Mechanisms of Pancreatic β-Cell Death in Type 1 and Type 2 Diabetes

Many Differences, Few Similarities

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Type 1 and type 2 diabetes are characterized by progressive β-cell failure. Apoptosis is probably the main form of β-cell death in both forms of the disease. It has been suggested that the mechanisms leading to nutrient- and cytokine-induced β-cell death in type 2 and type 1 diabetes, respectively, share the activation of a final common pathway involving interleukin (IL)-1β, nuclear factor (NF)-κB, and Fas. We review herein the similarities and differences between the mechanisms of β-cell death in type 1 and type 2 diabetes. In the insulitis lesion in type 1 diabetes, invading immune cells produce cytokines, such as IL-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ. IL-1β and/or TNF-α plus IFN-γ induce β-cell apoptosis via the activation of β-cell gene networks under the control of the transcription factors NF-κB and STAT-1. NF-κB activation leads to production of nitric oxide (NO) and chemokines and depletion of endoplasmic reticulum (ER) calcium. The execution of β-cell death occurs through activation of mitogen-activated protein kinases, via triggering of ER stress and by the release of mitochondrial death signals. Chronic exposure to elevated levels of glucose and free fatty acids (FFAs) causes β-cell dysfunction and may induce β-cell apoptosis in type 2 diabetes. Exposure to high glucose has dual effects, triggering initially "glucose hypersensitization" and later apoptosis, via different mechanisms. High glucose, however, does not induce or activate IL-1β, NF-κB, or inducible nitric oxide synthase in rat or human β-cells in vitro or in vivo in *Psammomys obesus*. FFAs may cause β-cell apoptosis via ER stress, which is NF-κB and NO independent. Thus, cytokines and nutrients trigger β-cell death by fundamentally different mechanisms, namely an NF-κB–dependent mechanism that culminates in caspase-3 activation for cytokines and an NF-κB–independent mechanism for nutrients. This argues against a unifying hypothesis for the mechanisms of β-cell death in type 1 and type 2 diabetes and suggests that different approaches will be required to prevent β-cell death in type 1 and type 2 diabetes. *Diabetes* 54 (Suppl. 2): S97–S107, 2005

Clinical definitions of disease often obscure different mechanistic subtypes. This is particularly relevant for complex diseases such as diabetes, where combinations of multiple genes and environmental factors eventually lead to loss of functional β-cell mass and hyperglycemia. The mechanisms leading to β-cell loss may be quite diverse in the various subtypes of the disease. As our knowledge of disease pathogeneses increases, better classifications of diabetes may be proposed.

The two main forms of diabetes are type 1 and type 2 diabetes (1). Both types are characterized by progressive β-cell failure. In type 1 diabetes, this is typically caused by an autoimmune assault against the β-cells, inducing progressive β-cell death. The pathogenesis of type 2 diabetes is more variable, comprising different degrees of β-cell failure relative to varying degrees of insulin resistance.

The genetics (i.e., HLA-related in type 1 diabetes vs. non–HLA-related in type 2 diabetes), putative environmental triggers (for instance viral infection in type 1 diabetes, obesity in type 2 diabetes), and natural history of the disease are different between type 1 and type 2 diabetes. Because these topics are covered in detail in other articles in this supplement issue, they will not be discussed further here.

In type 1 diabetes, β-cell mass is reduced by 70–80% at the time of diagnosis. Because of the variable degrees of insulitis and absence of detectable β-cell necrosis, it was suggested that β-cell loss occurs slowly over years (2). These pathology findings are in line with the progressive decline in first-phase insulin secretion in antibody-positive individuals, long before the development of overt diabetes (3). It was later shown that β-cell apoptosis causes a gradual β-cell depletion in rodent models of type 1 diabetes (rev. in 4). In type 2 diabetic subjects, initial pathological studies suggested a β-cell loss of 25–50% (2,5), but this was debated by others (6). Recent studies, which matched diabetic patients and control subjects for BMI, showed a significant reduction in β-cell mass (7,8) and a threefold increase in β-cell apoptosis (8). These observations suggest that β-cell mass is decreased in type 2 diabetes, secondary to increased rates of β-cell apoptosis, but it

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ATF, activating transcription factor; CHOP, C/EBP (CCAAT/enhancer binding protein) homologous protein; ER, endoplasmic reticulum; ERK, extracellular signal–regulated kinase; FACS, fluorescence-activated cell sorting; FFA, free fatty acid; GIIS, glucose-induced insulin secretion; IκB, inhibitory κB; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; NF, nuclear factor; SOCS, suppressor of cytokine signaling; TNF, tumor necrosis factor.

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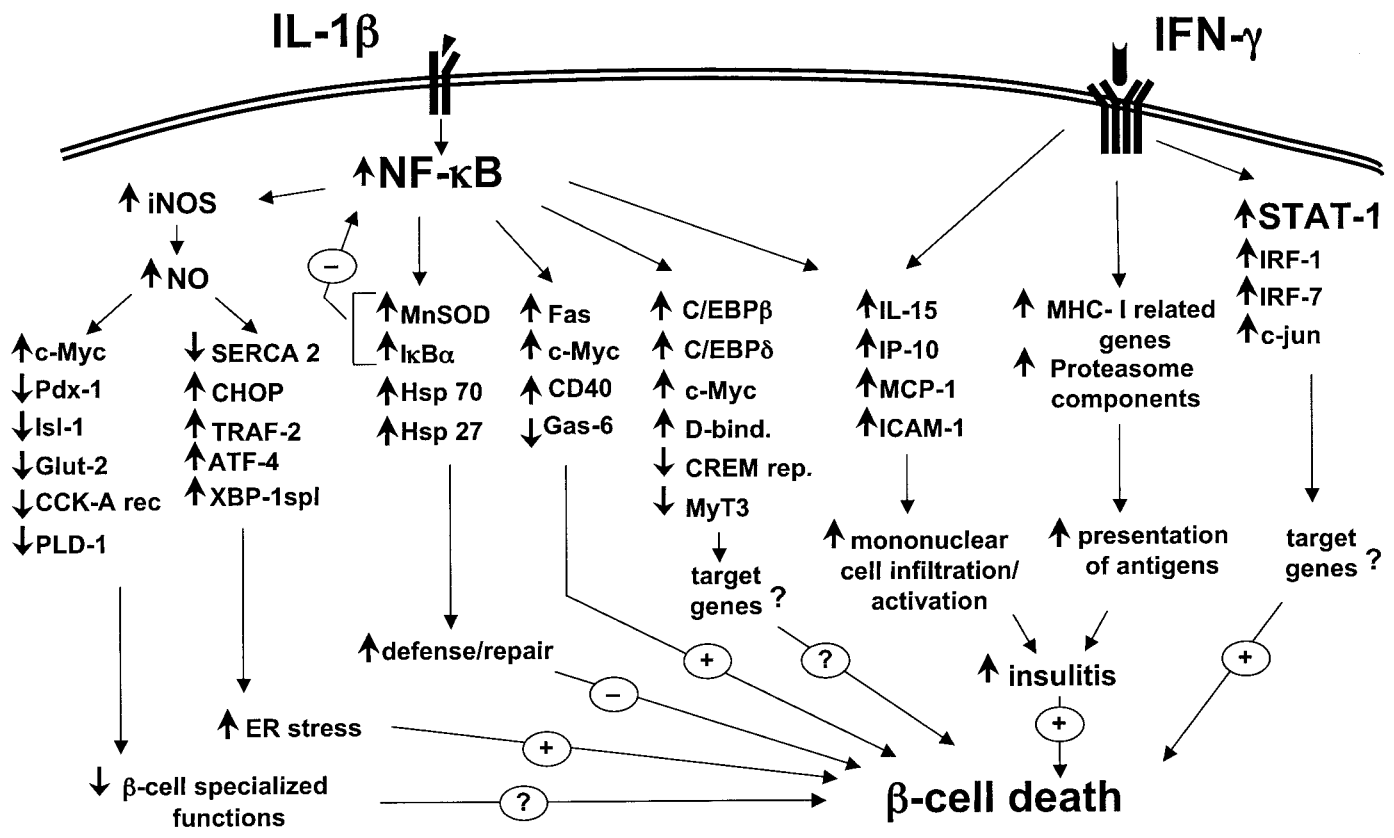

FIG. 1. The transcription factor and gene networks putatively involved in the cytokine-promoted β-cell "decision" to undergo apoptosis. The transcription factors NF-κB and STAT-1 are the main regulators of the pathways triggered by IL-1β and IFN-γ, respectively. The figure is based on Refs. 14–18. MHC-1, major histocompatibility complex 1.

remains unclear whether this explains the observed functional loss (9).

β-Cell apoptosis may thus be a common feature of type 1 and type 2 diabetes. Recent studies (rev. in 10 and 11) suggest that both forms of diabetes are characterized by intra-islet expression of inflammatory mediators (especially the cytokine interleukin [IL]-1β), triggering a final common pathway of β-cell apoptosis, progressive β-cell loss, and diabetes. Based on this hypothesis, a unifying classification of diabetes has been proposed (10). Against this background, we review herein the experimental evidence on the similarities and differences between the mechanisms of β-cell death in type 1 and type 2 diabetes.

MECHANISMS OF β-CELL DEATH IN TYPE 1 DIABETES

Pancreatic β-cells are the target of an autoimmune assault in type 1 diabetes, with invasion of the islets by mononuclear cells in an inflammatory reaction termed "insulitis," leading to loss of most β-cells after prolonged periods of disease (2). β-Cell death in the course of insulitis is probably caused by direct contact with activated macrophages and T-cells, and/or exposure to soluble mediators secreted by these cells, including cytokines, nitric oxide (NO), and oxygen free radicals (4). In vitro exposure of β-cells to IL-1β, or to IL-1β + interferon (IFN)-γ, causes functional changes similar to those observed in pre-diabetic patients, namely elevated proinsulin/insulin levels (12) and a preferential loss of first-phase insulin secretion in response to glucose, caused by an IL-1β–mediated decrease in the docking and fusion of insulin granules to the β-cell membrane (13). After prolonged exposure to IL-1β + IFN-γ and/or tumor necrosis factor (TNF)-α, but not to either cytokine alone, this functional impairment evolves to β-cell death (4).

Apoptosis, the main cause of β-cell death at the onset of type 1 diabetes, is a highly regulated process, activated and/or modified by extracellular signals, intracellular ATP levels, phosphorylation cascades, and expression of pro- and anti-apoptotic genes (4). Cytokines induce stress response genes that are either protective or deleterious for β-cell survival. In extensive microarray experiments (14–17), we have identified ~700 genes and expressed sequence tags that are up- or downregulated in purified rat β-cells or insulin-producing cells after 1–24 h of exposure to IL-1β and/or IFN-γ. The main findings of these studies are summarized in Fig. 1. A detailed review of the gene networks activated by cytokines in β-cells, and on the role of chemokines produced by β-cells in the build up of insulitis, is provided by Eizirik et al. (18), while the complete list of β-cell–expressed genes, as detected by our microarray analyses, is accessible at the Beta Cell Gene Expression Bank (http://t1dbase.org/cgi-bin/enter_bcgb.cgi) (19). IL-1β activates the transcription factor nuclear factor (NF)-κB (Fig. 1) in rodent and human islet cells (4), and prevention of NF-κB activation by an inhibitory κB (IκB) "super-repressor" (20,21) protects pancreatic β-cells against cytokine-induced apoptosis. In an additional microarray analysis, we studied IL-1β–treated β-cells whose NF-κB activation was blocked by adenovirus-mediated expression of the $I\kappa B^{(SA)2}$ super-repressor (16). A total of 66 cytokine-responsive NF-κB–dependent genes were identified, including genes coding for cyto-

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kines and chemokines and stress-related genes such as GADD153/CHOP [C/EBP (CCAAT/enhancer binding protein) homologous protein] (a mediator of endoplasmic reticulum [ER] stress-induced cell death; see below) and c-myc. NF-κB was also found to downregulate (probably indirectly, via NO production) the expression of other transcription factors responsible for β-cell differentiation and function (e.g., PDX-1 and Isl-1). NF-κB regulates expression of inducible nitric oxide synthase (iNOS) in β-cells (22), and ~50% of the β-cell genes modified after 12 h of cytokine exposure are secondary to iNOS-mediated NO formation (15). Of note, it has been recently described that transgenic expression of an NF-κB inhibitor under the control of the pdx-1 promoter (blocking NF-κB during β-cell development) causes defective GLUT2 expression and glucose-induced insulin secretion (GIIS) later in mouse life, suggesting that basal NF-κB is required for normal insulin release (23). In our hands, however, blocking basal NF-κB activity for 48–72 h in adult rat β-cells affected neither GLUT2 expression nor GIIS (16,21; A.K. Cardozo, D.L.E., unpublished data), suggesting that the putative physiological role of basal NF-κB activity is more relevant during fetal β-cell development than during adult life or is only detectable after prolonged NF-κB inhibition. In summary, IL-1β–induced NF-κB activation plays a crucial role in controlling multiple and distinct gene regulatory networks, which affect the β-cell–differentiated state and ER Ca^{2+} homeostasis, attract and activate immune cells, and directly contribute to β-cell apoptosis.

Exposure of purified human or rodent β-cells to IL-1β alone is not sufficient to induce apoptosis, but when IL-1β is combined with IFN-γ, ~50% of these cells undergo apoptosis after 6–9 days (4). This suggests that IFN-γ signal transduction must synergize with IL-1β signaling to trigger β-cell apoptosis (Fig. 1). IFN-γ binds to cell surface receptors and activates the tyrosine kinases JAK1 and JAK2. These kinases phosphorylate the transcription factor STAT-1, which dimerizes and translocates to the nucleus to bind to γ-activated sites of diverse genes (4). STAT-1 mediates the potentiating effect of IFN-γ on IL-1β–induced iNOS expression (22), and our recent observations show that fluorescence-activated cell sorting (FACS)-purified β-cells from STAT-1–deficient mice ($STAT\text{-}1^{-/-}$) are protected against IL-1β + IFN-γ–induced apoptosis (C. Gysemans, L. Ladriere, H. Callewaert, J. Rasschaert, D. Flamez, D.E. Levy, D.L.E., C. Mathieu, unpublished data). Because excessive activation of JAK/STAT signaling may lead to cell death, STAT transcriptional activity is regulated by multiple negative feedback mechanisms. These include dephosphorylation of JAK and cytokine receptors by SHP, and inhibition of JAK enzymatic activities by the suppressor of cytokine signaling (SOCS) family. Upregulation of SOCS-1 or SOCS-3 protects β-cells in vitro and in vivo against cytokine-induced death (24,25). SOCS-3 also protects insulin-producing cells against IL-1β–mediated apoptosis via NF-κB inhibition (26). The results summarized in Fig. 1 indicate that β-cell fate after cytokine exposure depends on the duration and severity of perturbation of key β-cell gene networks. The precise identity and regulation of these gene networks remain to be elucidated, but the available data suggest an important role for NF-κB and STAT-1.

It is of interest to understand how the cytokine-activated gene expression patterns described in Fig. 1 actually result in β-cell death. Some of the probable mechanisms are outlined in Fig. 2. They include the following: *1*) activation of the stress-activated protein kinases c-Jun NH_2-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and extracellular signal-regulated kinase (ERK); *2*) triggering of ER stress; and *3*) the release of death signals from the mitochondria.

JNK is a member of the MAPK family. Pancreatic β-cells exposed to IL-1β have an early and sustained increase in JNK activity, a phenomenon potentiated by IFN-γ or TNF-α (4,11). Cell-permeable peptide inhibitors of JNK prevent cytokine-induced apoptosis in insulin-producing cells (27), but this remains to be confirmed in primary β-cells. p38 MAPK and ERK are also activated by cytokines, and pharmacological inhibition of these MAPKs diminished cytokine-induced rat islet cell death (28,29), possibly by attenuating transcriptional activation of iNOS (28). However, when purified β-cells were exposed to IL-1β + IFN-γ for 6–9 days, ERK, but not p38, inhibitors provided partial protection against apoptosis (30), suggesting that some of the protection by MAPK inhibitors in whole islets is mediated via effects on other islet cells (such as resident macrophages). p38 may also increase the apoptotic propensity of the β-cell, since genetic downregulation of p38α results in a lowered sensitivity to cell death induced by the NO donor DETA/NONOate (N. Makeeva, J. Myers, N.W., unpublished data). In addition, the tumor suppressor p53 is activated in response to cytokine-induced NO production (31). It is conceivable that stabilization of the pro-apoptotic protein p53 lies downstream of the NO-induced activation of MAPKs.

Disruption of ER homeostasis, as induced by changes in ER Ca^{2+} concentrations, triggers accumulation of unfolded proteins and activation of a specific stress response, known as the ER stress response (32). This cellular response is a coordinated attempt to restore ER homeostasis and function, and it includes translational attenuation, upregulation of ER chaperones, and degradation of misfolded proteins. In case of prolonged and severe ER stress, the apoptosis program is activated and executed by the transcription factor CHOP, the MAPK JNK, and caspase-12 (although it remains unclear whether caspase-12 has a role in human cells) (32). Because of their high rate of protein synthesis, β-cells are particularly susceptible to ER stress (33), and NO donors trigger an ER stress response in β-cells leading to CHOP expression and apoptosis (34). We have recently shown that IL-1β + IFN-γ inhibit SERCA2b expression, via NF-κB activation and NO production, and deplete ER Ca^{2+} stores. This is followed by activation of diverse components of the ER stress response, including activation of IRE-1α and PERK/activating transcription factor (ATF)-4, xbp1 mRNA processing, and induction of CHOP (35). Different from the β-cell response to chemical SERCA2b inhibitors or free fatty acids (FFAs) (36), cytokines neither activate ATF-6 nor induce the ER chaperone BiP (35). This defective ATF-6 activation may deprive β-cells from a crucial defense against ER stress, contributing to their exquisite vulnerability to cytokines.

Mitochondria are key organelles for β-cell function and survival (37). Paradoxically, mitochondria also play an important role in triggering apoptosis (38). Members of the Bcl-2 protein family regulate the mitochondrial response to pro-apoptotic signals (38), preventing release of mitochondrial proteins such as cytochrome c, which, when liberated to the cytosol, sequentially activate caspase-9 and -3 and execute cell death (39). Cytokines disrupt the

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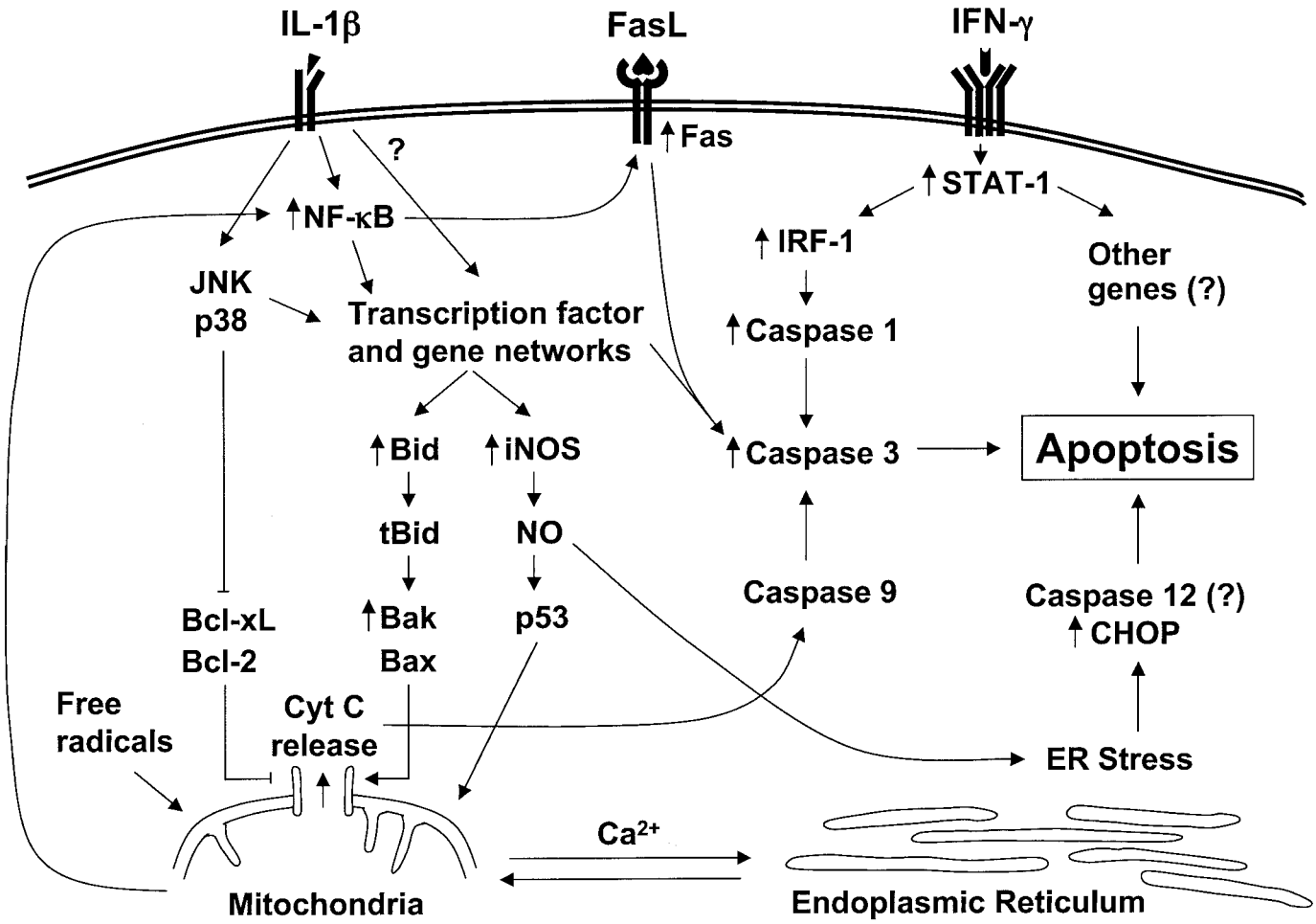

FIG. 2. Proposed model for the different pathways contributing to the execution of cytokine-induced β-cell apoptosis. Arrows indicate genes for which expression was modified by cytokines in a time course microarray analysis (15). β-Cell apoptosis is probably mediated by three main pathways—namely JNK, ER stress, and liberation of pro-apoptotic proteins from the mitochondria.

mitochondrial membrane potential in RINm5F cells, which is prevented by overexpression of the anti-apoptotic protein Bcl-2 (40). Overexpression of Bcl-2 partially protects mouse (41) and human (42) islets against cytokine-induced cell death, but does not prevent adenovirus-induced islet cell death (43) or spontaneous diabetes in nonobese diabetic (NOD) mice (44). This suggests that other mechanisms, bypassing Bcl-2, induce β-cell death in vivo and/or that Bcl-2–regulated mitochondrial events and caspase activation are late steps in the apoptosis process, occurring when the cell fate has already been decided. In line with the second possibility, blocking caspase-1 (induced in β-cells by cytokines [45]) decreases β-cell apoptosis after 4 days of exposure to IL-1β + IFN-γ, but it does not prevent their subsequent death by necrosis after 9 days (D. Liu, D.L.E., unpublished data). Other pro-apoptotic genes that are induced by cytokines, as detected by microarray analysis (15), are indicated in Fig. 2 and include Bid, Bak, and caspase-3. An intriguing possibility is that an early cytokine-induced "dialogue" between the nucleus, mitochondria, and ER influences the decision of the β-cell to undergo apoptosis or not. In favor of this hypothesis, overexpression of free radical scavenging enzymes in mitochondria, but not in the cytosol, prevents IL-1β–induced NF-κB activation (46).

MECHANISMS OF β-CELL DEATH IN TYPE 2 DIABETES

Insulin resistance, often associated with obesity, and insulin secretion defects are major risk factors for type 2 diabetes (9). A progressive decrease of β-cell function leads to glucose intolerance, which is followed by type 2 diabetes that inexorably aggravates with time (47). The alterations of GIIS in human type 2 diabetes may theoretically result from changes in β-cell function, β-cell mass, or both. A decrease in β-cell mass is likely to play a role in the pathogenesis of human type 2 diabetes (8,9) as it does in rodent models of the disease (48,49). However, in contrast with type 1 diabetes, the 25–50% reduction in β-cell mass measured postmortem in type 2 diabetic patients may not be important enough to account for the observed loss of GIIS. Because β-cell mass cannot be measured in vivo, it remains unclear whether type 2 diabetic patients had a lower β-cell mass early in life, failed to increase their β-cell mass in the face of insulin resistance, or had a progressive β-cell loss. The question whether the reduction in human β-cell mass results from increased β-cell apoptosis, reduced cell neogenesis/replication, or both also remains unsettled (49). Based on results obtained in rodent models of the disease and in cultured rodent and human islet cells, it seems reasonable to assume that dyslipidemia and hyperglycemia negatively affect β-cell mass by increasing β-cell apoptosis in human

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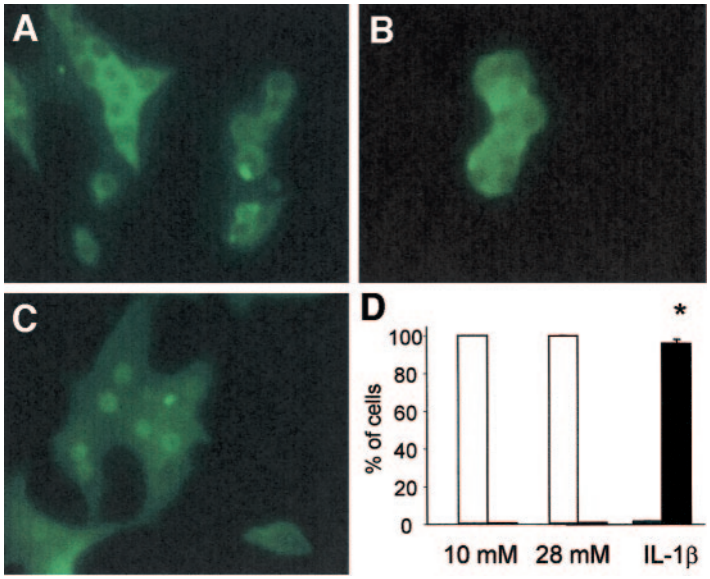

FIG. 3. High glucose (28 mmol/l) does not induce NF-κB activation and DNA binding in β-cells, as assessed by immunofluorescence using an antibody directed against the p65 NF-κB subunit. FACS-purified rat β-cells were exposed to 10 mmol/l (control, *A*) or 28 mmol/l glucose (*B*) for 12–24 h. The data at 12 h are shown here; similar observations were made at 24 h (not shown). As a positive control, cells were exposed to IL-1β (30 units/ml, in medium containing 10 mmol/l glucose [*C*]) during the last 30 min of culture. NF-κB is located in the cytosol at 10 mmol/l (*A*) or 28 mmol/l (*B*) glucose, whereas it translocates to the nucleus after exposure to IL-1β, indicating activation (*C*). Subcellular NF-κB localization was counted in 200–400 cells using the same experimental conditions as above (*D*). □, Cytoplasmic NF-κB localization; ■, nuclear localization. The results are means ± SE of three independent experiments. **P* < 0.001 vs. percent nuclear staining in the control by two-sided paired *t* test (*D*). Original magnification ×200.

type 2 diabetes (10,48). In the following paragraphs, we discuss recent hypotheses on the mechanisms of glucotoxicity and lipotoxicity. Readers are directed to another recent review (49) for information on other potential agents causing β-cell dysfunction/death in type 2 diabetes.

Glucotoxicity. Moderate or severe hyperglycemia cannot be the primum movens in the pathophysiology of type 2 diabetes, but it contributes to the reduction of GIIS (50). As such, it could contribute to the progression from glucose intolerance to overt type 2 diabetes (47). The mechanisms by which hyperglycemia negatively affects functional β-cell mass are still debated. Rodent β-cells chronically exposed to high glucose display several alterations of their phenotype, including changes in glucose stimulus-secretion coupling, gene expression, cell survival, and cell growth (48,49). These alterations could result from cytokine-, oxidative stress–, or ER stress–induced changes in gene expression and cell survival (10,32,51) or from functional changes that are not directly related to β-cell apoptosis, such as accumulation of glycogen (52).

Rodent β-cells display reduced expression of genes involved in GIIS in in vivo and in vitro models of prolonged exposure to high glucose. These include insulin, GLUT2, glucokinase and voltage-dependent Ca^{2+} channels, and the transcription factors that regulate their expression (53). These changes, which may play a role in the alterations of GIIS in rodent type 2 diabetes, have some similarities with those induced by cytokines (17,18). On the other hand, several genes expressed at low levels in normal β-cells are induced by hyperglycemia, including hexokinase 1, lactate dehydrogenase and glucose-6 phosphatase. In addition, pro- and anti-apoptotic factors, antioxidant enzymes, and some transcription factors are upregulated (53). Some of these genes, such as c-Myc, A20, and heme-oxygenase 1, are induced by hyperglycemia and cytokines, suggesting that both conditions share some common mechanisms to alter the β-cell phenotype. The suggestion that hyperglycemia increases β-cell production of IL-1β in human islets provides such a unified hypothesis for β-cell pathophysiology in type 1 and type 2 diabetes (10). However, the pattern of hyperglycemia-induced β-cell genes is only partly similar to that induced by cytokines (17,18,53). For instance, iNOS and IκBα, two NF-κB–dependent genes markedly induced by IL-1β, are not induced in rodent β-cells exposed to high glucose. Other genes, such as lactate dehydrogenase A, the mitochondrial uncoupling protein UCP-2, and the transcription factor CREM, are induced by hyperglycemia (53,54) and downregulated by cytokines (17,55). Furthermore, hyperglycemia induces β-cell hypertrophy, whereas cytokine treatment does not, and the induction of β-cell apoptosis by high glucose is much lower than that produced by cytokines. These differences raise questions about the role of IL-1β–induced NF-κB activation in β-cell glucotoxicity. These doubts are strengthened by our observations that, under various culture conditions, exposure of rat or human islets or FACS-purified rat β-cells to high glucose does not increase IL-1β mRNA expression or NF-κB DNA-binding activity (56; Fig. 3; see also below).

It is generally assumed that oxidative stress activates NF-κB activity in β-cells as in other cell types (53). This does not seem to be the case, since acute (57) or overnight exposure to low concentrations of hydrogen peroxide does not increase rat islet NF-κB activity and iNOS expression (56). Islet c-Myc and heme-oxygenase 1 expression are similarly induced by hydrogen peroxide and high glucose, and these effects are abrogated by the free radical scavenger *N*-acetyl-L-cysteine. This suggests that β-cell glucotoxicity may, at least in part, result from an increase in β-cell oxidative stress and subsequent JNK activation that is NF-κB independent (51,56). The main source of reactive oxygen species in the β-cell is probably the mitochondrial electron transport chain (58,59). It is therefore possible that chronic stimulation of insulin secretion in states of insulin resistance induces oxidative stress. Other possible explanations for changes in β-cell function and viability before overt hyperglycemia include activation of the ER stress pathway (also in the context of lipotoxicity; see below) and sustained elevation of cytosolic Ca^{2+} concentration (60).

It is well established that chronic hyperglycemia leads to β-cell degranulation and reduction in GIIS (48), but the effect of hyperglycemia on the β-cell sensitivity to glucose is controversial. A first group of studies indicates that the absence of a glucose-induced rise in ATP production, perhaps due to hyperglycemia-induced expression of uncoupling protein 2, is responsible for defective GIIS (61).

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These observations, conceptualized as β-cell "glucose desensitization," seem in contradiction with other studies showing that β-cells exposed to hyperglycemia become more sensitive to glucose for the stimulation of mitochondrial metabolism, proinsulin biosynthesis, and insulin secretion. This leads to maximal stimulation of triggering and amplifying pathways of GIIS at low glucose (a concept we refer to as "glucose hypersensitization") (62–64). This glucose hypersensitization, which results from higher ATP production at low glucose concentrations, may be due to the accumulation of glycogen in β-cells (52,64). Glucose hypersensitization was observed together with a paradoxical dissociation between glucose-induced Ca^{2+} influx and insulin secretion on one hand and a sustained elevation of cytosolic Ca^{2+} unaffected by glucose on the other, and it is associated with a strong reduction of GIIS between 5 and 10 mmol/l glucose. This concept of β-cell glucose hypersensitivity fits with the presence of fasting hyperinsulinemia in type 2 diabetic patients and the observation (based on autopsy material) that their β-cells are actively engaged in proinsulin synthesis (65).

Although both β-cell "glucose desensitization" and "glucose hypersensitization" may explain loss of GIIS at physiological glucose concentrations, these two hypotheses have different implications for the role of apoptosis in β-cell glucotoxicity. Thus, β-cell glucose desensitization is compatible with the concept that β-cell dysfunction (partly) results from β-cell apoptosis (10,51,61). In contrast, β-cell glucose hypersensitization may result from changes in the expression of glycolytic enzymes (decreased glucokinase and increased hexokinase 1 and lactate dehydrogenase expression), from the accumulation of glycogen at high glucose and its subsequent degradation at low glucose, or from other functional alterations of β-cells (48,62,64), but not from apoptosis. We have recently observed that overnight exposure of rat islets to low concentrations of hydrogen peroxide induces glucose desensitization and β-cell apoptosis that are both prevented by *N*-acetyl-L-cysteine. In contrast, a 1-week culture at 30 mmol/l glucose, compared with 10 mmol/l, induces a state of glucose hypersensitization and a modest increase in β-cell apoptosis that are both unaffected by *N*-acetyl-L-cysteine (66). These results suggest that the various facets of β-cell glucotoxicity may result from different pathophysiological mechanisms. Thus, after prolonged exposure to hyperglycemia, part of the surviving β-cells may still be "glucose hypersensitive" while apoptosis is already affecting a small proportion of these cells. These different pathophysiological mechanisms are compatible with the observations that *1*) after 3 weeks of diet-induced diabetes in the gerbil *Psammomys obesus*, a stage at which the β-cell mass is decreased (67), isolated islets were still glucose hypersensitive (62); and *2*) human islets transplanted under the kidney capsule of hyperglycemic nude mice and maintained in vivo for 4 weeks have severely impaired GIIS, which can be dissociated from impaired glucose oxidation or protein synthesis and, under some conditions, from depleted insulin content or cell death (68,69).

Lipotoxicity. Physical inactivity, energy-dense diets rich in saturated fat, and central obesity predispose individuals to type 2 diabetes. Prospective studies in subjects at risk for diabetes have shown that the development of abdominal obesity is correlated with loss of β-cell function and hence glucose intolerance (70; M.C., J. Vidal, R.L. Hull, K.M. Utzschneider, D.B. Carr, E.J. Boyko, W. Fujimoto, S.E. Kahn, unpublished data). Autopsy data suggest that the progressive decline in insulin secretion in type 2 diabetes is accompanied by a decrease in β-cell mass and that this is secondary to increased β-cell apoptosis. Thus, it is conceivable that circulating adipose tissue–derived products, such as FFAs and adipokines, play a direct role in pancreatic β-cell dysfunction and death. A high plasma concentration of FFAs is indeed a risk factor for the development of type 2 diabetes, independently of its effects on insulin sensitivity (71).

Circulating FFAs are solubilized and transported in millimolar concentrations, by virtue of their tight binding to albumin. Unbound FFA levels measure in the nanomolar range (5–20 nmol/l), a concentration at which they are rapidly taken up via a protein-mediated transport. FFAs acutely stimulate insulin secretion, but prolonged β-cell exposure to high FFA levels reduces GIIS in vitro (72) and in vivo, especially in individuals genetically predisposed to type 2 diabetes (73). Studies in the ZDF rat indicate that high circulating FFAs and triglyceride levels induce triglyceride accumulation in pancreatic islets (74). The associated rise in cytoplasmic FFA levels would increase ceramide formation and induce iNOS, resulting in NO-mediated β-cell apoptosis (75).

In our in vitro experiments (36,76,77), we used physiological concentrations of palmitate and oleate. FFAs are toxic to FACS-purified rat β-cells (36,76) and insulin-producing INS-1E cells (36). Cytotoxicity depends on the unbound FFA concentration and is greater for palmitate than oleate. FFA-induced cell damage results in apoptosis and, to a lesser extent, necrosis in β-cells (76) and mostly in apoptosis in INS-1E cells (36). The toxic effects of FFAs are potentiated when β-cells are concomitantly exposed to high glucose levels (78,79). FFA cytotoxicity does not depend on mitochondrial FFA oxidation, because etomoxir, an inhibitor of carnitine palmitoyltransferase I, did not alter FFA-induced β-cell death, and bromopalmitate, a nonmetabolizable analog, was as toxic as palmitate (76). FFA-induced cell death occurred in the absence of iNOS expression or NO production (36,76), and it was not counteracted by antioxidant or free radical scavenging compounds (76), suggesting that oxidative stress is not its main mediator. Moreover, oleate or palmitate did not activate NF-κB in INS-1E or β-cells (36), at low (6.1 mmol/l), medium (10 mmol/l), or high (28 mmol/l) glucose levels (36; I. Kharroubi, D.L.E., M.C., unpublished data). It was suggested that FFA cytotoxicity could be counteracted by the peroxisome proliferator–activated receptor-γ agonist troglitazone through lowering islet triglyceride content (80). In our hands, however, troglitazone did not improve survival of FFA-exposed β-cells, but rather sensitized them to necrosis and apoptosis at low FFA concentrations (77). Furthermore, FFA-induced cytoplasmic triglyceride accumulation was inversely correlated to β-cell death (76). A mixture of oleate and palmitate caused the lowest cell death and the highest triglyceride accumulation, whereas bromopalmitate, which did not increase cellular triglycerides, exerted the highest toxicity (76). These findings suggest that storage of excess FFAs as triglycerides protects the cell against accumulation of potentially deleterious fatty acyl-CoA.

FFA-induced β-cell toxicity might also occur at the ER level, where FFA esterification takes place. Using electron microscopy, we observed that the ER of FFA-exposed β-cells is dilated (M.C., unpublished data), and we therefore examined whether FFAs induce ER stress. Both

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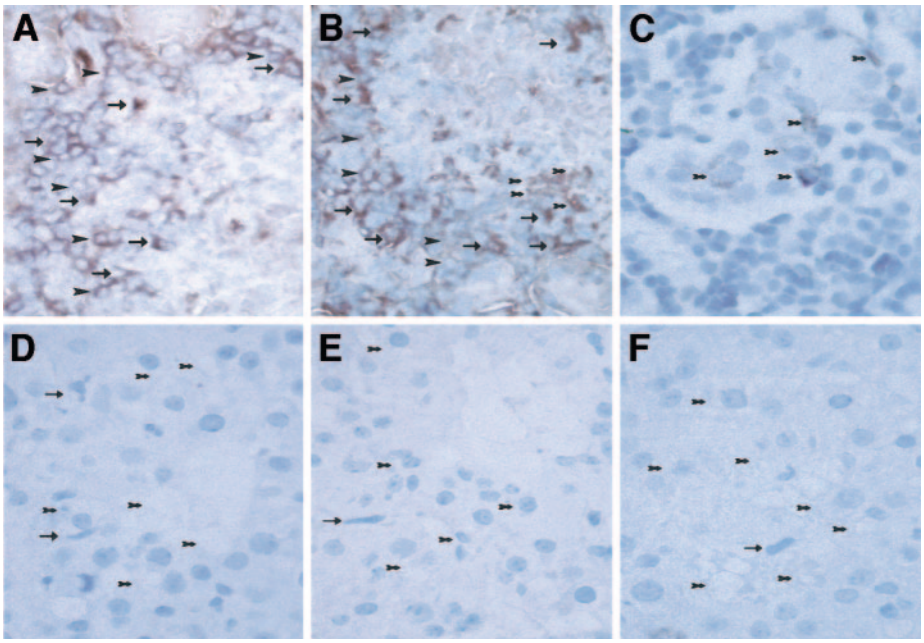

FIG. 4. Morphology of an islet from a diabetic IDDM (LEW.1AR1/Ztm-iddm) rat (*A–C*) exhibiting hyperglycemia (21.4 mmol/l) and hypoinsulinemia (0.5 ng/ml) 1 day after diabetes manifestation and of an islet from a type 2 diabetes *Psammomys obesus* (sand rat) (*D–F*) exhibiting hyperglycemia (17.5 mmol/l) and hyperinsulinemia (1.8 ng/ml) after 3 weeks on a high-energy diet. The sections were immunostained for IL-1β (*A* and *D*), iNOS (*B* and *E*), and activated caspase-3 (*C* and *F*) and show cytoplasmic immunoreactivities only in the infiltrated islets of the type 1 diabetic animal (*A–C*). Infiltrating immune cells in the diabetic IDDM rat (*A–C*) are mostly ED1$^+$ macrophages (arrows) and CD8$^+$ T-cells (arrowheads). These cells express immunoreactivity for IL-1β (*A*) and iNOS (*B*) but not for activated caspase-3 (*C*). Pancreatic β-cells undergoing apoptosis (thick arrows), in contrast, express immunoreactivity for iNOS and activated caspase-3, but not for IL-1β. The few infiltrating immune cells in the islet of a diabetic *Psammomys obesus* (*D–F*) are exclusively ED1$^+$ macrophages (arrows). These cells show no signs of immunoreactivity for IL-1β (*D*), iNOS (*E*), or activated caspase-3 (*F*). β-Cells (thick arrows) of *Psammomys* showed signs of necrotic destruction including intra- and intercellular vacuolization without expression of IL-1β (*D*), iNOS (*E*), or activated caspase-3 (*F*). These β-cells showed no signs of nuclear heterochromatin condensation. The same findings were made after 1 week of a high-energy diet. ED1$^+$ macrophages, CD8$^+$ T-cells, and pancreatic β-cells were identified by sequential sections immunostained with specific antibodies against the given cell type as previously described (67,86) (data not shown). Original magnification ×400.

oleate and palmitate caused alternative splicing of XBP-1, activation of ATF-6, and induction of the ER chaperone BiP in INS-1E cells (36). In addition to these specific ER stress markers, there was induction of ATF-4 and CHOP (36). It is thus conceivable that a high FFA load, that exceeds the β-cell's esterification capacity, impairs ER functions and triggers an ER stress response, thus contributing to β-cell toxicity. The mechanism by which FFAs cause ER stress remains to be elucidated, but (over)stimulation of FFA esterification in the ER might result in delayed processing and export of newly synthesized proteins, whereas saturated triglycerides may precipitate at their site of synthesis in the ER because of their high melting point. FFAs might also impair ER Ca^{2+} handling (81), whereas conditions that increase β-cell secretory demand, such as insulin resistance or high glucose, might amplify ER stress and β-cell death. ER stress has been recently proposed as the cellular/molecular mechanism linking obesity with insulin resistance (82,83). FFAs might thus be responsible for the ER stress response observed in the hepatocytes and adipocytes of obese mice (82,83), while hampering in parallel pancreatic β-cell function/viability (36). If that is the case, these intriguing novel observations (36,82,83) place ER stress as a common molecular pathway for the two main causes of type 2 diabetes—namely insulin resistance and loss of β-cell mass.

SIMILARITIES AND DIFFERENCES BETWEEN THE MECHANISMS OF β-CELL DEATH IN TYPE 1 DIABETES

The development of novel approaches to prevent β-cell death in diabetes depends on our knowledge of the mechanisms leading to β-cell demise. Thus, if the mechanisms of β-cell apoptosis were similar in type 1 and type 2 diabetes, it would be logical to search for common interventions in both forms of diabetes. Let us therefore examine the evidence pointing to the differences and similarities between the mechanisms of β-cell death and analyze whether sufficient information is available to support a similar "etiological" intervention in type 1 and type 2 diabetes.

Novel in vivo evidence for the in situ expression of mediators of β-cell death in animal models of diabetes. The NOD mouse and the BB rat are the most used animal models of type 1 diabetes (84), but a new model for type 1 diabetes has been recently described—the IDDM (LEW.1AR1/Ztm-*iddm*) rat (85,86). This latter model is of particular interest, since IDDM rats have a well-preserved cellular immune system, there is no sex bias in the incidence of diabetes, and detailed studies of the events leading to β-cell death are possible (see below).

ED1$^+$ macrophages are the predominant infiltrating immune cell species during the early stages of insulitis for all three animal models of type 1 diabetes. This is followed by an increasing infiltration by cytotoxic CD8$^+$ lymphocytes, predominating at the onset of diabetes. Other immune cells participating in the insulitis are CD4$^+$ lymphocytes, NK cells, and B-cells (rev. in 4,84). These immune cells are activated (87) and express proinflammatory cytokines such as IL-1β, TNF-α, and IFN-γ (88,89). IL-1β and TNF-α, but not IFN-γ, are detected in the infiltrating immune cells in the IDDM rat, but the pancreatic β-cells do not express these cytokines at any of the stages leading to overt diabetes (90; Fig. 4*A* and *B*; A.J., A. Günther, H.-J. Hedrich, D. Wedekind, M. Tiedge, S.L., unpublished data). Data from other models suggest that β-cells express chemokines such as MCP-1 and IP-10, which may contribute to the buildup of insulitis (91). Detailed morphological studies in the IDDM rat, using both in situ PCR and immunohistochemistry (A.J., A.

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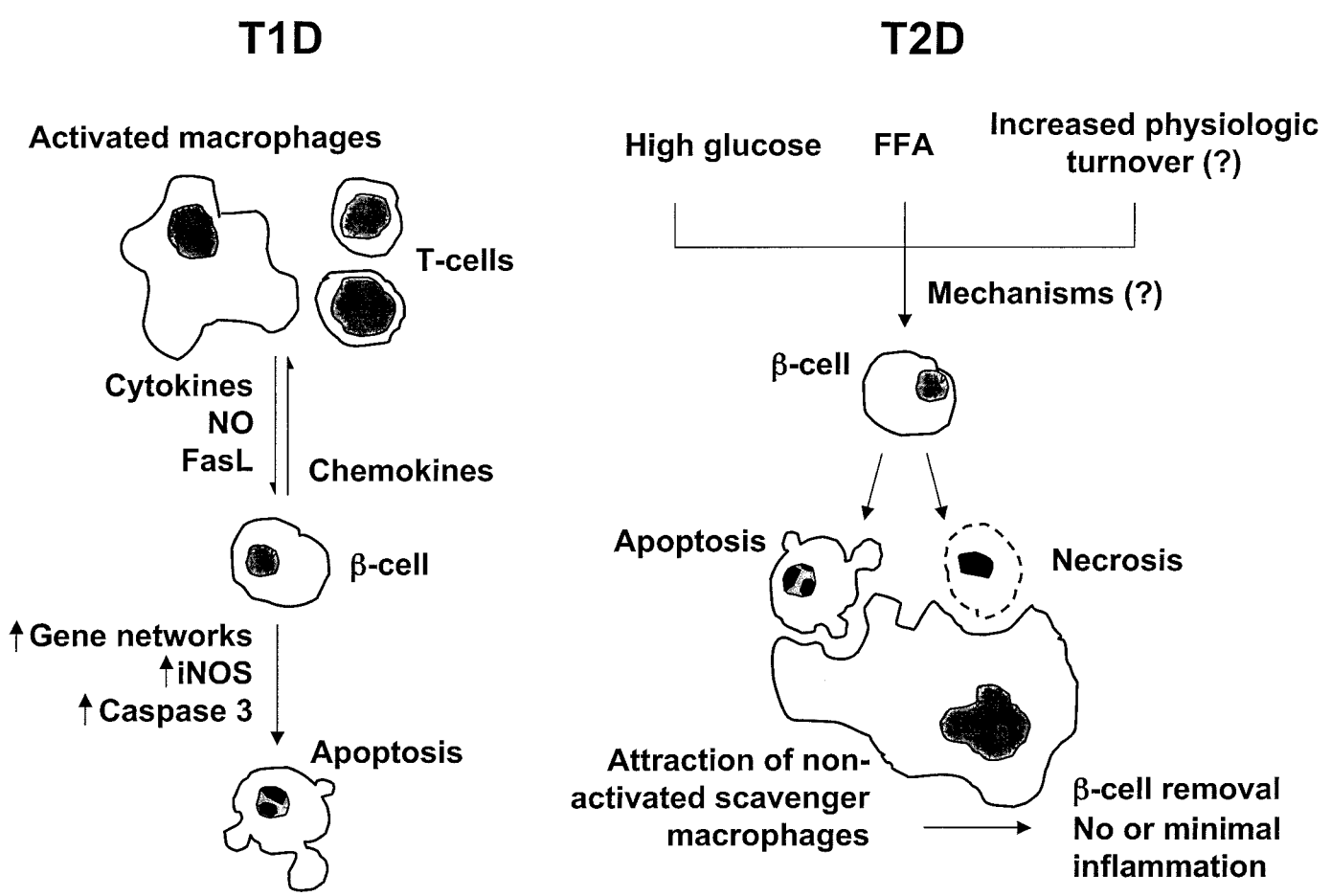

FIG. 5. Overview of the putative sequence of events leading to β-cell death in animal models of type 1 and type 2 diabetes. For additional information on the mechanisms of β-cell apoptosis in type 1 diabetes, see Figs. 1 and 2. T1D, type 1 diabetes; T2D, type 2 diabetes.

Günther, H.-J. Hedrich, D. Wedekind, M. Tiedge, S.L., unpublished data), suggest the following sequence of events: *1*) islets are initially infiltrated by macrophages, followed by $CD8^+$ and $CD4^+$ cells; *2*) this infiltration is accompanied by a high IL-1β (Fig. 4*A*) and TNF-α expression in the invading immune cells (but not in the β-cells) and iNOS expression (Fig. 4*B*) in both immune cells and β-cells; and 3) the β-cells under attack progressively express procaspase-3 (Fig. 4*C*) and undergo apoptosis. These observations suggest that proinflammatory cytokines are synthesized and released by the activated infiltrating immune cells, but not by the β-cells themselves, leading to apoptotic β-cell death in a paracrine fashion.

Loss of pancreatic β-cell mass is slow in type 2 diabetes, and there is no evidence for mononuclear cell infiltration (2). This is well documented in a number of type 2 diabetes animal models, including the *Psammomys obesus* (sand rat) (92) and the GK rat (93). When *Psammomys* are placed on a high-carbohydrate diet, they rapidly evolve to a diabetic state because of the loss of endocrine pancreas function and β-cell destruction (48,67). In contrast to the type 1 diabetes models (see above), β-cell demise occurs mostly by necrosis (67). The necrotic cells are removed by scavenger macrophages, which, at variance from the type 1 diabetes situation, are not activated and do not express the proinflammatory cytokines IL-1β, IFN-γ, or TNF-α (Fig. 4*D*; A.J., S.L., unpublished data). Importantly, the β-cells from these animals do not express IL-1β, iNOS, or caspase-3, as evaluated by immunohistochemistry (Fig. 4*E* and *F*) and in situ PCR over the course of high-energy diet-induced metabolic changes (1–3 weeks; A.J., S.L., unpublished data). As a positive control, IL-1β mRNA expression was confirmed by in situ PCR using immune cells of pancreas draining lymph nodes.

The same sequence of events seems to take place in the physiological situation, where β-cells undergoing apoptosis during their cell renewal cycle are removed by nonactivated macrophages (94). Even when the β-cell turnover rate is increased by administration of thyroid hormones (67,95), the increased demand for removal of apoptotic cells does not trigger macrophage activation or cytokine expression (A.J., S.L., unpublished data). These observations suggest a sequence of events that is different in type 1 and type 2 diabetes models. Thus, β-cells die by necrosis or apoptosis in type 2 diabetes, but the cause of death is not related to cytokine production by infiltrating mononuclear cells or the β-cells themselves. The dead β-cells attract scavenger macrophages, which in this case are *the consequence* rather than *the cause* of β-cell death (Fig. 5).

Analysis of the evidence for putative final common pathways of β-cell death in type 1 and type 2 diabetes. It has been recently suggested that β-cells exposed in vitro to high glucose produce IL-1β, thus activating NF-κB and Fas signaling and consequently triggering apoptosis (10,11). Another report indicated that FFAs also activate NF-κB in β-cells (96). Because both IL-1β and NF-κB are crucial mediators of β-cell death in type 1 diabetes (4), the IL-1β–NF-κB pathway was suggested as a "common final

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pathway" for β-cell death in both forms of diabetes (11), providing a rationale for revising and unifying the classification and treatment of diabetes (10).

As discussed above, exposure to IL-1β alone is not sufficient to kill human or rodent β-cells, and the signal transduction of IFN-γ is also required for β-cell demise. To exclude that exposure of β-cells to high glucose or FFAs induces the IFN-γ pathway, we reviewed the results of five different microarray analyses of human or rodent islets exposed to these nutrients (list of microarray studies provided upon request). We also contacted some of the authors to make sure that small changes in gene expression were not overlooked (D. Flamez, D. Melloul, G. Webb, personal communications). The data were compared with the gene expression patterns in rat (14) or human (P. Ylipaasto, B. Kutlu, S. Raisilainen, J. Rasschaert, T. Teerijoki, O. Korsgren, R. Lahesmaa, T. Hovi, D.L.E., T. Otokonski, M. Roivainen, unpublished data) islets exposed to IFN-γ. The mRNAs whose expression was most augmented by IFN-γ in β-cells were the transcription factors STAT-1, IRF-1, and IRF-7 and the chemokine CXCL 10 (IP-10). Glucose or FFAs modified none of these genes in β-cells, practically excluding the IFN-γ–STAT-1 pathway as a mediator of glucotoxicity or lipotoxicity. We therefore focused on IL-1β–NF-κB as the putative "common final pathway" for β-cell death.

As mentioned above, there is strong evidence that IL-1β contributes to β-cell death in type 1 diabetes via activation of NF-κB. Which is the evidence that FFAs induce IL-1β production or NF-κB activation in β-cells? We (36) and others (97) did not observe FFA-induced NF-κB activation in β-cells using three different techniques (gel shift, ELISA, and immunohistochemistry), and there are no reports of FFA-induced IL-1β expression in these cells. Moreover, FFAs do not induce expression of the NF-κB–dependent genes iNOS and MCP-1 in rodent β-cells (36,98). What about high glucose? Most of the in vitro data supporting glucose-induced IL-1β production and NF-κB activation were obtained by one group (rev. in 11). Based on their observations, this group initiated a clinical trial with the IL-1 receptor antagonist in type 2 diabetic patients (10). Of concern is that there is no in vivo evidence in animal models that blocking IL-1β protects β-cells against glucotoxicity. In addition, it has been difficult to reproduce the key findings of this "unifying hypothesis." Thus, we could not detect glucose-induced NF-κB activation or IL-1β expression in rat islets or FACS-purified β-cells (Fig. 3; 56). We examined whether this was due to a species difference between rat (56) and human (10,11) islets. Exposure of five preparations of human islets to increasing glucose concentrations (11 and 28 vs. 5.6 mmol/l) did not lead to the expression and release of IL-1β or other NF-κB–dependent genes, such as IκBα or MCP-1 (99). Of note, the concentration of IL-1β released by human islets exposed to 28 mmol/l glucose is negligible, i.e., <50-fold below the amount of IL-1β released by human monocytes (99). Moreover, there was no glucose-induced Fas mRNA expression (99), the proposed NF-κB–dependent mechanism by which glucose causes β-cell death (11). In line with our findings, islets isolated from mice deficient in either the IL-1 receptor or Fas were not protected against high glucose–induced β-cell death, and Fas was not detectable in wild-type mouse islets cultured at high glucose (100). As a whole, these observations argue against a role for IL-1β, NF-κB, or Fas in high glucose–induced β-cell death.

In conclusion, the suggestion that β-cells are killed by a similar mechanism in type 1 and type 2 diabetes is probably an oversimplification, not supported by convincing data. This oversimplification may bring confusion to a difficult and complex field and promote testing of novel therapeutic approaches in humans without adequate experimental support.

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NOTE ADDED IN PROOF

In agreement with the lack of IL-1β expression or release by human islets exposed to high glucose in vitro (as discussed in this review), recent data do not support a role for IL-1β in type 2 diabetes in vivo. Two studies, using respectively real-time RT-PCR and microarray analysis, demonstrate that IL-1β and Fas expression in islets isolated from type 2 diabetic patients is not increased as compared with islets from nondiabetic controls (99,101).

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