Mechanisms of β-Cell Death in Type 2 Diabetes

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A decrease in the number of functional insulin-producing β-cells contributes to the pathophysiology of type 2 diabetes. Opinions diverge regarding the relative contribution of a decrease in β-cell mass versus an intrinsic defect in the secretory machinery. Here we review the evidence that glucose, dyslipidemia, cytokines, leptin, autoimmunity, and some sulfonylureas may contribute to the maladaptation of β-cells. With respect to these causal factors, we focus on Fas, the ATP-sensitive K^+ channel, insulin receptor substrate 2, oxidative stress, nuclear factor-κB, endoplasmic reticulum stress, and mitochondrial dysfunction as their respective mechanisms of action. Interestingly, most of these factors are involved in inflammatory processes in addition to playing a role in both the regulation of β-cell secretory function and cell turnover. Thus, the mechanisms regulating β-cell proliferation, apoptosis, and function are inseparable processes. *Diabetes* 54 (Suppl. 2):S108–S113, 2005

For many years, the contribution of a reduction in β-cell mass to the development of type 2 diabetes was heavily debated. Recently, several publications have convincingly confirmed this hypothesis (1–3), leading to a rapid overemphasis of this etiological factor. Indeed, other mechanisms contributing to the failure of the β-cell to produce enough insulin appear more and more neglected. While we strongly believe that β-cell destruction is an important etiological factor in the development and progression of type 2 diabetes, in this review, we will highlight evidence that this is not dissociable from an intrinsic secretory defect. We will show that pathways regulating β-cell turnover are also implicated in β-cell insulin secretory function. It follows that adaptive mechanisms of function and mass share common regulatory pathways and will therefore act in concert. Depending on the prevailing concentration and the intracellular pathways activated, some factors may be deleterious to β-cell mass while enhancing insulin secretion, protective to the β-cell while inhibiting function, or even protective to the β-cell while enhancing function. It will become apparent that the failure of the β-cell in type 2 diabetes is akin to a multifactorial equation, with an overall negative result.

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ER, endoplasmic reticulum; FLIP, FLICE inhibitory protein; IL, interleukin; K_{ATP} channel, ATP-sensitive K^+ channel; NF, nuclear factor.

Thus, although we will review the factors and mechanisms regulating β-cell mass individually, only in a minority of diabetic patients does one single etiological factor underlie the failure of the β-cell. In addition to maturity-onset diabetes of the young, another example of this is autoimmune-mediated destruction of β-cells in young lean individuals. However, given that the incidence of type 1 diabetes increases with obesity (4), that insulin resistance is a risk factor for the progression of this condition (5), and that ~50% of the general population carry the same genetic predisposition (6), this example already implicates multiple etiological factors. Recognition of β-cell destruction not only in type 1 but also in type 2 diabetes led us to recently propose a unifying classification of diabetes (7). We believe it is important to revisit the current etiological classification of diabetes proposed by the American Diabetes Association, which still limits the notion of β-cell destruction to type 1 diabetes (8).

GLUCOSE AND THE INTERLEUKIN-1β–FAS–FLICE INHIBITORY PROTEIN PATHWAY: FROM ADAPTATION TO FAILURE

Glucose is the key physiological regulator of insulin secretion; therefore, it appears logical that it also regulates the long-term adaptation of insulin production by regulating β-cell turnover. Indeed, in all species, short-term exposure of β-cells to increasing glucose concentrations induces proliferation in a concentration-dependent manner (9–11). However, in *Psammomys obesus* and humans, the proliferative capacity of these cells is suppressed after a prolonged exposure to increased glucose concentrations. With regard to the role of glucose in β-cell apoptosis, the importance of the genetic background also appears crucial. In rodent islets, increasing glucose from a physiological concentration of 5.5 to 11 mmol/l decreases apoptosis (12). Further increases above 11 mmol/l will be either pro- or anti-apoptotic depending on the culture conditions, e.g., purified β-cells versus whole islets, or culture on matrix versus in suspension (10,12,13). The fact that rodent islets survive best at 11 mmol/l glucose is empirically reflected by the standard use of medium containing this concentration of glucose for optimal culture conditions. In contrast, in human and *Psammomys obesus* islets, an increase in glucose from 5.5 to 33 mmol/l induces a linear and much stronger increase in β-cell apoptosis (10,11,14) (Fig. 1). This difference in glucose sensitivity may explain why in animals genetically predisposed to diabetes, hyperglycemia increases rates of apoptosis, whereas in rats after 90% partial pancreatectomy, the incidence of β-cell apoptosis does not change despite increased glucose levels (15,16). It is probable that such differences also exist between humans with different genetic predispositions. Indeed, although glucose was capable of inducing β-cell apoptosis in most batches of human islets that we studied over the last few years, striking variations were observed in the

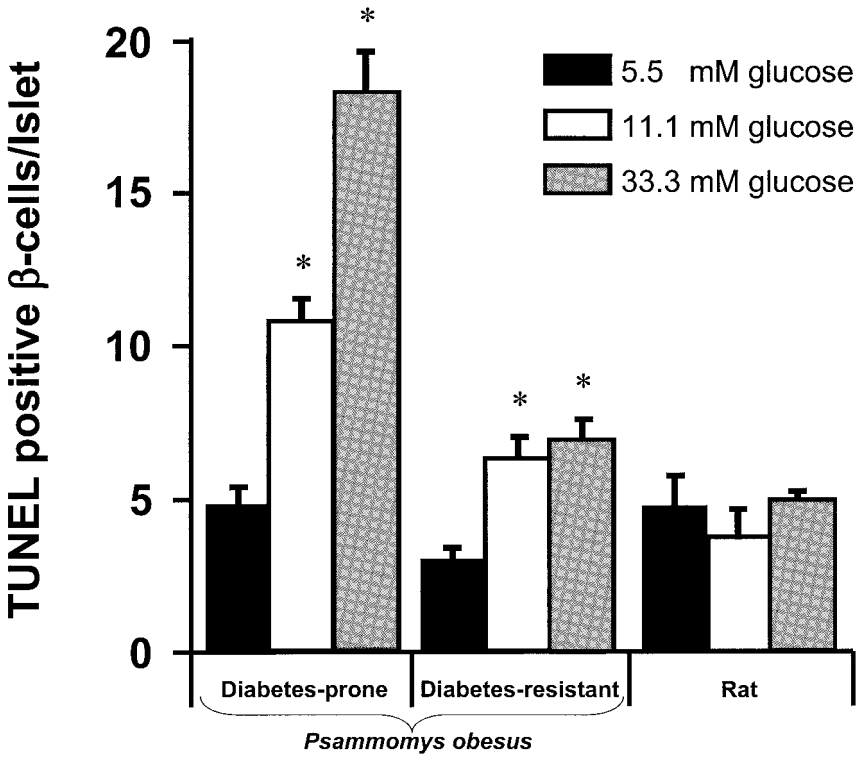

FIG. 1. Glucose-induced β-cell apoptosis in cultured islets of diabetes-prone and -resistant *Psammomys obesus* and in normal rat islets. Islets were cultured for 9–10 days in 5.5, 11.1, and 33.3 mmol/l glucose. *$P < 0.001$ relative to islets at 5.5 mmol/l glucose. Adapted from Donath et al. (10).

magnitude of this response (11,17–20). All of this points to a limitation of most studies performed in cell lines and rodent and human islets. Indeed, the above-mentioned differences in susceptibility to glucose-induced β-cell apoptosis between *Psammomys obesus* and rat islets highlight the importance in genetic background. Most studies testing agents potentially involved in the failure of the β-cells are performed with islets, which do not carry the genetic background predisposing to diabetes. With respect to this, it will be important to see whether human islets isolated from apparently healthy organ donors differ from diabetic islets.

β-Cell mass increases in conditions of increased demand such as in obesity, and its decrease leads to diabetes (21). This failure to adapt in diabetic patients could be explained by the effect of glucose on β-cell turnover, as detailed above. We propose a hypothesis for the underlying mechanisms by which glucose regulates β-cell mass (Fig. 2). According to this hypothesis, long-term adaptation of the β-cells to conditions of increased demand may be triggered by hyperglycemic excursions (9). These excursions elicit β-cell production of interleukin (IL)-1β (20), followed by Fas upregulation (11,22). In the presence of the caspase-8 inhibitor FLICE inhibitory protein (FLIP), Fas engagement is directed to proliferation. However, excessive glucose stimulation will decrease FLIP, switching this adaptive pathway toward deleterious signals and eventually to diabetes (17). In support of this hypothesis, IL-1β is found in the β-cells of type 2 diabetic patients (20), where there is a concomitant induction of Fas (11) and a decrease in FLIP protein expression (17).

Recently, two additional animal models of type 2 diabetes (the GK rat [23] and the human islet amyloid polypeptide transgenic rat [24; P. Butler, personal communication]) have confirmed pancreatic β-cell expression of IL-1β under hyperglycemic conditions. In addition, glucose-induced β-cell production of IL-1β was demonstrated by electron microscopy in sections from isolated human islets (Fig. 3). Finally, glucose-induced Fas expression has been confirmed in vitro and in vivo (22,25), thus supporting a molecular link between type 1 and type 2 diabetes.

In addition to its effect on β-cell turnover, hyperglycemia also impairs β-cell secretory function (7,26–28). This glucotoxic effect is evident before apoptosis leads to a significant decrease in β-cell mass. This is most striking in vitro, where a 4-day exposure of human islets to elevated glucose concentrations leads to almost complete ablation of β-cell secretory function, although <1% of β-cells are apoptotic (20). Because hyperglycemia regulates Fas expression (11,22), we hypothesized that the Fas pathway may not solely mediate glucose-induced changes in cell turnover, but also changes in β-cell secretory function. Recently, we demonstrated a novel role for the Fas

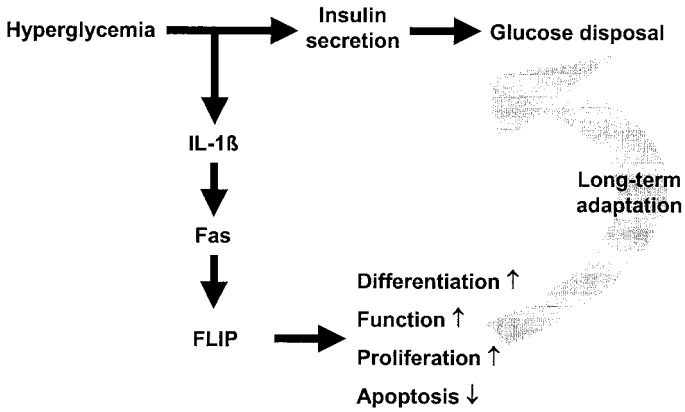

FIG. 2. Hypothetical model illustrating the consequence of hyperglycemia on β-cell production of IL-1β in parallel with insulin secretion. The paracrine effect of IL-1β induces Fas engagement, which in the presence of FLIP leads to β-cell proliferation, differentiation, and increased function.

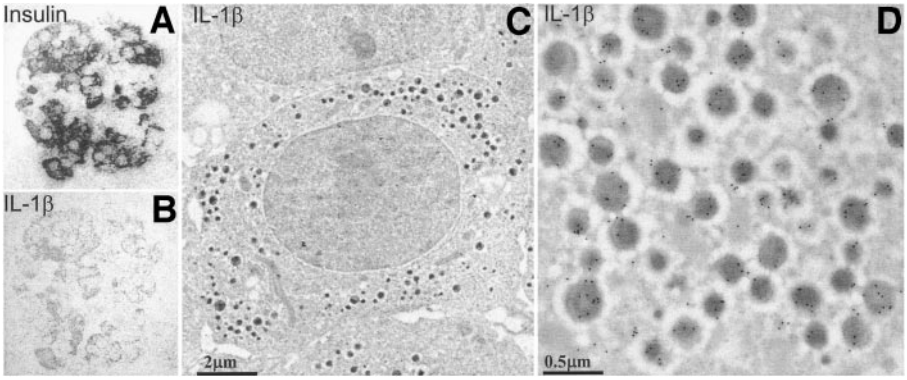

FIG. 3. High-glucose–induced expression of IL-1β by human β-cells. Cultured human islets were exposed for 4 days to media containing 5.5 (not shown) or 33.3 mmol/l glucose and gold-immunolabeled for insulin (*A*) or IL-1β (*B–D*) as previously described (19,20). Light microscopy of consecutive semi-thin sections of a human islet showing the high degree of coexistence of insulin (*A*) and IL-1β (*B*) is shown, as is electron microscopy of an ultra-thin section of a β-cell (*C* and *D*). At higher magnification, immunogold-labeled IL-1β (dense spherical particles) can be localized to the secretory granules as characterized by a dense core (*D*). Samples were prepared and evaluated by two investigators (E.E. and M.R.) blinded to the treatment conditions. IL-1β was detected only in β-cells exposed to high glucose.

pathway in regulating insulin production and release (D.M.S., K.M., I. Franklin, J.A.E., H.E., A. Gjinovci, M.O. Kurrer, B.R. Gauthier, Y. Iwakura, A.V. Chervonsky, C.B. Wollheim, M.Y.D., unpublished data). Clearly, the IL-1β–Fas pathway is not the sole mediator of glucotoxicity in pancreatic β-cells (26–28,29,30). Rather, we view this pathway as one example of multiple mechanisms regulating of β-cell mass and function.

DYSLIPIDEMIA, LEPTIN, AND INFLAMMATION: LINKING OBESITY TO β-CELL FAILURE

Obesity is the main risk factor for the development of diabetes. It is often part of the metabolic syndrome and is accompanied by dyslipidemia and increased circulating leptin and cytokine levels. All of these factors have been shown to modulate β-cell function and survival. The influence of dyslipidemia on the β-cells of an individual will depend on his or her specific lipid profile. Whereas some free fatty acids and lipoproteins have been shown to be pro-apoptotic for the β-cell, others are protective. Thus, long-term exposure to saturated fatty acids such as palmitate appear highly toxic, whereas monounsaturated fatty acids such as oleate protect against both palmitate- and glucose-induced β-cell apoptosis (18,31). It is of interest to note that similar toxic effects are also observed in non–β-cells such as cardiac cells (32). Lipoproteins may affect β-cell survival in a similar way, whereby VLDL and LDL are pro-apoptotic and HDL is protective (33,34). These lipotoxic effects may also be influenced by the prevailing glycemia (35,36).

Leptin was initially identified as an adipocyte-derived satiety factor. Recently, leptin has also been considered as a pro-inflammatory cytokine because of its structural similarity with other cytokines and its receptor-induced signaling pathways (37). Interestingly, leptin accelerates autoimmune diabetes in NOD mice (38), providing an additional link between type 1 and type 2 diabetes. Of note, leptin also promotes other autoimmune diseases including inflammatory bowel disease (39), multiple sclerosis (40), and rheumatoid arthritis (37). We have shown that leptin, in addition to its established effect on insulin secretion (41,42), induces β-cell apoptosis via increasing release of IL-1β and decreasing release of the IL-1 receptor antagonist in human islets (19). Other cytokines released by adipocytes, including tumor necrosis factor-α and IL-6, may also modulate β-cell survival, although it is unclear if the amount released into the circulation is sufficient to affect β-cells (6). Furthermore, it may well be that these cytokines are only effective in the presence of other cytokines. Finally, all the above-mentioned factors are also known to affect β-cell secretory function, as reviewed elsewhere (6).

INSULIN RECEPTOR SUBSTRATE 2, NUCLEAR FACTOR-κB, ENDOPLASMIC RETICULUM STRESS, MITOCHONDRIAL DYSFUNCTION, AND OXIDATIVE STRESS: REGULATORS OF BOTH β-CELL MASS AND INSULIN SENSITIVITY

Several recent reports point to common responsive elements in the pathways regulating β-cell turnover and peripheral insulin effects. A striking example is insulin receptor substrate 2, which promotes β-cell growth and survival and insulin secretion, in addition to its well-recognized role in insulin sensitivity (43,44). The role of insulin receptor substrate 2 in regulating β-cell fate has recently been extensively reviewed (44).

The transcription factor nuclear factor (NF)-κB is another example of such a common regulator. In the β-cell, it may induce apoptosis or promote survival, depending on the kinetics and mode of induction (6,45–47). NF-κB is also involved in the etiology of insulin resistance (48,49). Recent work has provided evidence that NF-κB signaling is involved in the low-grade inflammation that occurs in the liver of type 2 diabetic models, contributing to insulin resistance (50,51). Further, this signaling node may influence peripheral insulin resistance via actions in myeloid cells (51). Interestingly, nonsteroidal anti-inflammatory drugs enhance insulin sensitivity and protect the β-cell from apoptosis via inhibition of NF-κB (46–49). Finally, hyperglycemia and free fatty acids induce activation of NF-κB (11,52,53), whereas attenuation of NF-κB inhibits glucose-stimulated insulin secretion in pancreatic β-cells (54). All these findings support the concept that NF-κB may have a key role in causing both insulin resistance and impaired insulin secretion in type 2 diabetes (53).

Because of the high secretory demand, the endoplasmic reticulum (ER) is very well developed and highly active in the β-cell. This also likely increases the susceptibility of these cells to ER stressors. ER stress might produce signals mediating glucose-induced impairment of function and death (55–57). However, activation of the unfolded protein response in β-cells in response to ER stress may also provide protection to β-cells by attenuating the ability of cytokines such as IL-1β to signal and activate the expression of downstream targets (58). ER stress has recently been observed in obesity and linked to insulin resistance (59).

Mitochondrial dysfunction has also been proposed as a common feature of both impaired insulin responsiveness

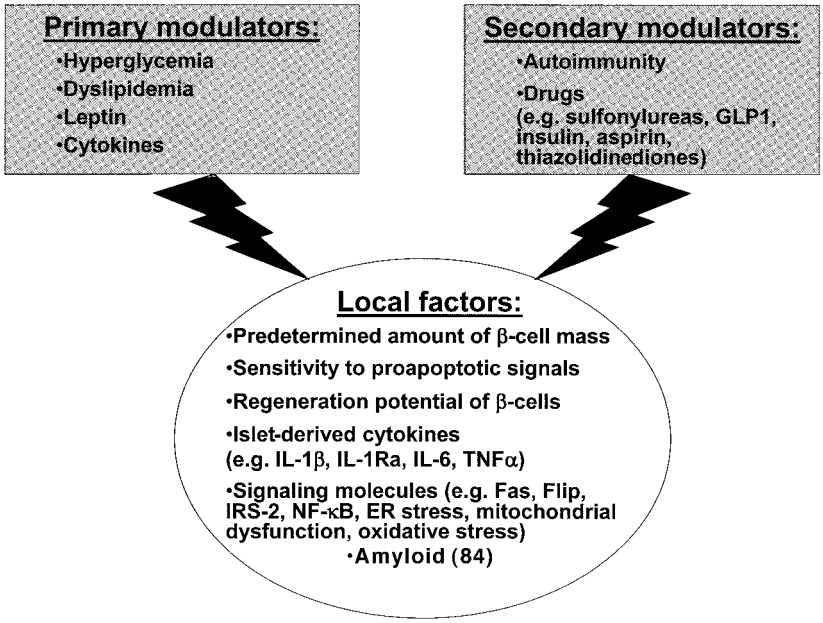

FIG. 4. Proposed model for mechanisms regulating β-cell mass in type 2 diabetes. Before onset of diabetes, insulin resistance may lead to transient postprandial hyperglycemic excursions. Other factors modulating β-cell mass may include dyslipidemia, leptin, and cytokines. Genetic predisposition to diabetes may include a predetermined amount of β-cell mass, as well as differences in the susceptibility to apoptotic signals and in the regenerative potential of the β-cell. Additionally, induction of local inflammatory mediators and cell death may activate the acquired immune system. Finally, drugs may protect or harm the β-cell. GLP1, glucagon-like peptide 1; IRS-2, insulin receptor substrate 2; TNFα, tumor necrosis factor-α.

of peripheral tissues and defective β-cell secretory function and survival (60). The central role of the mitochondria in insulin secretion and insulin sensitivity underlies this hypothesis. Recently, it was shown that pancreatic duodenal homeobox gene-1 (PDX-1) regulates insulin secretion via mitochondrial effects, whereas the role of the mitochondrial uncoupling protein-2 in insulin secretion is well established (61–63). It is interesting to note that PDX-1 is also a critical regulator of β-cell survival (64,65).

Finally, the pathogenic effect of glucose, possibly in concert with free fatty acids, is also mediated by oxidative stress and may not only affect insulin sensitivity, insulin secretion, and survival, but may also play a role in the development of the secondary complications of diabetes (66–68). These effects are mainly catalyzed by the generation of reactive oxygen species and reactive nitrogen species, which will ultimately activate stress-induced pathways (NF-κB, stress kinases, and hexosamines) to manipulate cell fate.

ANTI-DIABETIC DRUGS: BENEFICIAL OR HARMFUL?

Understanding that decreased β-cell mass is an important factor in the pathogenesis of type 2 diabetes raises a concern regarding the application of drugs potentially harmful to the remaining β-cells. Conversely, protection of β-cells from death presents itself as a new therapeutic target. In this context, modulation of the β-cell ATP-sensitive K^+ (K_{ATP}) channel (K_{ATP} channels are octamers composed of four inwardly rectifying K^+ channels [Kir 6.2] and four sulfonylurea receptors [SUR1]) appears particularly interesting. Indeed, closure of the K_{ATP} channels by the sulfonylureas tolbutamide and glibenclamide may induce Ca^{2+}-dependent β-cell apoptosis in rodent and human islets (12,69,70). This effect was observed only in vitro and not consistently (71). However, in an important recent clinical study comparing insulin and sulfonylurea treatment of type 2 diabetes, it was shown that treatment with insulin preserved β-cell function more effectively than glibenclamide (72). It remains to be established whether it is the beneficial effects of insulin per se or the possible β-cell toxicity of glibenclamide that accounts for this observation. Whereas a deterioration of insulin secretion was seen in patients treated with sulfonylureas in the U.K. Prospective Diabetes Study, those treated with insulin were not evaluated in this regard (73). Given the possible deleterious effect of some sulfonylureas, alternatives to these as well as alternative insulin secretagogues may have to be considered. When applied for their respective circulating half-lives in vitro, repaglinide and nateglinide do not appear to have an apoptotic effect on human islets (70). In contrast to sulfonylureas, K_{ATP} channels' channel openers may exert protective effects on β-cells (74,75). In 1976, Greenwood et al. (76) were the first to report an improvement in insulin secretion after administration of diazoxide to diabetic subjects for 7 days. Similar protective effects were observed more recently in patients classified with type 1 and type 2 diabetes (77,78). Finally, other antidiabetic drugs that have emerged as protectors of β-cells from apoptotic stimuli include thiazolidinediones, glucagon-like peptide 1 analogs, and, last but not least, insulin (47,70,79,80).

SUMMARY AND PROPOSED INTEGRATED VIEW OF β-CELL DEMISE IN TYPE 2 DIABETES

We have presented evidence in support of numerous factors that are potentially deleterious to β-cells. Additionally, some individuals may have limited β-cell mass early in life because of genetic factors predisposing them to diabetes. However, the demise of insulin-producing cells can be compensated for to a certain degree by regeneration. We propose that glucose plays a central role among those factors contributing to β-cell "burnout." While transient postprandial hyperglycemic excursions may predominantly induce β-cell proliferation in insulin-resistant individuals, this adaptive mechanism may fail in the long term and be overridden by β-cell apoptosis. However, it is unlikely that glucotoxicity acts alone, and the negative contribution of saturated fatty acids, lipoproteins, leptin, and circulating and locally produced cytokines will further burn out the β-cells (Fig. 4). These factors will induce apoptosis and/or necrosis, which in the presence of proinflammatory cytokines may activate specific immunological phenomena, which ultimately result in autoimmunity

(6,81–83). Finally, evidence now exists that therapeutic agents may influence, for the good or the bad, the fate of the β-cells.

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