamide-induced acceleration of diabetes (36). Moreover, administration of IL-12 antibodies to NOD mice at the age of 5-30 weeks prevents the development of diabetes in NOD mice (37). These observations suggest that macrophage-derived IL-12 may be involved in the pathogenesis of IDDM. However, IL-12 antibody treatment of NOD mice at an early age of 2 weeks does not prevent the development of diabetes (37). Similarly, IL-12 knockout NOD mice are still capable of developing diabetes (38). These studies suggest that inhibition of IL-12 production at very early stage does not prevent the development of IDDM. Besides IL-12, macrophages are demonstrated to secrete IL-18 (39). IL-18 may also induce T-cell differentiation (40). High levels of IL-18 have been detected in the serum of individuals at high risk of developing IDDM (41). Therefore, IL-18 production may be responsible for the macrophage-mediated destruction of beta cells in the absence of IL-12. Based on these observations, it may be suggested that simultaneous inhibition of IL-12 and IL-18 formation may be a therapeutic strategy for IDDM at a relatively early stage of disease onset. However, macrophages may not be the final effectors of beta cell destruction in IDDM because late- and short-term silica treatment of NOD mice does not prevent the destruction of beta cells (42).

$CD4^+$ T cell-derived cytokines: facilitators of beta cell death

IL-12 is well documented to induce differentiation of the Th1 subset of $CD4^+$ T cells (31, 43-45). IL-12 mRNA expression in islet infiltrating mononuclear cells is reported to correlate with the expression of Th1 $CD4^+$ T cell-derived cytokines, such as IFN-γ and IL-2 (33). Therefore, presentation of MHC class II restricted beta cell specific autoantigens by macrophages and/or dendritic cells to $CD4^+$ Th cells may represent the next step for destruction of beta cells in IDDM (46, 47) (Fig. 2). Moreover, large number of T cells have been detected in the human pancreas at the onset of IDDM (48, 49). Neonatal thymectomy (50) and administration of antilymphocyte serum (50), corticosteroid or cyclosporine (51) have been reported to prevent the development of IDDM in BB rats. In addition, IDDM does not develop in athymic or scid NOD mice (52, 53).

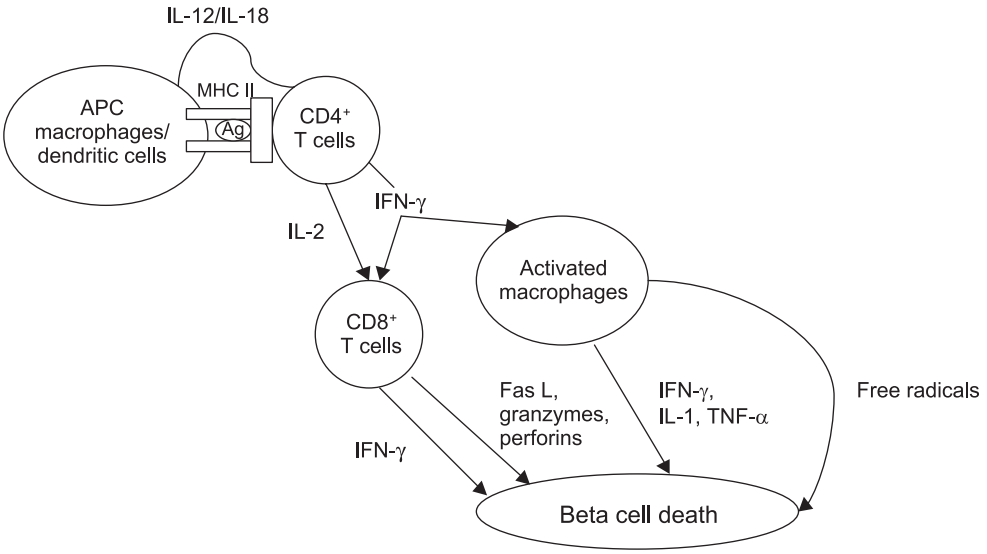

Fig. 2. Proposed sequence of leukocyte and cytokine involvement in autoimmune mediated destruction of beta cells.

IFN-γ and IL-2 are the 2 cytokines secreted by $CD4^+$ T cells (54). IFN-γ expression in the pancreas of NOD mice is reported to occur as a consequence of IL-12 and IL-18 production (55, 56). IFN-γ secreting splenic $CD4^+$ T cells obtained from diabetic NOD mouse are noted to increase the destruction of beta cells in recipient nondiabetic NOD mice (57, 58).

Expression of IL-2 in the mononuclear leukocytes infiltrating pancreatic islets is in parallel with IFN-γ expression (33, 59). A decrease in pancreatic IL-2 mRNA after administration of anti-CD2 monoclonal antibodies directed against $CD4^+$ T cells, confirms that $CD4^+$ T cells are the source of IL-2 (60). Exogenously administered [^{125}I]-labeled IL-2 is reported to accumulate in the pancreas of prediabetic and diabetic NOD mice (61). The level of soluble IL-2 receptor, an endogenous scavenger of IL-2, has been demonstrated to be increased in patients with IDDM (62). Moreover, anti-IL-2 receptor antibodies, IL-2 receptor-targeted fusion toxin and an anti-IL-2 chimeric fusion protein have been observed to suppress insulitis and to reduce the incidence of diabetes in NOD mice (63-65). Transgenic nondiabetes-prone NOD mice with a heterozygous overexpression of IL-2 in beta cells develop benign insulitis (66), whereas mice with homozygous overexpression of IL-2 gene become diabetic (67, 68). However, IL-2 overexpression-induced diabetes does not involve $CD4^+$ T cells and it has been ascribed to nonspecific beta cell destruction by macrophages (68). Systemic administration of IL-2 in NOD mice is reported to decrease the incidence of diabetes (18, 69). It is interesting to note that IL-2 increases and decreases the incidence of diabetes in strains of BB rats with low and high incidence of disease, respectively (69). Overall, it is difficult to implicate the role of IL-2 alone in pathogenesis of IDDM.

$CD4^+$ T-cell clones do not destroy beta cells *in vitro* but attach closely to them (46). Moreover, splenic $CD4^+$ T cells from prediabetic NOD mice transfer insulitis but not IDDM in SCID NOD mice (70). Transgenic NOD mice with overexpression of the gene encoding for the receptor of diabetogenic Th cells have an increase in the incidence of diabetes but not in the onset of disease (71). Anti-$CD4^+$ immunoglobulin does not prevent diabetes in NOD mice (72). These observations suggest that $CD4^+$ T cells may not be the main effectors of antigen specific beta cell destruction and are in accordance to the above mentioned equivocal role of IL-2, a $CD4^+$ T cell-derived cytokine, in pathogenesis of IDDM. However, a few studies indicate that $CD4^+$ T cells obtained from diabetic NOD mice produce beta cell destruction when administered to NOD mice (57, 73). Nonantigen specific beta cell destruction through enhanced cytokine production and macrophage activation by $CD4^+$ T cells may be responsible for this noted destruction of beta cells in NOD mice.

$CD8^+$ T cell-derived cytokines: executioners of beta cell death

IL-2 and IFN-γ produced by $CD4^+$ T cells are documented to activate $CD8^+$ cytotoxic T cells and macrophages respectively (2). Thus, the activated $CD8^+$ T cells and macrophages may serve as final effectors to destroy beta cells (see Fig. 2). MHC class I- or $CD8^+$ T cell-deficient NOD mice do not develop IDDM (74, 75). Transgenic NOD mice with an overexpression of $CD8^+$ cytotoxic T cells have been reported to exhibit an accelerated onset of IDDM (76). Anti-$CD8^+$ immunoglobulin is reported to prevent diabetes onset in transgenic mice (72). Moreover, islet specific $CD8^+$ T-cell clones were

demonstrated to selectively destroy beta cells *in vitro* (46). $CD8^+$ T cells have a direct cytotoxic effect on beta cells in association with class I MHC restricted antigen (77). $CD8^+$ T cells derived from BB rats and NOD mice are essential to produce diabetes in normal mice (46, 78). $CD8^+$ T cells may also render osmotic destruction of beta cells by perforin (cytolysin), a tubular protein homologous to membrane attacking complex of complement C9 (79), granzyme A, now identified as IL-1β-converting enzyme (ICE) (80), and Fas ligand (81).

IFN-γ has been detected by immunohistochemistry in lymphocytes infiltrating islets of human subjects with recent onset IDDM (82). Besides $CD4^+$ T cells, $CD8^+$ T cells and activated macrophages are demonstrated to be the major source of IFN-γ (23, 83). IFN-γ is reported to upregulate MHC class I expression in both rodent and human beta cells (84) and an increase in MHC class I molecule is consistently observed in the insulitis lesions of laboratory animals (85) and newly diagnosed IDDM patients (49, 86). Transgenic expression of IFN-γ in the beta cells of nondiabetes-prone mice results in progressive and severe lymphocytic infiltration of islets and subsequent development of IDDM due to beta cell destruction (87). Transgenic mice deficient in IFN-γ and expressing lymphocytic choriomeningitis virus (LMCV) nucleoprotein or glycoprotein in their beta cells, do not develop IDDM despite the generation of LMCV-specific $CD8^+$ T-lymphocytes (88). Anti-IFN-γ polyclonal and monoclonal antibodies are reported to delay the incidence of diabetes in BB rats (89) and transgenic diabetic mice (90). Recombinant murine soluble IFN-γ receptor treatment prevents diabetes in NOD mice when treatment is started even at the age of 16 weeks (91). Therefore, pharmacological modulation of IFN-γ or its receptors may be a clinically viable target site for IDDM and may offer protection even after manifestation of IDDM. Selective deletion of IFN-γ receptor gene from beta cells of NOD mice does not prevent IDDM (92) where as its knockout from all cells significantly inhibits IDDM (93) suggesting a more important role for IFN-γ receptors present on lymphocytes than those expressed on beta cells.

Activated macrophage-derived cytokines: executioners of beta cell death

Besides IFN-γ, activated macrophages also secrete IL-1 (2). IL-1 is reported to have an inhibitory effect on mitochondrial energy production in islet cells (94). IL-1 produces a decrease in the overall rate of protein synthesis, specifically biosynthesis of preproinsulin (95). IL-1 damages islet cell DNA and reduces its content (96, 97) and decreases viability of islet cells (104). Within a specified dose range and time frame, IL-1 is selectively toxic to beta cells of cultured islets (98, 99). It is worthwhile to note that, besides IL-1, no other single cytokine is capable of producing beta cell cytotoxicity (10, 100). Other cytokines such as IFN-γ and TNF-α augment the cytotoxic effects of IL-1β on beta cells (10, 83, 101). Administration of human recombinant IL-1 to rats produces transient diabetes characterized by hyperglycemia and hypoinsulinemia (102). A polyclonal anti-IL-1β antibody and soluble IL-1 receptor significantly decrease the incidence of cyclophosphamide-accelerated diabetes in male NOD mice (103, 104). Administration of a IL-1ra, a natural IL-1 receptor antagonist, delays onset of diabetes in BB rats (105) and inhibits diabetes recurrence after syngeneic islets transplantation in diabetic NOD mice (106). This evidence suggests that IL-1 is one of the main contributors to beta cell death in IDDM and may emerge as a major target for salvage of beta cells in IDDM.

Serum TNF-α levels are higher in prediabetic NOD mice and BB rats as compared to nondiabetes-prone controls (107, 108). Troglitazone, an inhibitor of macrophage TNF-α secretion, and MDL-201, a 499A transcriptional inhibitor of TNF-α, are reported to prevent type I diabetes in NOD mice and mice treated with multiple low-dose STZ (109-111). However, transgenic expression of TNF-α in control and NOD mice produces severe insulitis but does not lead to diabetes (112, 113). Permanent neutralization of TNF-α by transgenic expression of a soluble chimeric TNF receptor antibody is reported to decrease insulitis and protect from spontaneous diabetes as well as from accelerated onset of diabetes caused by transfer of NOD spleen cells or cyclophosphamide injections in NOD mice (114). Systemic administration of TNF-α, on the contrary, is reported to prevent diabetes development in NOD mice (115, 116). When TNF-α is administered systemically, cell-mediated immunity is preferentially inhibited (117) and expression of type I cytokines such as IFN-γ, IL-2 and TNF-β may be downregulated thus accounting for its protective effect (118). Neonatal islet specific expression of TNF-α in NOD mice promotes diabetes (119). Moreover, TNF-α molecules present on activated $CD8^+$ T cells is reported to assist $CD8^+$ T cells in mediating acute autoimmune diabetes independent of perforin and Fas cytotoxic pathways (120). TNF-α is also reported to potentiate the inhibitory effect of IL-1 on insulin secretion (83) and glucose metabolism in rat pancreatic islets (121). The combination of IL-1 and TNF-α is cytotoxic to rodent islet beta cells (83). Collectively, these reports suggest some role for TNF-α in the pathogenesis of IDDM. However, it may not act as a single effector but instead offer an additive effect together with IL-1 in the autoimmune destruction of beta cells.

Th2 $CD4^+$ cell-derived cytokines: protectors of beta cell death

The Th2 subset of $CD4^+$ Th cells is reported to produce IL-4 and IL-10 (54) (Table I). Increased expression of IL-4 and IL-10 has been reported in islets of NOD mice protected from diabetes development by various treatments such as insulin (122), complete Freunds adjuvant (123) and a noncytolytic IL-10/Fc fusion protein (124). Exogenous administration of IL-4 and IL-10 has also been shown to have a protective effect against beta cell

Table I: Various cell types implicated in IDDM and the cytokines secreted by these cells.

Cell Type	Cytokine	Putative Effect
APCs	IL-12, IL-18	Beta cell cytotoxic
Th1 $CD4^+$ T cells	IL-2, IFN-γ	Beta cell cytotoxic
$CD8^+$ T cells	IFN-γ	Beta cell cytotoxic
Activated macrophages	IFN-γ, IL-1, TNF-α	Beta cell cytotxic
Th2 $CD4^+$ T cells	IL-4, IL-10	Beta cell cytoprotective

destructive insulitis (125, 126) Anti-CD28 monoclonal antibody treatment prevents insulitis and IDDM in NOD mice and this protective effect is accompanied by an increase in IL-4 production (127). Systemic administration of IL-4 and IL-10 in combination is also reported to delay autoimmune diabetes recurrence in NOD mice transplanted with syngeneic islets (128, 129). IL-4 alone has been reported to prevent diabetes development in NOD mice after systemic administration and intraislet transgenic expression by beta cells (130, 131). Transgenic overexpression of IL-10 in nondiabetes-prone NOD mice is also reported to prevent insulitis or diabetes but does not inhibit periislet inflammation (132). Moreover, IL-10 monoclonal antibody treatment started at age 3 weeks prevents insulitis in NOD mice (133). However, late treatment started after 10 weeks of age does not affect the course of disease in NOD mice (134).

Expression of another cytokine, TGF-β, correlates with IL-10 and IL-4 in mice protected from diabetes by oral insulin administration (122). TGF-β is a product of the Th3 subset of Th cells (135). Interestingly, islets obtained from diabetes-prone BB rats treated orally with bacterial extract were also reported to have increased levels of TGF-β and IL-10 (136). Transgenic overexpression of TGF-β, in beta cells is reported to prevent beta cell death but induces chronic pancreatitis with fibrosis in nondiabetes-prone mice (137).

Cytokine downstream signaling in beta cell death

The cytokine IL-1β alone is cytotoxic to rat/human pancreatic beta cells in monolayer cultures and exhibits synergistic effects with IFN-γ and additive effects with TNF-α (83, 101, 138). These 3 cytokines induce beta cell apoptosis and/or necrosis and are proposed as important effector molecules in the pathogenesis of IDDM (139).

Beta cells of NOD mice express IL-1 receptors (140). An IL-1 receptor monoclonal antibody and IL-1 receptor antagonist are documented to prevent the deleterious effects of IL-1β and IL-1α on rat and mouse islets (114, 142). Moreover, the density of the IL-1 receptor sharply decreases with ongoing beta cell death suggesting that beta cells are the main source of IL-1 receptors in islets (140). Activation of IL-1β receptors leads to assembly of several IL-1β receptor-associated proteins such as MyD88, IRAK1/2 (143) and TRAF-6 (144). These proteins activate nuclear factor κ B (NFκB) inducing kinase (NIK) which subsequently activates NFκB (145) and p38, a member of the ERK subfamily of MAP kinases (145). IL-1β is demonstrated to activate these intracellular signaling molecules in beta cells of islets (146, 147). These kinases further phosphorylate c-jun, ATF-2 and the IL-1 receptor itself (10). IL-1β is also reported to activate transcription of c-fos and c-jun (148). Moreover, these factors combine together to form the AP-1 complex. IL-1β is noted to induce iNOS expression in beta cells (149, 150) which may be mediated through activation of MAPKinase through NIK and consequent activation of AP-1 complex transcription factors (150, 151). This contention is supported by the observation that VK-19577, a p38 MAP kinase inhibitor, or PD-098059, an ERK1/ERK2 inhibitor, prevents IL-1-induced increases in iNOS mRNA expression in rat pancreatic islets (146). NFκB activation by IL-1β is also reported to induce iNOS mRNA expression and it is prevented by PDTC, a NFκB inhibitor (152). Besides increases in NO production, IL-1β evoked activation of ERK1/ERK2 and p38 MAPKinase signaling has been recently reported to induce expression of mononuclear chemoattractant protein-1 (MCP-1) mRNA in rat and human islet cells (153). MCP-1 may be a key contributor in the recruitment of mononuclear cells into pancreatic islets in early type I diabetes.

TNF-α has been noted to moderately induce iNOS expression in beta cells (154, 155) which is also mediated through NIK-induced activation of NFκB. TNF-α also potentiates iNOS expression induced by IL-1β which may account for the TNF-α-induced potentiation of the cytotoxic effect of IL-1β on beta cells (147).

IFN-γ is demonstrated to potentiate TNF-α-induced NFκB activation through STAT-1 resulting in iNOS generation and cell destruction in the insulin secreting cell line, INS-1 (156). Persistent activation of STAT-1 by IFN-γ in beta cells is also associated with potentiation of IL-1-induced iNOS expression (157).

The polymorphic region idd4 of chromosome 11 is reported to be associated with IDDM in spontaneously diabetic NOD mouse (158). iNOS gene has been located in the middle of this idd4 region (159). Furthermore, iNOS mRNA and NO have been detected in islets of NOD mice and BB rats around the time of onset of diabetes (160, 161). iNOS knockout mice have been shown to be relatively resistant to diabetes induced by multiple subdiabetogenic injection of STZ (162). iNOS inhibitors protect rat islets against cytokine-induced cell damage (163, 164). NO is reported to inhibit the mitochondrial enzyme aconitase in rodent islets by nitrosylation of the enzyme's Fe-S groups (165, 166) and consequently decreases

oxidative metabolism and ATP formation. NO produces nuclear DNA damage, breaks in DNA strands (97) and activates DNA repair enzyme, *i.e.*, poly(ADP ribose) polymerase (PARP) (167). All these effects may account for the beta cell cytotoxic effect of NO. *N*-Acetyl cysteine and ebselen, H_2O_2 scavengers, prevented NFκB and iNOS induction in cultured insulin producing cell lines (168). Deforxamine, an inhibitor of the iron-dependent conversion of H_2O_2 into highly reactive OH radical, is also reported to protect islet cells from immune destruction, both in multiple low-dose STZ-induced diabetes and islet allograft rejection (169). Peroxynitrite is a highly reactive oxygen species produced by the combination of superoxide $O_2^{\cdot-}$ and NO (170) and is believed to be a more potent cytotoxic mediator than NO or $O_2^{\cdot-}$ (171). Rodent and human islets have been reported to be highly sensitive to peroxynitrite-induced damage (172). Formation of peroxynitrite in islet beta cells of acutely diabetic NOD mice has also been reported (173). Recently, guanidinoethyldisulphide, a selective inhibitor of iNOS and scavenger of peroxynitrite, has been shown to prevent diabetes in NOD mice and terminate production of NO and nitrotyrosine induced by a mixture of cytokines (IL-1β, TNF-α and IFN-γ) *in vitro* (174). Therefore, it may be suggested that oxygen free radicals also may amplify the deleterious effects of cytokine-induced NO formation in beta cell.

Binding of the Fas ligand (FasL) present on $CD8^+$ and $CD4^+$ T cells to the Fas receptor on beta cell causes beta cell destruction (1) through activation of NFκB (145). However, the role of the Fas gene in genetic susceptibility for type 1 diabetes mellitus has been ruled out (175). IL-1β is a potent inducer of the Fas receptor on mouse and human islets (176, 177). Fas expression is also reported to correlate with expression of other proinflammatory cytokines such as IL-1α, TNF-α and IFN-γ in islet grafts (178). Therefore, proinflammatory cytokines may also exert beta cell cytotoxic effect via a Fas-FasL-mediated NO-independent pathway. However, NO may play a role in priming beta cells for Fas-mediated destruction in IDDM (177). A biologically active recombinant fusion protein coupling mouse Fas to the Fc portion of human IgG1 (Fas Fc) has been reported to block Fas-FasL (*in vivo*) interaction and protect NOD mice from cyclophosphamide-induced diabetes (179). The protective effect in this study was attributed to inhibition of the cyclophosphamide effect on T cells rather than blockade of the Fas-FasL interaction. Fas ligand mechanisms are reported to play a important role in promoting leukocyte infiltration of islets and subsequent beta cell destruction in NOD mice (180). On the other hand, NOD islet grafts lacking Fas expression (NOD-*ipr*/*ipr* mice) are protected only marginally from autoimmune attack when grafted into diabetic NOD mice (181) suggesting a relatively minor role of Fas in beta cell death.

Conclusions

Cell-mediated autoimmune destruction of beta cells is a well accepted hypothesis for the pathogenesis of IDDM. Immune-mediated destruction of beta cells is thought to precede the overt clinical expression of IDDM by several years (182). Therefore, theoretically, it is thought that it may be possible to arrest autoimmune destruction of beta cells before progression to IDDM. Cytokines are important mediators secreted by immune cells involved in beta cell destruction and thus may serve as attractive therapeutic target sites. Several cytokine-based therapies such as monoclonal antibodies directed against cytokines or their receptors, soluble cytokine receptors and cytokine receptor antagonists, which block the production/action of pro-inflammatory cytokines (IL-1β, TNF-α, IFN-γ) may be investigated. Immunostimulatory agents directed at specific augmentation of the Th2 subset (IL-4, IL-10) or Th3 subset (TGF-β) of $CD4^+$ Th cells may offer an alternative approach.

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