Repair of Pancreatic β-Cells
A Relevant Phenomenon in Early IDDM?

DÉCIO L. EIZIRIK, STELLAN SANDLER, AND JERRY P. PALMER

Most studies dealing with the pathogenesis of IDDM have emphasized the immune assault against β-cells. In this perspective, we review the data that suggest that the β-cell destruction of IDDM depends on a balance between β-cell damage and repair. The progressive β-cell damage leading to IDDM seems to follow markedly different temporal courses in individual patients. Some individuals at high risk for developing IDDM, and presenting with impaired β-cell function, appear to recover β-cell function when followed prospectively. Moreover, after the clinical onset of IDDM, most patients experience a transitory period of improved insulin secretion. In vivo and in vitro experimental data suggest that β-cells are indeed able to repair themselves after damage. Dispersed β-cells or whole islets can survive and regain their function after a toxic assault. Furthermore, the abnormal insulin release and glucose oxidation of islets isolated from NOD mice during the prediabetic period is completely restored after 1 wk in tissue culture. Finally, treatment of NOD mice with monoclonal antibodies directed against infiltrating T-cells reverses the altered glucose metabolism of β-cells. Note that β-cell repair after exposure to different toxic agents can be enhanced both in vivo and in vitro. Potential enhancers of β-cell repair are nicotinamide, glucose, protein-rich diets, and branched chain amino acids. A basic question that remains to be answered is the nature of the repair mechanisms.

In IDDM, tolerance to the pancreatic β-cells is lost, and immunological effector mechanisms are selectively directed against this cell type. Classical cell-mediated and humoral immune responses to β-cell antigens are involved. Many of the cytokines released by activated immune cells have direct toxic effects on islet cells and other cell types, including eosinophils, NK-cells and macrophages may also actively participate in the IDDM process. But β-cells are probably not passive to this attack. Considerable evidence suggests that in response to injury, protective and/or repair mechanisms are activated within the β-cells. Moreover, there may also be limited β-cell regeneration. In this perspective, we focus on these protective and/or repair mechanisms, review the potential cellular and molecular bases for these mechanisms, and discuss some clinical observations suggesting that the degree of β-cell damage in IDDM is in part determined by the balance between β-cell injury and β-cell defense and repair and/or β-cell proliferation (Fig. 1). This latter issue has been reviewed previously (1,2) and will not be discussed further herein.
EXPERIMENTAL EVIDENCES FOR β-CELL REPAIR UNDER IN VITRO AND IN VIVO CONDITIONS

Experimental evidence for β-cell repair after injury came initially from in vitro studies (3,4). Thus, when purified β-cells were acutely exposed to different cytotoxic substances, i.e., the diabetogenic drugs STZ or ALX, the free-radical generator t-butylhydroperoxide, or β-cell surface antibodies plus complement, some of these cells were able to survive (3). The observation that cell mortality varied with the culture conditions prevailing between the initial toxic insult and the assessment of cell death (performed after a 20-h culture period) suggested that defense mechanisms in pancreatic β-cells may have been activated (3). Additional observations supporting this hypothesis came from studies in which whole rodent islets of Langerhans were exposed to MMS (5), nitroso-N-methylurea (5), ALX (6), IL-1 (7–9), or elevated temperatures (heat shock) (10). In all of these cases, the β-cells went through an initial phase of suppressed glucose metabolism and decreased insulin release in response to glucose. However, after 3–7 days of culture in the absence of the toxic agent, the surviving β-cells were able to completely regain their function (5–10). Moreover, purified β-cells exposed for 20 h to IL-1 also regained their function after an additional 3-days culture in the absence of the cytokine (11). The exception was pancreatic islets exposed to STZ (12–14). Under these circumstances, long-lasting damage to β-cells was characterized by a persistent impairment in glucose metabolism at the mitochondrial level and a defective insulin response to glucose (13–15).

These in vitro observations are more likely attributable to β-cell repair rather than compensatory β-cell regeneration. The replicatory capacity of adult β-cells under culture conditions is <2% for both rat and mouse islets (1,2), and this value is further decreased in cultured purified β-cells (16). Moreover, β-cell proliferation does not increase significantly after in vitro islet exposure to STZ (17,18).

β-cell repair also has been demonstrated after in vivo β-cell damage. Perhaps one of the best suited experimental models for IDDM is the NOD mouse (19,20). Starting around 5 wk of age, these mice develop a progressive mononuclear cell infiltration in and around their pancreatic islets. This infiltrate will eventually evolve into a destructive insulitis, and by 18–24 wk of age most female mice are overtly diabetic (21). When pancreatic islets with insulitis were isolated from female NOD mice 5–7, 8–11, or 12–13 wk of age, the islets showed a deficient glucose-induced insulin release that progressively worsened with age. The observed β-cell dysfunction had a close correlation to the severity of the mononuclear cell infiltration and was accompanied by defective glucose metabolism (22,23). However, when these islets were cultured for 7 days, during which the islet mononuclear cell infiltrate was mostly depleted, both insulin release and glucose oxidation completely recovered (22,23) (Fig. 2). In a subsequent series of experiments, female 12- to 13-wk-old NOD mice were treated with monoclonal antibodies directed against infiltrating T-cells, and their islets isolated after a 10-day period (24). Islets obtained from control NOD mice, treated in parallel with vehicle alone or with normal rat IgG, had a severe mononuclear cell infiltration and altered glucose metabolism. The monoclonal antibody treatment markedly reduced the islet inflammatory reaction and restored islet glucose metabolism (24). These data suggest that in prediabetic NOD mice, a population of suppressed and/or partially damaged, but still viable, β-cells exist. If the immune assault is arrested, either by removing the islets from the in vivo environment (22,23) or by eliminating the invading T-cells with specific monoclonal antibodies (24), these cells can use repair mechanisms and regain normal function. Thus, both in vitro and in vivo experimental data point to the existence of efficient β-cell repair mechanisms.

POTENTIAL ENHANCERS OF β-CELL REPAIR

The vitamin B₃-derived compound nicotinamide has attracted considerable interest as a potential therapeutic
agent that might affect the development of IDDM (25–27). Nicotinamide can counteract STZ-induced diabetes in rodents (28), even when administered for up to 2 h after injection of STZ (29). It has been proposed that the protective effect of nicotinamide may be mediated by inhibition of STZ-induced PARP activation after DNA alkylation (30–32). Addition of nicotinamide to the culture medium immediately after exposure of isolated rat β-cells to either STZ or t-butylhydroperoxide or, to a lesser extent, ALX, inhibited cytotoxicity (3). It is likely that these in vitro effects of nicotinamide are accomplished by PARP inhibition, because the nicotinamide concentration was ≤10 mM. However, when using nicotinamide at a concentration >10 mM, the compound might also scavenge free oxygen radicals (33,34). At these higher concentrations, nicotinamide can also block the toxicity of chemically generated nitric oxide and of IL-1 to islet cells (35,36). In vivo experiments suggest that nicotinamide administration can prevent the outbreak of diabetes in NOD mice, attenuate the symptoms of already diabetic NOD mice (37), and counteract islet allograft destruction in NOD mice (38). The exact role of nicotinamide in β-cell recovery remains to be defined, but the combination of retarded activation of PARP and scavenging of free radicals might enhance the efficiency of other cellular repair mechanisms (see below).

Glucose at high concentrations (20–56 mM) has been shown to partly counteract damage to rodent β-cell in vitro, when present after the exposure of islet cells to various types of stress. Thus, culturing in high glucose limited the deleterious effects of ALX (3), t-butylhydroperoxide (3), STZ (3,39), β-cell surface antibodies plus complement (3), IL-1 (40–42), and cryopreservation (43). The mechanisms behind these beneficial effects of glucose remain unclear, but they may be related to acutely providing an energy source that allows the β-cells to activate energy-dependent processes involved in cellular repair. Indeed, the islet ATP contents declined after exposure to STZ (13,44,45) and IL-1 (42,46). Conversely, hyperglycemia may also exacerbate the IDDM disease process, because in vitro exposure of human islets to high glucose levels leads to both β-cell dysfunction (47,48) and increased expression of islet autoantigens, including glutamic acid decarboxylase (49–51). The interactions of glucose with β-cells in early IDDM are complex and multifactorial; which of these effects predominate and consequently whether the net effect of the hexose on the IDDM disease process is positive, negative, or neutral will require additional investigation.

From experiments with rats fed a high-protein diet (52,53) or a balanced diet supplemented with branched chain amino acids (54), we and others have shown that STZ-induced diabetes can be alleviated. This effect was associated with increased pancreatic insulin content and serum insulin levels (53), which could not be explained by increased β-cell proliferation (D.L.E. A. Hadad, and R.H. Migliorini, unpublished observations). Interestingly, diet supplementation with branched chain amino acids decreased the severity of encephalomyocarditis virus-induced diabetes in mice (55). These findings suggest that some amino acids can modulate β-cell repair, although peripheral effects on glucose homeostasis cannot be excluded.

β-CELL RESPONSES TO INJURY

HSPs. One of the best characterized responses of eukaryotic cells to noxious stimuli is the heat shock or stress response (56–58). This response features a rapid and coordinated increase in the expression of a group of proteins referred to as heat shock or stress proteins. A decrease in the synthesis of other cell proteins occurs simultaneously. Under physiological conditions, several HSPs function as molecular chaperones, i.e., they mediate the folding and assembly of other polypeptides and help direct them to their correct intracellular location (58,59). Under conditions of cell stress, HSPs (mostly HSP60 and HSP70) may stabilize denatured proteins and allow for either their subsequent refolding or disposal (56,59,60). Indeed, HSPs seem to be essential for the survival of cells exposed to environmental insults (58,61).

Pancreatic islets exposed either to high temperatures (heat shock; 42°C) (10,62), the cytokine IL-1 (62–65), or STZ (64), express three of these proteins, with ~32,000, 70,000–72,000, and 90,000 M, The 70,000-M, protein was identified by Western blot analysis as a member of the HSP70 family (62,64,65), whereas the 32,000-M, protein was identified as heme oxygenase (62). Heme oxygenase has antioxidant properties (66). Neonatal rat islets exposed to IL-1 present increased expression of this protein, and this was interpreted as a β-cell defense response to cytokine-induced oxygen free radical generation (62).

The major HSP70 seems to be of special interest in the context of diabetes. First, when purified HSP70 proteins are introduced into pancreatic islet cells by the liposome technique, they protect β-cells against the deleterious effects of IL-1β (67). Second, HSP70 genes have been localized within the HLA class III region of the MHC in humans (68). The HSP70 genes also have been located in the rat MHC (69) and the mouse H-2 complex (70). In humans, the duplicated locus encoding the HSP70 is located between the genes for complement and TNF-α (71). This area of the genome displays polymorphism, and differences in the frequencies of HSP70 and TNF-β genotypes between IDDM patients and control populations have been reported (71–74). The cytokine TNF potentiates the deleterious effects of IL-1 on β-cells (75,76). Because TNF may contribute to β-cell damage in early IDDM and HSP70 may have a role in β-cell defense, it is conceivable that susceptibility to IDDM is enhanced by unfavorable combinations of these genes (72). Clearly, this intriguing possibility deserves further investigation.

A recent study using immunocytochemical localization demonstrated the presence of the HSP60 in pancreatic islets obtained from both control SJL mice and prediabetic female NOD mice (77). In control mice, HSP60 was associated mostly with mature insulin granules and mitochondria, whereas in NOD mice with advanced insulinitis, the HSP60 protein was also localized in the cytoplasm.
and cell membranes (77). As described above, β-cells of prediabetic NOD mice have a decreased insulin release and impaired mitochondrial function (22,23). HSP60 may have a role in preventing protein denaturation, both in cytosol and mitochondria (58,60). It is thus possible that immune-mediated β-cell dysfunction may be a signal for redistribution of the HSP60, perhaps as part of activation of a defense mechanism in the β-cell.

**DNA repair.** Another possible consequence of different cell assaults is induction of DNA damage. Repair of DNA involves a network of enzymatic reactions catalyzed by a group of interacting gene products (78,79). Note that enzymes related to both DNA replication and repair are less prevalent in nonreplicating cells (79,80). Moreover, although it is well known that DNA damage in proliferating cells may lead to tumorogenesis, the impact of similar damage in cells with low rates of proliferation, like β-cells and neurons, still remains unknown (80).

The most well-characterized β-cell response to DNA damage, specially induced by alkylating agents such as STZ, is activation of the enzyme PARP (81). The enzyme uses NAD as substrate and catalyzes intracellular formation of poly(ADP-ribose) (81). Several roles for PARP in DNA repair have been suggested, such as inhibition of a Ca<sup>2+</sup>/Mg<sup>2+</sup>-dependent endonuclease, regulation of chromatin structure, dissociation and reassociation of histones with DNA (so-called DNA shutting), linkage between DNA and nuclear matrix, reversible blockade of free ends of DNA, and regulation of gene transcription (82–84). Furthermore, when the cell faces widespread DNA damage, it has been suggested that PARP may elicit cellular suicide to prevent tumorogenesis (85).

When exposed to an acute assault, most cellular activities nonrelated to actual cell repair are decreased (56). One of the mechanisms by which this decrease is effected is by synthesis of negative modulators of gene transcription and cell replication. Two genes related to this function are the growth arrest and DNA damage inducible genes 45 and 153 (86,87). The gadd genes were isolated after induction by DNA damage in CHO cells (88). Later, induction of gadd genes was observed after different noxious stimuli, like serum reduction (89), covalent modification of proteins (90), increase in intracellular Ca<sup>2+</sup> (91), or hypoxia (92). We have observed recently that rat pancreatic islets and HIT cells, a clonal hamster insulin-secreting cell line, increase the expression of gadd 45 and gadd 153 genes in response to the alkylating agents STZ and MMS (93). This increased expression is especially marked after MMS treatment. Because β-cells can recover their function completely after MMS exposure, but not after STZ administration (5,13,14,94), it may be worthwhile to further elucidate the role of gadd genes in repair of islet cell damage.

Finally, it must be mentioned that DNA repair in insulin-producing cells is not a homogeneous process. Thus, after an alkylation injury, active genes, such as the insulin gene, seem to be preferentially repaired compared with less active genes (95).

**Manganese superoxide dismutase.** A protein that may also be involved in β-cell repair and/or protection against subsequent injury is Mn-containing superoxide dismutase, an enzyme that catalyzes scavenging of toxic free oxygen radicals. After islet exposure to IL-1β, both Mn-containing superoxide dismutase mRNA and enzyme activity increase (96). Such an increase might be beneficial to β-cells in early IDDM, a situation in which these radicals are probably generated by cytokines and immune-effector cells in the islets.

**Possible β-cell outcome after injury.** A model for the β-cell outcome after different assaults is provided in Fig. 3. After damage, these cells undergo an initial stage of impaired function, characterized by decreased insulin release in response to glucose and a defective substrate metabolism at the mitochondrial level (4,14,97). During this phase, different repair mechanisms may be activated. Depending on the type and intensity of the cell assault, and on the effectiveness of β-cell repair mechanisms, the cells may or may not survive. In most cases, surviving β-cells regain their function and completely recover once the initial aggression is arrested (4). However, it appears that some forms of DNA damage, such as that induced by the alkylating agent STZ, lead to permanent β-cell dysfunction (4,12–14). This dysfunction is also characterized by a selective defect in glucose-induced insulin release and impaired islet nutrient metabolism (13,14,97). A similar selective defect in glucose-stimulated insulin release is observed in NIDDM (98) and in nondiabetic subjects during the preclinical stage of IDDM (99), suggesting that this may be a common response of β-cells to damage.

**CLINICAL OBSERVATIONS**

The fundamental, underlying biochemical lesion of IDDM is insulin deficiency. It is commonly assumed that this is a result of β-cell destruction and, based primarily on partial pancreatectomy experiments (100), that hyperglycemia only occurs after ~90% of the β-cells have been lost. Although nearly complete β-cell loss does characterize the final stages of the IDDM disease process (101), in the preclinical period and early after diagnosis, it is likely that β-cell loss is <90% and that much of the insulin deficiency present at these times is a result of functional inhibition of insulin secretion. In the context of this perspective, this concept is very important, because
one cannot postulate recovery from cell death, only from injury. Within 3–6 mo after the initial diagnosis of IDDM and subsequent treatment, most patients experience some recovery of β-cell function as reflected by increased C-peptide levels (102). Sometimes sufficient recovery of β-cell function allows patients to maintain normal or near-normal glucose levels temporarily, without the need for exogenous insulin. Moreover, cyclosporin A treatment of recently diagnosed IDDM patients results in more frequent and more prolonged remissions (103). These observations strongly suggest that at the time of IDDM diagnosis, some of the insulin deficiency is caused by functional inhibition of insulin release and that this inhibition is at least partially reversible. As discussed previously, data from the NOD mouse also supports the concept that early in the IDDM disease process much of the β-cell dysfunction is caused by a reversible lesion. β-cell function is markedly impaired when islets are isolated from mice with severe insulitis, but this β-cell function recovers with experimental procedures that reverse or block the insulitis process in vivo or in vitro (22–24) (Fig. 2).

Two additional observations provide further support for the concept that functional inhibition of insulin release is an important component of the islet lesion of IDDM. In vitro exposure of cultured islets or islet cells to cytokines such as IL-1, TNF, and interferon-γ, especially in combination, results in profound inhibition of insulin release and cytotoxicity, but these two cytokine effects can be discriminated, depending on the particular cytokine examined, the concentration used, and the combinations tested (104–106). By measuring insulin release and cytotoxicity separately, we have recently found that for some doses and combinations of IL-1 and TNF, the inhibition of insulin release is associated with islet cell death. Other doses, in contrast, cause loss of insulin secretion with little or no cytotoxicity (V.K. Metha, W. Hao, B.M. Brooks-Worrel, J.P. Palmer, unpublished observations). Similarly, Ling et al. (11) found that IL-1 caused both cytotoxicity and inhibition of insulin release from cultured islets, but only inhibition of insulin release from cultured purified β-cells.

The second observation comes from our studies correlating in vivo β-cell function with β-cell mass and pancreatic β-cell content in baboons receiving various doses of STZ. All three measurements were highly correlated, but pancreatic insulin content and in vivo measures of β-cell function approached zero when 40–50% of β-cell mass was still detectable histologically (107). These in vivo β-cell function tests are the same as those used to evaluate β-cells in human preclinical IDDM. Therefore, if these observations post-STZ are relevant to the islet lesion of human IDDM, ~50% rather than only 10% of β-cells may remain when high-risk individuals develop severely abnormal β-cell function tests. As we learn more about how to stimulate β-cell recovery, treatment may become available that can stimulate these remaining but insulin-deficient β-cells to regain normal or near-normal secretory function.

Since the discovery of ICAs in newly diagnosed IDDM patients in 1974 (108) and the subsequent observation that nondiabetic individuals were frequently ICA+ years before the onset of clinical IDDM, several groups initiated programs to identify and prospectively evaluate nondiabetic individuals at increased risk of subsequent clinical IDDM. As part of these studies, β-cell function tests are periodically performed, and in some individuals, a progressive decline in β-cell function is observed before the onset of IDDM. Initially, investigators from the Joslin Diabetes Center (Boston, MA) proposed that the fall in β-cell function was linear (109); therefore, the time to clinical IDDM could be accurately predicted (110). Subsequent studies by our group and others have observed more variable β-cell function over time (111–116). When β-cell function has been measured over several years in ICA+ first-degree relatives of IDDM patients, we have uncommonly observed a progressive decline in β-cell function. As illustrated by 4 separate individuals from the Seattle Family Study (Fig. 4), a few individuals with low β-cell function when first evaluated have had a further decrease and subsequently developed clinical IDDM; in many others, β-cell dysfunction is relatively stable and/or shows considerable fluctuation over time. We have proposed previously that in individuals with slightly impaired but stable β-cell function, this is likely the result of a prior β-cell insult from which the cells have partially recovered (113). The wide fluctuations in β-cell function may well represent transient episodes of immune attack followed by periods of relative quiescence and β-cell recovery. Our finding that glucose tolerance during the intravenous glucose tolerance test is strongly correlated with β-cell function in first-degree relatives of diabetic patients suggests that these changes in β-cell function over time are not normal physiological changes.

Perhaps the data that best support the concept of β-cell repair and recovery of function come from Kobayashi et al. (117). They evaluated a group of ICA+ diabetic patients who subsequently became ICA+. This change in ICA status was associated with an improvement in both insulin secretion and carbohydrate tolerance. In contrast, β-cell function continued to deteriorate

**FIG. 4. β-cell function, measured as the AIR to glucose and expressed in percentiles, compared with the normal population, from 4 ICA+ nondiabetic individuals followed prospectively in the Seattle Family Study. (X), developed clinical IDDM after 28 mo.**
FUTURE STUDIES

Up to now, most studies dealing with the pathogenesis of IDDM have been directed toward the possible mediators of the immune assault against β-cells. The observations described above suggest that attention must also be paid to the repair mechanisms activated by β-cells after injury. Indeed, a detailed understanding of the cellular and molecular mechanisms mediating β-cell damage and repair may lead to the development of effective early treatments to prevent β-cell destruction in IDDM. The genes and proteins discussed above are probably only a minor part of a complex network of repair mechanisms. As new repair mechanisms are discovered in other cell systems, it will be of importance to evaluate their presence both in rodent and human β-cells and to correlate their expression after cell damage with β-cell survival and functional outcome. Moreover, in vitro manipulation of these systems, both by introducing the desired proteins into β-cells with liposome techniques (67) or by cell transfection with the gene of interest (119), can provide direct evidence for the role of the potential protecting agent against different β-cell toxins. Such experiments may be extended to in vivo models by preparing transgenic mice, in which putative protective agents, e.g., HSP70 or Mn-containing superoxide dismutase, are linked to the insulin promoter. Nearly all transgenic mice designed to study diabetes pathogenesis to date have been related either to expression of β-cell antigens or to expression of potential mediators of β-cell destruction, like cytokines (120,121). Thus, it would be of interest to induce expression of β-cell defense proteins and test the resistance of these animals to drug, viral, or immune-mediated β-cell destruction. Finally, it will be extremely important to characterize whether some or all of the putative repair mechanisms are expressed in β-cells during the prediabetic period, using both NOD mice and BB rats. This may be achieved by immunocytochemical and in situ hybridization techniques. Both techniques have already been used successfully to study expression of gene transcripts and proteins involved in immune-mediated β-cell destruction in early diabetes in rodents (122,123).

Once these experimental studies provide a clearer picture of relevant repair mechanisms expressed by β-cells, they may be extended to human IDDM. In this respect, the first step will probably be to assess whether a polymorphism exists in the expression of genes encoding these proteins and whether any correlation exists between specific alleles and the prevalence of IDDM. Perhaps to succeed in solving the IDDM puzzle we must follow G.K. Chesterton's advice from *The Man Who Was Thursday:*

You only see the tree by the light of the lamp. I wonder when you would ever see the lamp by the light of the tree.

Indeed, it may be time to start seeing IDDM not only by the light of the immune effector cells, but by the light of the β-cells.

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