

β -Cell Apoptosis Stimuli and Signaling

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Pancreatic β -cells are sensitive to a number of proapoptotic stimuli. Thus, apoptosis is an important part of the physiological neonatal remodeling of the endocrine pancreas, and a number of pathological stimuli involved in type 1 and type 2 diabetes have been shown to elicit β -cell apoptosis. Factors of relevance to type 1 diabetes include proinflammatory cytokines, nitric oxide, and reactive oxygen species as well as Fas ligand. Recent findings that free fatty acids, glucose, sulfonylurea, and amylin cause β -cell apoptosis in vitro suggest that programmed cell death may also be involved in the pathogenesis of type 2 diabetes. Furthermore, there is evidence favoring a convergence in signaling pathways toward common effectors of β -cell apoptosis elicited by stimuli implicated in the pathogenesis of type 1 and type 2 diabetes. Therefore, recent studies involving the stimuli and signaling pathways of β -cell apoptosis—in particular, mitogen- and stress-activated protein kinases—will be reviewed. It is concluded that immunological, inflammatory, and metabolic signals cause β -cell apoptosis, and the possibility that these signals converge toward a common β -cell death signaling pathway should be investigated further. *Diabetes* 50 (Suppl. 1):S58–S63, 2001

Given the vital necessity of insulin, but also the hazards of release in excess of needs, β -cell function and β -cell mass are tightly regulated. This evolutionary pressure has resulted in a highly specialized cell that is receptive to replicatory but also apoptotic stimuli to fulfill the need for dynamic regulation. Apoptosis, or programmed cell death, is a physiological mode of remodeling tissue during organogenesis but is also involved in physiology later in life, for example, in relation to thymic involution. It has become clear that the endocrine pancreas is actively remodeled postpartum and that apoptosis is an important mechanism for this (1).

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Received for publication 15 May 2000 and accepted 15 August 2000.

T.M.-P. is employed by, is a member of the Development Committee for, and has received grant support from Novo Nordisk.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Les Laboratoires Servier.

ATF-2, activating transcription factor-2; ERK, extracellular signal-regulated kinase; FFA, free fatty acid; IB-1, islet brain 1; IFN- γ , γ -interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; JBD, JNK binding domain; JNK, c-Jun NH₂-terminal kinase; MAPK, mitogen-activated protein kinase; NF κ B, nuclear factor κ B; NO, nitric oxide; PD, Parke-Davis; SAPK, stress-activated protein kinase; SB, SmithKline Beecham; TNF, tumor necrosis factor.

Apoptotic cell death is an energy-requiring process that involves de novo protein synthesis. The process is characterized by morphological changes including condensation of nuclear chromatin, cellular shrinkage, membrane blebbing, and the formation of apoptotic bodies that are membrane-surrounded cellular constituents and undergo phagocytosis before leakage of intracellular contents, thereby avoiding the stimulation of an immune response, hence an autoimmune reaction.

In many chronic degenerative diseases, apoptosis leads to inappropriate deletion of cells. The apoptotic cascade may be elicited by a number of varying stimuli, including intracellular events, such as metabolic imbalance, cell cycle perturbation, or DNA damage, and extracellular factors, such as activation of death receptors (Fas and tumor necrosis factor [TNF] receptors) and withdrawal of growth factors, metabolic factors, certain hormones, and inflammatory mediators such as cytokines. Intracellular signals involve ceramide; increases in intracellular calcium; free oxygen and nitric oxide (NO) radicals; and protein kinases such as mitogen-activated protein kinase (MAPK), stress-activated protein kinase (SAPK), and protein kinase C. Finally, common effector pathways are the activation of cysteine proteases (caspases) and endonucleases that cleave nuclear DNA into oligosomal fragments.

There is increasing evidence that apoptosis is the main mode of β -cell death leading to type 1 diabetes (2), but there is also some evidence, particularly from animal models, that β -cells undergo apoptosis in type 2 diabetes (3). In this article, the evidence supporting this notion will be treated separately for type 1 and type 2 diabetes; then, signaling and in particular the involvement of mitogen- and stress-activated protein kinases in β -cell apoptosis will be reviewed.

APOPTOSIS IN TYPE 1 DIABETES

Studies in several animal models of immune-mediated diabetes have shown that apoptosis may be the main mode of β -cell destruction (4–6). It has been established that macrophages as well as CD4⁺ and CD8⁺ T-cells are needed to produce insulinitis and diabetes in animal models. There are therefore several theoretical possibilities of how the immune system can elicit β -cell destruction.

- CD8⁺ T-cells cause β -cell destruction via the perforin/granzyme pathway, which involves the insertion of tubular perforin complexes into the cell membrane and osmotic cell death, i.e., a nonapoptotic death form. However, this explanation has been strongly questioned in perforin-deficient T-cell receptor transgenic NOD mice, which develop diabetes even more frequently than their perforin-competent littermates (7).
- Activated CD4⁺ and CD8⁺ T-cells express FasL, which after binding to the Fas receptor causes β -cell apoptosis. In sup-

port of this hypothesis, *in vitro* studies have shown that cytokines, such as interleukin (IL)-1 upregulate Fas expression on β -cells in an NO-dependent fashion (8,9). Furthermore, Fas expression correlates with β -cell destruction in NOD mice (10). However, contradictory results have been reported from *in vivo* studies. Thus, NOD mice lacking Fas were resistant to both spontaneous diabetes and diabetogenic T-cells (11,12). In contrast, islets from NOD mice lacking Fas were protected only marginally from an immune attack when grafted into diabetic mice (13). In addition, anti-FasL antibody failed to inhibit diabetes (14). Recently, however, the apparent controversy was solved by the finding that the Fas-deficient NOD mice have abnormal FasL-expressing lymphoid cells that exert apoptosis on adoptively transferred lymphocytes and thereby inhibit the development of diabetes (15). Taken together, these findings seriously question the role of the perforin and Fas/FasL systems in β -cell destruction. Rather, the role of the CD4⁺ and CD8⁺ cells is to feedback activate macrophages upon antigen stimulation and costimulation; these activated macrophages facilitate islet destruction by an NO synthesis-dependent pathway (16). The implication of humoral mediators in immune-mediated β -cell destruction is underlined by the observation that insulinitis and β -cell destruction can proceed in the absence of islet β -cell surface antigen recognition by T-cells in chimeric mice, in which the bone marrow-derived antigen-presenting cells, but not islet β -cells, are capable of presenting antigen to T-cells (17).

- Apart from macrophage-dependent NO synthesis, macrophages and T-cells could affect β -cell viability via the pro-inflammatory cytokines IL-1 β , TNF- α , and γ -interferon (IFN- γ). There is extensive evidence that these cytokines cause β -cell destruction *in vitro* (18), including in human islets (19). There are recent *in vivo* studies to support this concept. First, circulating receptors and neutralizing antibodies against IL-1 prevent type 1 diabetes with no apparent effect on the T-cell immune system (20,21). Second, transgenic NOD mice expressing the soluble TNF receptor resulting in neutralization of TNF or NOD mice deficient for the type 1 TNF receptor are protected against diabetes (22–24). Third, anti-IFN- γ antibodies prevent diabetes in BB rats, and diabetes in NOD mice is prevented by IFN- γ receptor α -chain mutations or in IFN- γ -deficient mice (25–27).

Taken together, the studies reviewed above suggest that β -cell destruction and type 1 diabetes depend on interaction between macrophages, CD4⁺ cells, and CD8⁺ T-cells that establish a chronic inflammatory lesion, in which soluble mediators such as NO and cytokines are important effector molecules.

APOPTOSIS AND TYPE 2 DIABETES

Type 2 diabetes manifests itself clinically when the β -cell mass cannot compensate for insulin resistance with increased insulin release. In most animal models of type 2 diabetes, β -cell compensation is achieved by β -cell hyperplasia. In humans, it is unclear whether the β -cell mass is progressively reduced once diabetes is overt. Several studies have found either normal or reduced β -cell mass in type 2 diabetic patients (28–32). In this context, it should be kept in mind that the normal β -cell mass when compared with that of nondiabetic non-insulin-resistant individuals may in fact indicate a relative reduction compared with the expected hyperplasia needed

for compensation in obese insulin-resistant individuals. As shown by Sempoux et al. (32a), there was a reduction in β -cell mass in insulin-treated type 2 diabetic patients. It can be argued that this is the consequence of insulin administration because severe hypoglycemic hyperinsulinemia, for example, in animals transplanted with insulinomas causes β -cell mass reduction (33). However, it should be noted that improvement of β -cell function is common in insulin-treated recent-onset type 1 diabetic subjects and is sufficient to maintain non-insulin-requiring remission in ~20% of recent-onset type 1 diabetic subjects (as in the Canadian-European cyclosporine study [34]). Taken together, though still an unresolved issue, several studies point at reduced β -cell mass as a contributor to β -cell decompensation in human type 2 diabetes.

There is more conclusive evidence from animal studies that apoptosis may be involved in β -cell failure in type 2 diabetes. Thus, in the Zucker diabetic fatty *fa/fa* rat, islets contain 100-fold higher levels of free fatty acids (FFAs) than those in lean rats. Culture of Zucker prediabetic rat islets in 1 mmol/l FFA led to a fourfold increase in apoptosis. This effect was associated with an increase in the sphingomyelinase product ceramide, and blocking ceramide synthesis prevented FFA-induced apoptosis. Interestingly, FFAs also induced NO synthase expression and a fourfold increase in NO production, and inhibitors of inducible NO synthase (iNOS) minimized the loss of insulin secretion. Further, the antiapoptotic factor Bcl-2 was strongly reduced by FFAs and disappeared in *fa/fa* islets cultured in fatty acids. This suppression of Bcl-2 was prevented by leptin, which thereby prevented apoptosis in these islets (3,35,36).

Intriguingly, long-term incubation with sulfonylurea triggers pancreatic β -cell apoptosis in *ob/ob* mice islets in a calcium-dependent manner (37)—an effect also found when exposing human hepatoblastoma cells to sulfonylureas (38). High glucose concentrations have been found to cause β -cell dysfunction and glucose desensitization and even induce apoptosis in *ob/ob* mouse islets (37), although glucose may also exert protective actions. Finally, islet amyloid polypeptide induces apoptosis in cloned β -cells (39).

Thus, there is ample evidence from *in vitro* and animal model studies that metabolic factors involved in type 2 diabetes pathophysiology and pharmacological factors used to treat type 2 diabetes may cause β -cell apoptosis.

ROLE OF NITRIC OXIDE IN β -CELL DESTRUCTION

The role of NO in autoimmune- and cytokine-mediated β -cell destruction has been extensively reviewed (18,40,41). In rat β -cells, it seems that increased production of NO, due to induction of iNOS via the nuclear factor κ B (NF κ B) transcription factor, is an important signal in cytokine-induced cell death, because inhibition of iNOS has been shown to prevent cell death (42). NO may cause β -cell toxicity via different mechanisms (18): 1) NO inactivates the Krebs cycle enzyme aconitase by nitrosylation of Fe-S groups, thereby preventing mitochondrial glucose oxidation and ATP generation; 2) NO damages cellular DNA by causing DNA strand breaks, thereby activating DNA repair mechanisms including the enzyme poly(ADP-ribose) polymerase, which can cause cell death through depletion of cellular nicotinamide adenine dinucleotide (NAD⁺); 3) the NO-induced DNA strand breaks may be sufficient to induce apoptosis through activation of the tumor suppressor protein p53 (43) (and/or p73); and 4) NO may function as a redox mediator in the cytokine-induced apoptotic pathway.

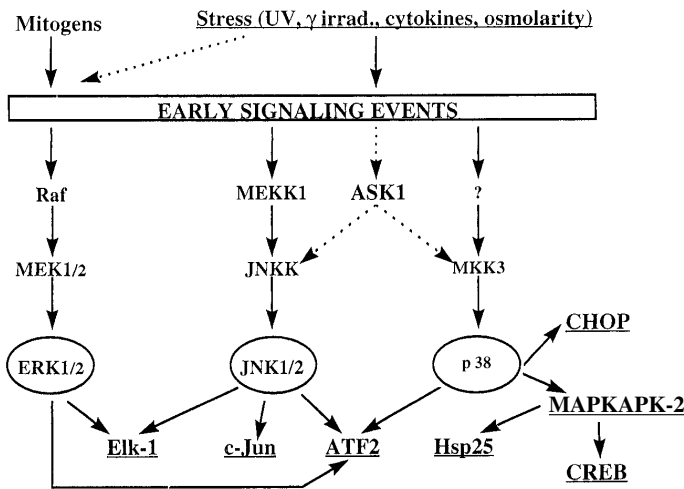


FIG. 1. Simplified scheme showing activation pathways and substrates of the three mammalian main groups of MAPK/SAPK. CHOP, C/EBP family of proteins; CREB, cyclic AMP response element binding protein; γ irradiat., γ irradiation; Hsp25, heat shock protein 25; JNKK, JNK kinase; MAPKAPK-2, MAPK activated protein kinase-2; MEK, mitogen activated protein external-signal regulated kinase; MEKK1, MEK kinase; MKK, MAPK kinase; UV, ultraviolet light.

Although increased NO production seems necessary but not sufficient for IL-1 β -induced cell death of rat β -cells, experiments with human islets indicate that cytokine-induced cell death is probably independent of NO generation (19). Therefore, in human β -cells, other mechanisms for cytokine-induced apoptosis must exist or, more likely, the relative importance of the different intracellular pathways leading to apoptosis is different from that in rat β -cells.

Even though there is *in vivo* evidence that peroxynitrite, a highly reactive oxidant species produced by the reaction of the free radical superoxide and nitrite oxide, can be detected in β -cells in acutely diabetic NOD mouse islets, and although transgenic mice overexpressing iNOS in pancreatic β -cells develop type 1 diabetes without insulinitis (44,45), it should be noted that NOD mice deficient in iNOS have normal diabetes development (24) and that iNOS inhibitors only modestly delay the onset of type 1 diabetes in animal models (46,47). Taken together, these data indicate that although NO is capable of killing pancreatic islet cells, there is still a lack of con-

vincing evidence that this is a relevant mechanism of β -cell destruction *in vivo*, and the data point to NO-independent mechanisms for β -cell apoptosis.

MITOGEN- AND STRESS-ACTIVATED PROTEIN KINASES

Mitogen- and stress-activated protein kinases (MAPK/SAPK) are a group of serine/threonine-specific kinases that are activated by dual phosphorylation on threonine and tyrosine residues in response to a variety of extracellular stimuli. MAPK/SAPK constitute a highly conserved cellular signal transduction system that is crucial for regulating cell growth, differentiation, and protective responses to extracellular stimuli, as well as cell death. There are three main groups of MAPK/SAPK (Fig. 1): 1) the extracellular signal-regulated kinases (ERKs), 2) the c-Jun NH₂-terminal kinase (JNK) or SAPK, and 3) the p38 group of protein kinases. Several isoforms of the MAPK/SAPK family have been identified (ERK-1, -2, -3, -4, -5, and -7; p38 α , β , γ , and Δ), and a total of 12 isoforms have been derived from three JNK genes (JNK-1, -2, and -3). The classic MAPK pathway involving ERK is mainly activated by growth factors and mitogens but also by many cellular stressors. The JNK and p38 pathways are mainly activated by cellular stress, including cytokines such as IL-1, IL-2, IL-7, IL-17, IL-18, and TNF- α (48,49). As shown in Fig. 1, MAPK/SAPK phosphorylate a number of substrates, many of which are common to the three classes of kinases, and many are transcription factors or precursors to transcription factors. The phosphorylation of heat shock protein 25 via the downstream kinase MAPK-activated protein 2 (MAPKAPK-2) is a specific indication of p38 activation, whereas c-Jun phosphorylation is specific for JNK activity.

MAPK/SAPK AND APOPTOSIS

As illustrated in Fig. 2, the balance between the ERK and the JNK/p38 pathways determines whether a response to an extracellular stimulus results in growth and differentiation or in stress responses and eventually apoptosis. Thus, in several cell systems, dominant and prolonged activation of JNK/p38 target cells leads to apoptosis, whereas selective activation of ERK prevents apoptosis and ensures cell survival (48). It should be emphasized, however, that the differential role of MAPK is not absolute. It is generally believed that JNK/p38 executes apoptosis via the caspase cascade. Of importance for the exploitation of MAPK/SAPK as drug targets, both nat-

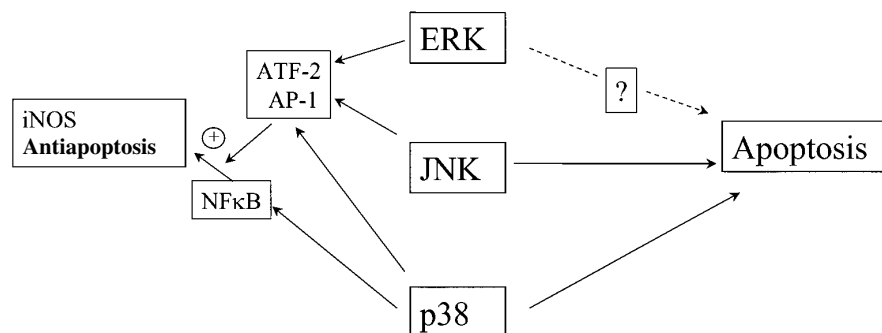


FIG. 2. Schematic representation of the importance of the balance between the ERK on one side and the JNK/p38 kinases on the other side determining whether extracellular stimulation of cells leads to apoptosis or antiapoptotic responses. For further explanation, see the text.

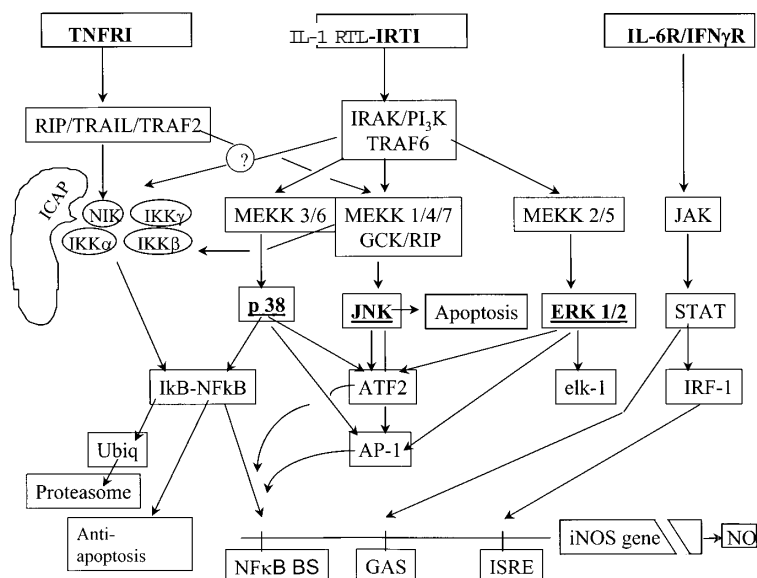


FIG. 3. Model of signaling pathways for cytokine-induced β -cell NO synthesis, apoptosis, and protective responses. AP, activating protein; GAS, interferon γ activated site; GCK, germinal center kinase; I κ B, inhibitor of NF κ B; IKK, I κ B kinase; IL-1 RTL, interleukin-1 type 1 receptor; IRAK, interleukin-1 receptor activating kinase; IRF-1, interferon regulatory factor-1; ISRE, interferon α stimulated response element; JAK, Janus kinase; NIK, nuclear factor κ B inducing kinase; PI $_3$ K, inositol triphosphate kinase; RIP, receptor-interacting protein; STAT, signal transduction and activators of transcription; TNFRI, tumor necrosis factor type 1 receptor; TRAF, TNF-receptor associated factor; TRAIL, TNF-related apoptosis inducing ligand; Ubiqu, ubiquitination.

urally occurring and pharmacological inhibitors have been discovered. Thus, heat shock protein 72, estrogen, selenite, the quinone reductase inhibitor dicumarol, and a Cephalon compound 1347 all indirectly inhibit JNK activation. Similarly, inhibitors of upstream activation of ERK (the Raf kinase inhibitor protein and the Akt protein), as well as the synthetic Parke-Davis (PD) compound, inhibit ERK activity by effects on the upstream Raf-mitogen activated protein external-signal regulated kinase (Raf-MEK) pathway. Similarly, a highly specific pharmacological inhibitor that directly binds to p38 (SmithKline Beecham [SB] compound) inhibits p38 α and β but not γ or Δ activation.

Effective transmission of signals from upstream kinases to MAPK/SAPK may be facilitated by the formation of scaffolding complexes that ensure effective interaction by the cascading components. JNK scaffolding proteins such as islet brain 1 (IB-1) (50) and JNK interacting protein-3 (JIP-3) (51) have also been identified in insulin-producing cells (50). The JNK-binding domains of the scaffolding proteins serve as specific and effective inhibitors of JNK (52). Intriguingly, the IB-1 protein was recently found to be involved in the regulation of insulin mRNA expression and to be a missense mutation located in the coding region of the protein segregated with type 2 diabetes in one family. In vitro, this mutation was associated with the ability of IB-1 to prevent apoptosis induced by activation of the JNK pathway (53).

MAPK/SAPK AND β -CELL APOPTOSIS

As mentioned above, ceramide has been implicated as an important signal of TNF-mediated apoptosis. Ceramide analogs were shown to mimic the effects of IL-1 on fetal islet insulin release and DNA synthesis (54). This study prompted Welsh (55) to investigate the downstream signaling events induced by ceramide. IL-1 induced a rapid (2–3 min) transient increase in ceramide, and a synthetic ceramide analog

induced JNK and activating transcription factor-2 (ATF-2) activity. It was suggested that IL-1-stimulated formation of ceramide might contribute to JNK-1 and ATF-2 transcription factor activation which, via NF κ B, might be necessary but not sufficient for β -cell NO synthase induction (55). Later studies have, however, questioned the role of ceramide in cytokine-induced β -cell death (56). We investigated the importance of the ERK and p38 kinases in the regulation of β -cell NO synthesis (57). We were able to demonstrate activation of the p38 kinase within 30 s of IL-1 exposure in intact rat islets and after 2.5 min of ERK, and the activation of these kinases was also found in clonal β -cells. Using the PD and SB selective inhibitors of ERK and p38 kinases, respectively, we demonstrated that, although each inhibitor individually attenuated IL-1-induced NO synthesis and iNOS messenger RNA expression, combination of the two inhibitors almost completely blocked these events. Interestingly, although osmotic shock induced similar MAPK responses, this stimulus was

Type 1	Type 2
IL-1, TNF α , IFN γ Ceramide \uparrow ?, bcl-2 \downarrow	Fatty acids, amylin, TNF α ? Ceramide \uparrow , bcl-2 \downarrow
JNK	JNK ?
Effectors (NO, caspases) APOPTOSIS	Effectors (NO, caspases?) APOPTOSIS

FIG. 4. Proposed differential stimulation of common signaling pathways and effectors leading to β -cell apoptosis in type 1 and type 2 diabetes.

unable to cause NO synthesis, demonstrating that the ERK and p38 kinases are necessary but not sufficient for islet NO synthesis. Subsequent preliminary studies have indicated that inhibition of JNK by means of the JNK binding domain (JBD) inhibitor did not affect NO synthesis or iNOS expression (M. Nikulina, unpublished data). We therefore investigated if inhibition of ERK and p38 affected cytokine-induced apoptosis in purified rat pancreatic β -cells (58). Although we confirmed that the combination of the two inhibitors together, but not individually, significantly inhibited purified primary β -cell NO synthesis and ERK/p38 enzymatic activity, there was only a minimally reducing effect of the ERK inhibitor on cytokine-induced apoptosis (58). Using the IB-1 and JBD inhibitors of JNK activity, it has been shown that JNK inhibition prevents apoptosis in clonal pancreatic β -cells (59,60). Interestingly, glucose is a signal of ERK and p38 kinase activity (61,62), and several secretagogues inhibit serine threonine protein phosphatases contributing to a prolonged MAPK signal (63). This observation may be relevant for the understanding of glucose toxicity in type 2 diabetes. Further, because the metabolic activity of β -cells regulates the sensitivity of the cells to cytotoxic cytokines, MAPKs may signal this effect, and preliminary studies confirm this notion (A. Egnös, unpublished data).

CONCLUSIONS

β -Cells may undergo apoptosis to metabolic and immunological stimuli in vitro and in animal models of type 1 and type 2 diabetes. We suggest that MAPK and SAPK are central in signaling cytokine-induced β -cell NO synthesis as well as apoptosis but that this pathway is also involved in protective responses, as depicted in Fig. 3. The ERK-1, ERK-2, and p38 MAP kinases are necessary but not sufficient for β -cell NO synthesis and iNOS expression but only play a minor role in cytokine-induced apoptosis. In contrast, the JNK pathway seems to be a major signal for cytokine-induced β -cell apoptosis. The possibility that JNK also serves as a signaling link between metabolic stimuli and apoptotic effectors in type 2 diabetes deserves further attention, as suggested in Fig. 4. The in vivo relevance of these signaling pathways remains to be established.

ACKNOWLEDGMENTS

Lisbeth Brandt-Møller is gratefully thanked for preparing the manuscript.

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