Role of Pancreatic β-Cells in the Process of β-Cell Death

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Studies on the pathogenesis of type 1 diabetes have mainly focused on the role of the immune system in the destruction of pancreatic β-cells. Lack of data on the cellular and molecular events at the β-cell level is caused by the inaccessibility of these cells during development of the disease. Indirect information has been collected from isolated rodent and human islet cell preparations that were exposed to cytotoxic conditions. This article reviews in vitro experiments that investigated the role of β-cells in the process of β-cell death. β-Cells rapidly die in necrosis because of toxic levels of oxidizing radicals or of nitric oxide; they progressively become apoptotic after prolonged culture at low glucose or with proinflammatory cytokines. Their susceptibility to necrosis or apoptosis varies with their functional state and thus with the environmental conditions. A change in cellular phenotype can alter its recognition of potentially cytotoxic agents and its defense mechanisms against cell death. These observations support the view that β-cells are not necessarily passive victims of a cytotoxic process but can actively participate in a process of β-cell death. Their role will be influenced by neighboring non–β-cells, which can make the islet internal milieu more protective or toxic for the β-cells. We consider duct cells as potentially important contributors to this local process. Diabetes 50 (Suppl. 1):S52–S57, 2001

Type 1 diabetes is caused by a massive and selective death of pancreatic β-cells (1). The loss of β-cells is associated with local and circulating signs of an autoimmune reactivity against β-cell antigens (1,2). In reviewing reports on the islet pathology in human diabetes, we noticed that not all β-cells were equally affected by the pathogenic process (3). At clinical onset, a large proportion of β-cells had indeed disappeared, but all cases still exhibited functional β-cells (3,4). Beyond the first year after onset, virtually no β-cells were left in patients younger than 7 years of age, but this was not as common in older individuals; when onset was diagnosed above the age of 20 years, long-term persistence of β-cells was rather the rule than the exception (3). These observations correlate with the occurrence of insulitis: at the time of, or shortly after, onset, a lymphocytic islet infiltration was found in virtually all patients younger than 14 years of age, although it was not detectable in all islets; its presence was less frequent in adolescents and rare when onset was diagnosed above age 20 years (3). The β-cell destructive process in humans is thus not affecting all cells simultaneously; although it may proceed over many years, it leaves a detectable number of β-cells intact in many adolescents and most adults. The reasons for this heterogeneity in β-cell death can be multiple, as are the factors involved in its pathogenesis. They are expected to cause a variability in either the inflammatory or immune aggression of the β-cells or in the susceptibility of these cells to potentially damaging conditions. If the biologic basis of this variability can be understood and manipulated, methods might emerge for counteracting the processes leading to β-cell depletion in type 1 diabetic patients and in individuals at risk for developing this disease. The obvious obstacle is of course the inaccessibility of the β-cells in their normal habitat, and, a fortiori, in the pancreas of patients who develop the disease. Pieces of information are therefore gathered from experimental models. In this article, we will review in vitro studies that have indicated that islet β-cells can play an active role in the process of β-cell death.

MODELS FOR STUDYING β-CELL DEATH

The process of β-cell destruction has been investigated mainly in isolated rodent islets and cells (5–7). These models face several limitations. First, they carry the disadvantages of any in vitro model that is used to examine an in vivo process with fluctuating variables. Second, the amount of isolated tissue is often insufficient for molecular analysis of a cell death process. Cell lines can fulfill these quantitative needs but are not representative for β-cells undergoing destruction or protection. Third, tissue is almost never available from the pathologic phase in humans. The few cases that could be investigated at the islet level have generated interesting data (8–10) but have not really clarified the process of β-cell death. Animal models of type 1 diabetes have provided insights into possible immune components, but have, so far, not unraveled the molecular events at the β-cell level. Furthermore, these models might not be sufficiently representative for the human disease, in view of the differences in their clinical and biologic presentation. These considerations do not cast doubt on the usefulness of studies conducted in these experimental models but caution against extrapolating their data to the human disease process without due attention to their inherent limitations.
We have used isolated β-cells to investigate whether β-cells can play an active role in their own destruction (11). The purpose was to examine whether the properties of β-cells influence their susceptibility to potentially damaging conditions and to cell death in general. This in vitro model certainly faces the same limitations as others with respect to its representativeness for the β-cell destructive process in type 1 diabetes. However, compared with other models, it offers the advantage that the number of dead cells can be counted and thus used as a direct index for detecting and monitoring cytotoxic or protective conditions. It also allows analysis of β-cells without interference of other cell types. In isolated islets, cell death cannot be quantified, and the presence of other endocrine and nonendocrine cells may variably influence the in vitro process without being necessarily involved in vivo (Fig. 1). Furthermore, their changing cellular composition and increasing rate of cell death makes cultured islets rather inadequate for studying chronic influences in the process of β-cell death.

Cell death is easily quantified in single-cell preparations that are cultured in polylysine-coated cups. Under these experimental conditions, both dead and living cells remain attached to the bottom and are distinguished by vital staining with neutral red (11) (Fig. 2). A fluorescence assay can be used to count the percentage of cells in necrosis or apoptosis (12) (Fig. 2).

SUSCEPTIBILITY OF β-CELLS TO NECROSIS
Most studies on β-cell–specific death have used alloxan and streptozotocin, two diabetogenic toxins that cause a rapid and specific destruction of rodent β-cells. Early work has shown that the β-cell toxicity of these drugs can be reduced by glucose and nicotinamide, respectively (6). Later experiments with isolated β-cells indicated that both toxins cause a rapid and specific necrosis of β-cells, which can be counteracted by cellular defense mechanisms that are stimulated by glucose or nicotinamide (11,13,14). This mode of cell death is in agreement with the proposed action mechanism of these drugs, namely a rapid toxicity through formation of oxidizing radicals and/or ATP depletion (13,15,16). Necrosis of rat β-cells also occurred when oxidative phosphorylation was blocked (12); addition of an oxidizing agent such as t-butylhydroperoxide caused necrosis in both rat and human β-cells—an effect that was prevented by nicotinamide when given during the subsequent 24 h (11,14). Islet β-cells are thus susceptible to rapid necrosis when exposed to oxidizing radicals. It has been proposed that these cells are particularly vulnerable to oxidation as a result of their low activity in radical scavenging enzymes (17). This relative deficiency might have negative consequences only when the cells exhibit low rates of mitochondrial metabolism, such as at low glucose levels. This would then be compatible with the findings that the cellular susceptibility to oxidative damage varies with their metabolic responsiveness to glucose and with the prevailing metabolic condition. The β-cell–specific kinetics of glucose metabolism (18) may thus well protect the cells against oxidative damage. Consequently, cells with dysregulated glucose recognition become more vulnerable to oxidative influences than other cells and thus undergo a cell-specific destruction.

Over the last years, cytokines have become the major agents in studies on mechanisms of β-cell death (6,7). Their use was initiated after the observation that addition of interleukin (IL)-1β to isolated rat islets leads to loss of β-cell function and damage to β-cells (6). The toxic effects of IL-1β are more pronounced in islets cultured at high glucose levels, again suggesting a variability in cellular susceptibility (6). They have been attributed to the elevated nitric oxide (NO) levels that are produced after an IL-1β–induced expression of inducible nitric oxide synthase (iNOS) in β-cells (19). High NO concentrations cause necrosis of both rat and human β-cells but also of other islet cell types (20). This result explains why the massive IL-1β–induced and NO–dependent necrosis in isolated rat islets is not specific for the β-cells (20,21). The interstitium of these statically cultured islets is indeed expected to reach high concentrations of released products such as NO, which can then affect any surrounding cells. When dispersed β-cells are submitted to the same IL-1β dose, they exhibit the same NO production as β-cells in intact islets. The released NO probably diffuses faster so that its extracellular concentration remains lower than that inside cultured islets and therefore fails to induce necrosis (20,21). The question is now whether human β-cells are also capable of producing NO and whether this can lead to toxic concentrations in the vicinity of β-cells in situ, causing their necrosis in the intact pancreas. Several laboratories have demonstrated that cytokine combinations can induce iNOS expression and NO production in isolated human islet preparations (22–24). In a study by Arundel et al. (25), iNOS has been localized in insulin-containing β-cells; in our cytokine-treated preparations, we have not yet succeeded in identifying an iNOS immunoreactivity in insulin-positive cells or in eliciting an NO-dependent necrosis of these cells (24). We did, however, notice an iNOS positivity and NO production in nonendocrine pancreatic duct cells (24) (Fig. 3). It is still unclear whether this difference is due to the use of another iNOS antibody (Fig. 3 uses an antibody described by Singer et al. [26]) or of cultured (27) instead of freshly isolated islets. It is certainly important to clarify the reasons for this apparent discrepancy. However, the obser-
RATION that cytokines can induce production of NO in duct cells that are adjacent to human β-cells (24) is also compatible with the possibility that NO might cause β-cell destruction in humans (6, 7, 25)—in particular, when the normal islet flow is so severely impaired that locally released products rapidly accumulate.

SUSCEPTIBILITY OF β-CELLS TO APOPTOSIS

Islet β-cells can also die in apoptosis, as illustrated by in vitro and in vivo observations in the rat (12, 28). This form of cell death can be induced by a sustained inhibition of protein synthesis, which suggests that it is normally suppressed by antiapoptotic proteins in β-cells with adequate protein synthetic activity (12). Glucose, a well-known activator of protein synthesis in β-cells, suppresses apoptosis in cultured rat β-cells (12). The antiapoptotic effect of glucose is expected to vary with the glucose sensitivity of the cells. Intercellular differences in glucose sensitivity (29) can explain the intercellular differences in susceptibility to apoptosis (12). Thus, β-cells with a reduced sensitivity to glucose may be less protected against apoptosis. This raises the almost philosophical question as to whether a sustained reduction in the glucose sensitivity of a β-cell, and thus in the purpose of its existence, prepares for its death by apoptosis.

The concept that glucose-responsive β-cells are protected by glucose against apoptosis bears the consequence that a loss of the typical β-cell phenotype should alter the cellular susceptibility to apoptosis. Such a phenomenon was noticed when isolated rat β-cells were cultured with IL-1β. Under this condition, β-cells survived but lost their characteristic phenotype (27, 30). The reduced expression of glucose-inducible proteins is associated with a loss of glucose protection against apoptosis: addition of γ-interferon with or without tumor necrosis factor-α resulted in apoptosis, irrespective of the glucose concentration (20). This combination also induced apoptosis in human β-cells (14, 31), against which nicotinamide failed to protect.

ACTIVE ROLE OF β-CELLS

It is still unknown which death-promoting factors cause the loss of β-cells in type 1 diabetes, but they apparently do not affect all cells simultaneously. Their effect will probably depend not only on their concentration at the β-cell level, but also on the susceptibility of these cells to undergo necrosis or apoptosis when exposed to such agent(s). This cellular susceptibility might vary as shown by in vitro studies on isolated β-cells. The functional state of the target cells was found to influence their sensitivity to potential toxins and their ability to survive and
generate defense reactions. This functional state varies among β-cells and fluctuates with their environmental condition. It might thus determine, in a variable way, the toxicity and the cell specificity of a pathogenic condition. This view is supported by experimental observations. The β-cell specificity of the alloxan and streptozotocin toxicity depends on the differentiated state of the cells; disappearance of their characteristic phenotype makes them markedly less susceptible to these drugs (30). On the other hand, differentiated rat β-cells possess defense mechanisms that can be stimulated to counteract necrosis and apoptosis (11–14). Glucose is a potent activator of this cellular property. An alteration in β-cell phenotype, or in its glucose sensitivity, will have consequences on the cellular susceptibility to necrosis or apoptosis.

Whereas in vitro studies on isolated β-cells do not clarify the events that occur during development of type 1 diabetes, they do indicate that β-cells are not necessarily passive victims of cytotoxic agents but could actively participate in this process. Their role may only be operative in certain pathogenic conditions. It is probably not identical for all cells in view of their intercellular heterogeneity (29). Because protective mechanisms can be stimulated by environmental conditions, they could be considered as targets in intervention strategies. However, development of such a strategy remains difficult because so little is known about β-cell destruction in vivo. In addition to the inability to detect and monitor this process before the onset of diabetes, it is still unclear whether the in vivo destruction occurs by necrosis or apoptosis, or by a combination of both. More insights in the underlying pathways are needed. They can direct attempts to prevent production of death-promoting factors and indicate ways to counteract their effects. Prospective trials with nicotinamide are presently undertaken in individuals at risk of a β-cell–destructive process (32). When tested in vitro, this agent can indeed protect human β-cells against radical-induced necrosis but not against cytokine-induced apoptosis (14). In rodents, nicotinamide was shown to protect against both streptozotocin-induced diabetes and the spontaneous autoimmune form of the disease (33,34). The experimentally used concentrations are, however, much higher than those used in humans. This underlines the need to further examine the mode of action of agents with a beneficial effect in the laboratory and to search for more specific compounds that operate at lower doses (35).

**ROLE OF NEIGHBORING ENDOCRINE AND NONENDOCRINE CELLS**

Like other cell types, islet β-cells will undergo influences by neighboring cells, with possible effects on their functions and their survival. It is generally accepted that such interac-
tions exist between β-cells and infiltrating immunocytes. There is also indirect evidence that β-cells can locally interact with cells other than those of the immune system.

In the normal pancreas, it is still unclear which cell types locally communicate with β-cells, be it via cell junctions or via their secreted products. Direct contacts with β-cells are not infrequent, as judged from the number of tightly coupled somatostatin-containing D-cells in rat islet dissociates (36) and from the number of closely associated duct cells in the human pancreas (37) (Fig. 3). They have so far not been studied for their possible influences on β-cell survival. Indirect contacts via the blood stream have not been described. According to the vasculature in rat islets, β-cells are not irritated by glucagon-rich blood (38); however, it remains possible that locally released glucagon reaches a number of β-cells via the islet interstitium. The hormone may then serve as a cytoprotective agent. Glucagon, as well as other agents that increase the β-cell cyclic AMP levels, was found to facilitate the survival of cultured β-cells (39).

When islets are infiltrated by inflammatory or immune cells, their local milieu is probably markedly altered. Vascular and interstitial flow are likely perturbed, with profound changes in the concentration of regulatory substances, including those that influence functions and survival of β-cells. Infiltrating macrophages may release oxidative radicals and cytokines that can damage adjacent cells (6,25,40). Activated T-cells may generate an antigen-driven cytotoxicity. These mechanisms can account for a rapid β-cell destruction in heavily infiltrated islets, in which β-cell protective mechanisms may have little success. If β-cells can influence the in vivo process, they are more likely to do so in earlier stages and in islets with only a mild or peripheral infiltration. In these conditions, other islet cells might also become actively involved and influence the role of the β-cells. Studies in islets of prediabetic BB rats have indicated the existence of an endothelial dysfunction and of activated macrophages (40,41). Experiments with human pancreatic islets have shown that IL-1β release from activated macrophages helps induce iNOS expression in β-cells (25). Our observations suggest that associated duct cells may participate in the inflammatory process. The use of cytokeratins as a duct cell marker allowed us to identify a close anatomic association between a significant proportion of human β-cells and duct cells (37). This association results in comigration of endocrine and duct cells during the isolation process and in a considerable nonendocrine contamination of our cultured human islet preparations (24,27,42) (Fig. 3). A number of these associated duct cells respond to cytokines by expressing major histocompatibility complex II molecules, and others respond by expressing iNOS with the production of NO; in contrast, we did not detect such responses in the adjacent human endocrine islet cells (24,43) (Fig. 3). Under our experimental conditions, the NO production by the duct cells apparently did not generate sufficiently high concentrations at the level of neighboring β-cells to cause their necrosis (24). It is of course not excluded that these concentrations can be reached in vivo (Fig. 1). Nontoxic NO concentrations have been found to influence the properties of β-cells, including their susceptibility to cytotoxic conditions (30). It is not known whether these concentrations can exert such action on human β-cells and whether this could reflect an interaction with adjacent duct cells. Clearly, more work is needed to substantiate the role of other islet cells in the β-cell destruction process.

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