β-Cell Apoptosis and Defense Mechanisms
Lessons From Type 1 Diabetes

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Increased evidence suggests that apoptosis is the main mode of β-cell death in early type 1 diabetes. Cytokines mediate β-cell apoptosis, and in this article, we discuss some of the cytokine-modified genes that may contribute to β-cell survival or death. The gene encoding for the inducible form of nitric oxide synthase is induced by interleukin (IL)-1β or IL-1β plus γ-interferon in rodent and human islets, respectively. This leads to nitric oxide (NO) formation, which contributes to a major extent to β-cell necrosis and to a minor extent to the process of β-cell apoptosis. The main mode of cell death induced by cytokines in human β-cells is apoptosis, whereas cytokines lead to both necrosis and apoptosis in rat and mouse β-cells. It is suggested that the necrotic component in rodent islets is due to NO-induced mitochondrial impairment and consequent decreased ATP production. Human islets, possessing better antioxidant defenses, are able to preserve glucose oxidation and ATP production, and can thus complete the apoptotic program after the death signal delivered by cytokines. We propose that this death signal results from cytokine-induced parallel and/or sequential changes in the expression of multiple proapoptotic and prosurvival genes. The identity of these "gene modules" and of the transcription factors regulating them remains to be established. *Diabetes* 50 (Suppl. 1):S64–S69, 2001

Hyperglycemia in type 1 diabetes probably results from a long-term negative balance between immune-mediated β-cell damage (1) and β-cell repair/regeneration (2). Once macrophages and T-cells have been attracted to the islets and activated, they secrete soluble mediators such as cytokines, oxygen free radicals, and nitric oxide (NO), which probably contribute to β-cell dysfunction and death (3–5). Under in vitro conditions, interleukin (IL)-1β, in combination with interferon (IFN)-γ and/or tumor necrosis factor (TNF)-α, induces severe functional suppression and death by apoptosis in rodent pancreatic islet cells (3,6,7). Human islets are less sensitive to the deleterious effects of cytokines, but their exposure for several days to IL-1β plus TNF-α plus IFN-γ induces β-cell functional impairment and death by apoptosis (4,8). Interestingly, neither IL-1β nor IFN-γ alone induces apoptosis in human β-cells, whereas the combination of these cytokines directly induces β-cell death (8,9). A potential indirect effect of cytokines is upregulation of the β-cell Fas receptor, increasing the susceptibility of these cells to apoptosis mediated by Fas ligand (FasL), expressed on the surface of invading T-cells and macrophages (10–12). Recent data indicate that Fas expression and apoptosis are also present in human β-cells in the early stages of type 1 diabetes (11,13).

Apoptosis is apparently the main mode of β-cell death in NOD mice (14). In this species, β-cell apoptosis precedes massive T-cell infiltration (15), reinforcing a putative role for inflammatory mediators produced by early infiltrating cells such as macrophages and dendritic cells (3–5). In line with this possibility, expression of IL-1, IFN-γ, and the inducible form of nitric oxide synthase (iNOS) has been detected in the endocrine pancreas of prediabetic NOD mice (4,5). Moreover, membrane TNF, borne by activated $CD8^+$ cells, mediates autoimmune diabetes in the absence of perforin or Fas (16).

Apoptosis is a highly regulated form of cell death (17), affected by both extracellular inducing signals and intracellular ATP levels, phosphorylation cascades, and expression of apoptotic and antiapoptotic genes and proteins (17,18). These observations emphasize that events at the β-cell level will be decisive for their survival or death in early type 1 diabetes (19,20). Cytokine-treated β-cells modify their expression of several genes potentially involved in β-cell survival or death. Recent reviews dealt with the morphology, detection methods, and effectors of β-cell apoptosis under in vivo or in vitro conditions (14,19,20). This brief review will focus on the regulation and effects of β-cell genes potentially involved in the process of β-cell death, comparing data from rodent and human islets whenever possible. Because of the limitation of space, mostly review articles and a few key original articles are quoted.

iNOS ROLE FOR β-CELL DEATH

After the original study by Southern et al. (21; subsequent studies in the field were reviewed by Eizirik et al. [4]), describing the crucial role for NO in cytokine-induced suppression of rat islet function, there was a trend to consider this radical as a common final pathway for β-cell death. The concept of a "single-gene-that-explains-it-all" is indeed appealing, and NO was presented as the main mediator of cytokine-induced β-cell destruction in mouse, rat, and human β-cells (22), as the intermediary for IL-1β–induced Fas expression in human islet cells (11), and even as

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FACS, fluorescence-activated cell sorter; FasL, Fas ligand; FFA, free fatty acid; HO-1, heme oxygenase 1; hsp, heat shock protein; IFN, interferon; IL, interleukin; iNOS, inducible form of nitric oxide synthase; MnSOD, Mn superoxide dismutase; NO, nitric oxide; PLD-1, phospholipase D-1; RT-PCR, reverse transcriptase–polymerase chain reaction; SPI-3, serine protease inhibitor 3; TNF, tumor necrosis factor; wt, wild-type.

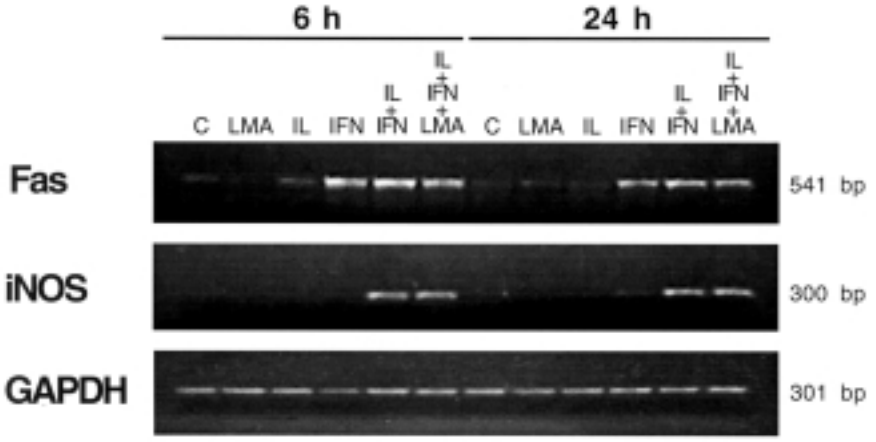

FIG. 1. RT-PCR analysis of Fas and iNOS mRNA expression by human islets exposed for 6 or 24 h to control condition (C; no cytokine or iNOS blocker added), the iNOS blocker N^G-monomethyl-L-arginine (LMA) (1 mmol/l), IL-1β (50 U/ml), and/or IFN-γ (1,000 U/ml). The cDNAs were amplified in parallel with GAPDH-specific primers, confirming similar loading in all lanes. Human islet isolation and culture and RT-PCR were performed as previously described (33). The figure is representative for four similar experiments (33).

the mediator of free fatty acid (FFA)-induced β-cell death in type 2 diabetes (23), thus providing a link between the mechanisms of β-cell death in both types of diabetes.

The first indication that the picture was somewhat more complex came from studies showing that cytokine-induced human β-cell dysfunction and death is mostly NO-independent (4). Moreover, the findings that IL-1β alone does not induce iNOS expression in human islets (24) and that FFA fails to induce iNOS expression or NO production by islets or β-cells isolated from lean rats (25,26) challenged the concept that NO is the mediator of IL-1β–induced Fas expression in human islets or FFA-induced β-cell death in rat islets.

A possible explanation for these conflicting results is the use of relatively nonspecific blockers of iNOS. Pancreatic islets express, under basal conditions, a constitutive isoform of NO synthase (nNOS), which may play a role in β-cell function (27,28). Nonspecific iNOS blockers could confound the functional data obtained by blocking both enzyme isoforms. Furthermore, some of the pharmacological agents used to inhibit the iNOS enzyme interfere directly with β-cell function (29,30). The development of mice genetically iNOS-deficient (iNOS knockout, or $iNOS^{-/-}$) (31) helped to clarify these issues. Using islets isolated from these mice, it was first shown that absence of iNOS prevents IL-1β–mediated inhibition of glucose-induced insulin release (32). Moreover, $iNOS^{-/-}$ mice were relatively resistant to diabetes induced by multiple subdiabetogenic injections of streptozotocin, an experimental model for combined toxic- and immune-mediated β-cell damage (32). The first surprise from this model came when two independent groups reported that cytokine-treated $iNOS^{-/-}$ islets express similar amounts of Fas mRNA (33) and surface Fas protein (34) compared with wild-type (wt) mice and that they are equally susceptible to cell death after surface cross-linking of Fas with soluble FasL (34). Similarly, human islets exposed to cytokines in the presence of iNOS blockers also preserve Fas mRNA expression (33) (Fig. 1). The data shown in Fig. 1 and in the study by Liu et al. (33) confirm that IL-1β plus IFN-γ, but not IL-1β or IFN-γ alone, induces iNOS expression by human islets. Moreover, they indicate that the main inducer of Fas expression by human islet cells is IFN-γ and not IL-1β, as previously suggested (11). Because IFN-γ alone does not induce iNOS expression or NO production by human islets (present data; 24,35,36), it is clear that cytokine-induced Fas expression is NO-independent.

Experiments using fluorescence-activated cell sorter (FACS)-purified β-cells from the $iNOS^{-/-}$ mice provided an additional argument against the "NO-explains-all" model for cytokine-induced β-cell death. Thus, we showed that absence of iNOS expression delayed, but did not prevent, β-cell apoptosis induced by a combination of IL-1β plus IFN-γ plus TNF-α (33). It is noteworthy that whereas combinations of cytokines induce mostly apoptosis in human β-cells (8), they lead to β-cell death by both necrosis and apoptosis in cells isolated from wt mice (7,33) or rats (9). The necrotic component in mouse β-cells was nearly completely prevented by the lack of iNOS expression (33). Moreover, in whole mouse islets, in which necrosis seems to preponderate because of a high local concentration of NO in the poorly perfused islet center (9), cytokine-induced cell death was markedly decreased in $iNOS^{-/-}$ islets compared with wt islets (28 vs. 93% dead cells in $iNOS^{-/-}$ and wt islet cells, respectively, after a 9-day exposure to IL-1β plus IFN-γ plus TNF-α) (33). These observations suggest that cytokine induction of NO predominantly results in mouse β-cell necrosis, with only a minor effect on the apoptotic pathway. This does not exclude that NO, synthesized in a different time course and/or quantity, could play a relevant role for β-cell apoptosis. Indeed, NO production is required for the induction of apoptosis in FACS-purified rat β-cells by combinations of viral products and cytokines (D. Liu, D.L.E., unpublished data).

One important question that remains to be answered is why combinations of cytokines, inducing similar NO production by murine and human islets (4), predominantly lead to cell death by apoptosis in human β-cells, while they induce both apoptosis and necrosis in mouse and rat β-cells (see above). Progression to necrotic or apoptotic cell death may depend on the cellular ATP content: if ATP levels decrease below a critical threshold following a proapoptotic insult, necrotic death ensues; if ATP levels are at least partially preserved, the apoptotic program can be completed (18). A 24- to 48-h exposure of mouse islets to IL-1β plus IFN-γ plus TNF-α decreases glucose oxidation by 70%, an effect that can be

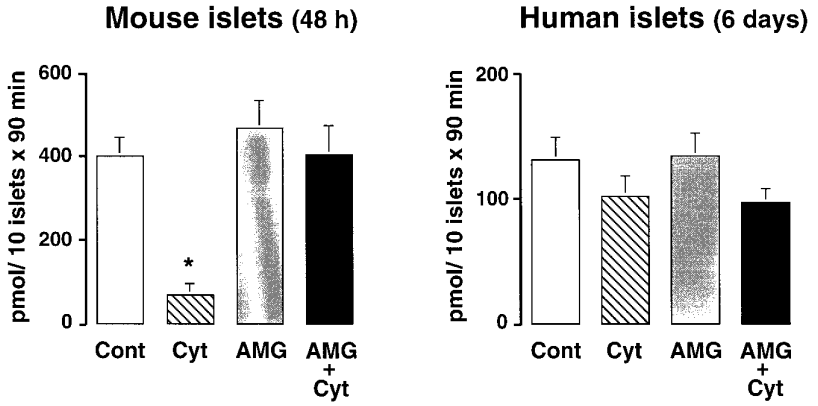

FIG. 2. Glucose oxidation by mouse or human pancreatic islets exposed for 48 h or 6 days to IL-1β (50 U/ml) plus IFN-γ (1,000 U/ml) plus TNF-α (1,000 U/ml) and/or the iNOS blocker aminoguanidine (AMG) (5 mmol/l). The data shown are derived from two previous publications by our group: Cetkovic-Cvrlje and Eizirik (37), mouse islets; Eizirik et al. (29), human islets. Cont, control; Cyt, cytokines.

prevented by the iNOS inhibitor aminoguanidine (4,37) (Fig. 2). On the other hand, exposure of human islets for a longer period (6 days) to the same cytokines induces only a minor decrease in glucose oxidation (Fig. 2), probably because human islet cells have better defense mechanisms against oxidative stress (38). We suggest that a preserved mitochondrial function in human β-cells allows them to maintain ATP production, even in the face of a prolonged "death signal" delivered by cytokines, enabling β-cells to complete the apoptotic program (Fig. 3). Rodent islet cells, exposed to the same death signal, develop an early (probably NO-mediated) mitochondrial dysfunction (4), resulting in decreased ATP production. Under these conditions, some rodent β-cells may preserve sufficient ATP to go through apoptosis, whereas others face a more severe ATP depletion and undergo necrosis. This heterogeneity in the fate of individual β-cells exposed to an immune-mediated assault is in line with the finding that IL-1β induces different degrees of functional impairment in distinct β-cell subpopulations (39). Because the main cytokine-induced death signal (Fig. 3) in human and murine β-cells is probably not NO, could it be the product of another single gene still to be discovered? As discussed in the following section, this is probably not the case.

GENE EXPRESSION PATTERNS AND THE NON–NITRIC OXIDE COMPONENT OF β-CELL APOPTOSIS

Exposure of β-cells or whole islets to cytokines leads to a complex pattern of gene response. By using the "candidate gene" approach (24) and differential display by reverse transcriptase–polymerase chain reaction (RT-PCR) (40), we and other groups have described more than 20 genes that are either up- or downregulated by IL-1β and/or IFN-γ (Fig. 4). The number of β-cell genes known to be induced or suppressed by cytokines has recently increased to >100 by the use of DNA microarrays (41). The upregulation of Fas (see above), caspase 1 (35), iNOS (see above), argininosuccinate synthetase (AS) (which supports NO synthesis through regeneration of arginine from citrulline [24]), cyclooxygenase (which may exacerbate the local inflammatory response [24]), and macrophage chemoattractant protein-1 (which may contribute to mononuclear cell homing into the islets [40]), together with the low expression of the anti-apoptotic gene *bcl-2* (38,42), can contribute to β-cell dysfunction and death. On the other hand, the induction of the free radical scavengers Mn superoxide dismutase (MnSOD) and heme oxygenase 1 (HO-1), heat shock protein (hsp) 70 (24), serine protease inhibitor 3 (SPI-3) (43), and the anti-apoptotic gene *A20* (44) are likely part of a defense/repair mechanism activated by β-cells in response to immune-mediated damage (2,24). This up- and downregulation of "damage and repair" genes is paralleled by the inhibition of genes related to differentiated β-cell functions, such as insulin (24), pancreatic and duodenal homeobox gene 1 (PDX-1) (45), GLUT2 (45), glucokinase (46), and the (pro)insulin converting enzymes prohormone convertase (PC)-1 and PC-2 (36,45). Their inhibition, together with the impaired mitochondrial ATP production, can explain the cytokine-induced β-cell dysfunction, which precedes cell death by several days (4). Changes in gene expression of Fas (see above), caspase 1 (35), iNOS (24), AS (24), MnSOD (47), PC-2 (36,45), interferon regulatory factor 1 (IRF-1) (48), SPI-3 (43), phospholipase D-1 (PLD-1) (40,49), and PDX-1 (45) are a direct result of cytokine exposure, whereas induction of other mRNAs, such as hsp 70 (33,50,51) and HO-1 (24,45), is probably secondary to cytokine-induced iNOS expression and NO production.

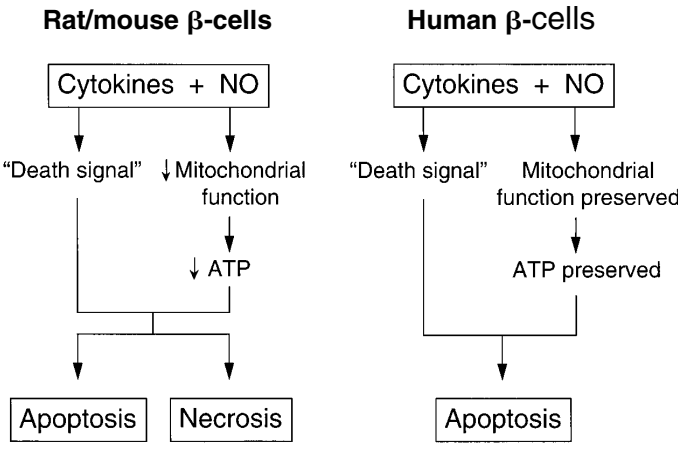

FIG. 3. Proposed model for cytokine-induced cell death in rat/mouse or human β-cells. See the text for description of the model.

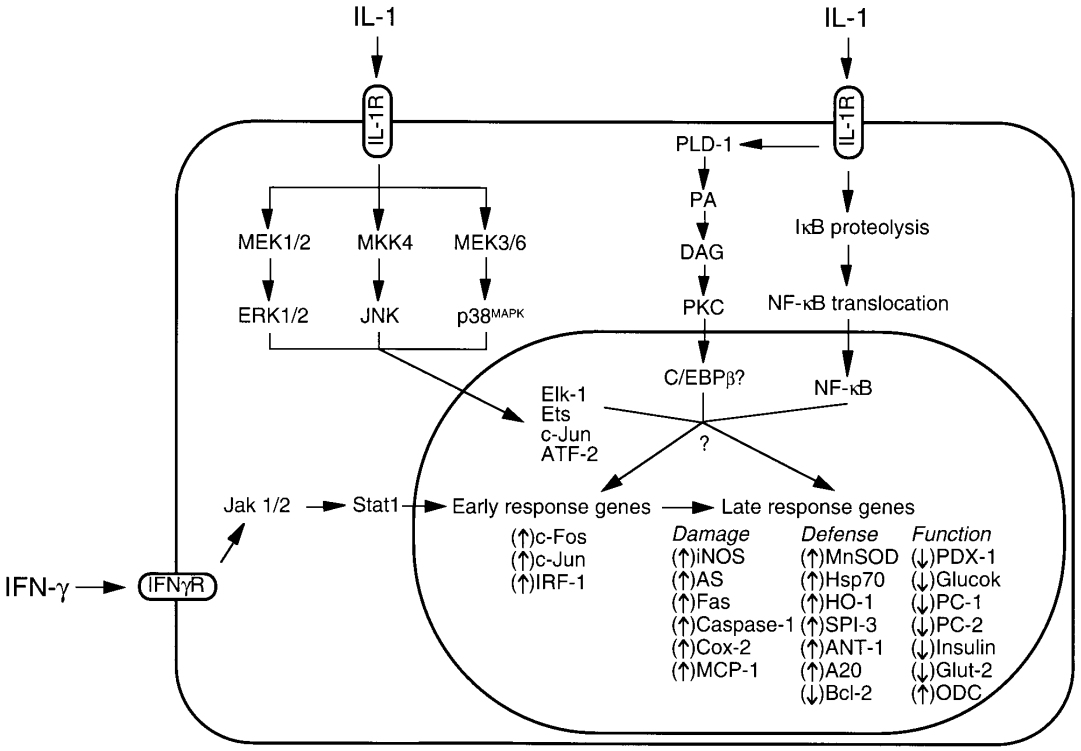

FIG. 4. IL-1β– and/or IFN-γ–induced genes in pancreatic β-cells. IL-1 binding to its receptors (R) in β-cells has been shown to act via at least three pathways: *1*) IκB degradation and NF-κB nuclear translocation; *2*) PLD-1 expression, diacylglycerol (DAG) generation, and protein kinase (PKC) activation; and *3*) activation of the mitogen-activated protein (MAP) kinases ERK1/2, JNK, and p38^{MAPK}, followed by activation of the transcription factors Elk-1, Ets, c-Jun, and ATF-2. IFN-γ binding and activation of its receptor leads to enhanced activity of the protein tyrosine kinases Jak 1 and 2 and consequent phosphorylation, dimerization, and nuclear translocation of the transcription factor Stat1. The above-described transcription factors induce or decrease the expression of early (30–60 min) and late (3–24 h) response genes, which ultimately transduce the cytokine effects on β-cells. These late response genes may participate in β-cell damage/apoptosis and defense/repair or may modify β-cell function. The figure is adapted from the study by Eizirik et al. (24). References and description of abbreviations are indicated in the text or in the studies by Eizirik et al. (24), Chen et al. (24,40), and Pavlovic et al. (55).

Interestingly, after a 24-h exposure of FACS-purified β-cells to either IL-1β alone or low doses of the NO donor GEA, expression of "defense" genes prevails and renders the β-cells more resistant to a subsequent insult by streptozotocin, alloxan, or high doses of an NO donor (45). On the other hand, prolonged exposure of β-cells to combinations of cytokines (see above) tilts the balance toward cell death. We suggest that under these conditions, the β-cell fate—either death by apoptosis or survival with or without complete functional recovery (2,20)—does not depend on one or two genes, but on the intricate pattern of dozens of genes up- or downregulated in parallel and/or sequentially. We now need to identify these "prosurvival" or "proapoptosis" patterns of gene and protein expression by DNA microarray and proteomic analysis, respectively, and next, elucidate the nature of the transcription factors regulating these "gene modules." The transcription factor NF-κB, for instance, seems to play a central role in the regulation of several cytokine-induced genes in β-cells (24,52,53), including the "NO-formation module" consisting of upregulated iNOS and AS (24) and, possibly, downregulated arginase (54). The identification of these modules and their controlling transcription factors—a formidable task—would permit interventions aimed at blocking cytokine-induced deleterious β-cell genes while preserving induction of defense/repair genes. Accordingly, inhibitors of the mitogen-activated protein kinases (MAPKs) ERK1/2 (extracellular signal regulated kinases 1 and 2) and p38^{MAPK} have been shown to prevent IL-1β–induced iNOS, but not MnSOD, expression in rat β-cells (55).

There is a growing recognition in the biomedical research community that reductionist biologic experiments do not provide an accurate picture of complex biologic phenomena and that biology seldom produces simple questions (56). The process leading to β-cell apoptosis in type 1 diabetes is a complex biologic phenomenon, and only interrogation of the entire system by DNA microarrays and proteomics technology will allow us to fully understand it. It is our hope that a better understanding of this process will open the door for the prevention of β-cell destruction in the early stages of type 1 diabetes.

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