The Potential for Stem Cell Therapy in Diabetes

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ABSTRACT: Both type 1 and type 2 diabetes are characterized by a marked deficit in beta-cell mass causing insufficient insulin secretion. Beta-cell replacement strategies may eventually provide a cure for diabetes. Current therapeutic approaches include pancreas and islet transplantation, but the chronic shortage of donor organs restricts this treatment option to a small proportion of affected patients. Moreover, recent evidence shows a progressive decline in beta-cell function after islet transplantation so that most patients have to revert to insulin treatment within a few years. In this article recent progress in the generation, culture and targeted differentiation of human embryonic stem (ES) cells is reviewed, and some of the issues surrounding their use as a source of beta-cells are discussed. (*Pediatr Res* 59: 65R–73R, 2006)

A SOURCE OF BETA-CELLS, THE NEED

Glucose homeostasis requires finely regulated insulin secretion by pancreatic beta-cells present in islets of Langerhans (1). In health, under fasting basal conditions insulin is secreted at a rate of ~ 2 pmol/kg/min (2,3) and after meal ingestion this rate increases by as much as ~ 5 – 10 -fold (4). To accomplish this requires not only normally functioning beta-cells, but also a sufficient number of beta-cells, collectively often referred to as beta-cell mass. In health, the human pancreas contains approximately one million islets, each containing approximately two thousand beta-cells (5–8). Thus, the beta-cells constitute $\sim 1.5\%$ of the total pancreatic mass (1–2 g in total) (8). While in humans, an up to 40% loss of beta-cells can be tolerated without a significant deterioration of glucose tolerance (9), a further reduction in beta-cell mass leads to hyperglycemia.

Type 1 diabetes is caused by autoimmune-mediated destruction of beta-cells (10–16). Longitudinal studies of insulin secretion in humans at risk for type 1 diabetes show declining first phase insulin secretion years before the onset of hyperglycemia which has been interpreted as being due to declining beta-cell mass (17,18). Once hyperglycemia develops as much as 90% of beta-cells have been lost (12–14,19,20). Some residual insulin secretion may persist in people with long-standing type 1 diabetes (21–24), consistent with autopsy

studies reporting scattered beta-cells and occasional islets with beta-cells present in patients with long standing type 1 diabetes (12–14,25). Indirect evidence suggests that these beta-cells may be present because of ongoing beta-cell formation, although the source of these cells remains unknown (25).

Type 2 diabetes is also characterized by an $\sim 65\%$ decrease in beta-cell mass (9,26), associated with a ~ 10 -fold increase in beta-cell apoptosis (9,27). In contrast to type 1 diabetes this increased apoptosis is not thought to be due to autoimmune disease. Toxic oligomers of human islet amyloid polypeptide (hIAPP) (9,28,29), glucose and FFA-induced toxicity have all been implicated (30–34). A comparable reduction in beta-cell mass in pigs, dogs and non-human primates also leads to hyperglycemia (35–39). Taken together, these data highlight the importance of beta-cell mass for the maintenance of normoglycemia. Therefore, beta-cell replacement is a potential therapy that might reverse rather than simply palliate both type 1 and type 2 diabetes. Pancreas transplantation is effective, improving quality if not duration of life in people with type-1 diabetes (40–42). However, limited organ availability and the risks associated with relatively major surgery and life-long immunosuppression limit the use of this option (41,42). Islet transplantation overcomes the need for major surgery but is far from being a risk-free procedure; it does not overcome the limitation of organ availability and is much less successful than pancreas transplantation at accomplishing sustained insulin independence (43–46). The continued need for an alternative means of replacing beta-cells in people with diabetes has fostered scientific and public interest in the potential of embryonic stem (ES) cells as a potential therapy.

WHY EMBRYONIC STEM CELLS?

Human ES cells are derived from the inner cell layer of the blastocyst (Fig. 1) (47,48). These cells subsequently give rise to all differentiated cells in the adult through a series of cell fate choices that involve self-renewal and differentiation (47–51). Therefore they can theoretically be differentiated into any definitive cell type, including pancreatic beta-cells, when exposed to the appropriate signals in the correct sequence and

Received November 18, 2005; accepted December 6, 2005.

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Supported by grants from the US Public Health Service NIH DK 59579 and 61539 [P.C.B.] and DK68763 [A.B.], the Larry L. Hillblom Foundation, the Juvenile Diabetes Research Foundation (7-2005-1152 [P.C.B.], 1-2005-1174 [A.B.] and 5-2006-330 [A.B.]) and the Deutsche Forschungsgemeinschaft (Me 2096/2-1).

DOI: 10.1203/01.pdr.0000206857.38581.49

Abbreviations: ES cells, embryonic stem cells; FACS, fluorescence-activated cell sorting; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; hIAPP, human islet amyloid polypeptide; NGN3, neurogenin3; XIAP, X-linked inhibitor of apoptosis protein

Figure 1. Generation of human embryonic stem cells. A few-celled embryo gives rise to the blastocyst, a structure comprised of an outer cell layer, the trophoectoderm, and the inner cell mass. The inner cell mass is harvested and plated on feeder cells to yield a population of embryonic stem cells. (Figure adapted from Landry *et al.*, J Clin Invest 114:1184–1186, copyright © 2004 by the American Society for Clinical Investigation, with permission.)

over the appropriate time periods (pluripotency) (48). Moreover, if the source of such cells was truly unlimited, it would be possible to provide new treatments from time to time as required if the cells were not self renewing once terminally differentiated. To obtain an unlimited supply of human ES cells it is necessary to establish human ES cell lines that behave like primary ES cells.

OBSTACLES ASSOCIATED WITH THE USE OF EMBRYONIC STEM CELLS

Despite the obvious appeal of ES cell-based beta-cell replacement therapy for diabetes, there are several major obstacles that must be overcome before this approach can be realistically considered as a therapeutic option.

Ethical and religious sensitivities. There are ethical and religious sensitivities concerning use of human embryos that still hamper the broader use of ES cells for research purposes, and many governments ban or at least highly restrict this kind of research (52,53). The Roman Catholic Church has repeatedly demanded an international ban of human ES cell research, since this requires the destruction of human embryos, and in their view, the human embryo has all the moral rights and protection as any other human being (54,55). Owing to these moral concerns, the United States Congress has enacted a broad ban on federal funding for human ES cell research. Later, this ban was loosened to allow research on human stem cell lines that already existed. A highly debated question is whether stem cells from human embryos should be used to generate stem cell lines, given that they were not suitable for fertilization programs and therefore would otherwise be discarded. The standpoint of the religious authorities on this matter is well exemplified by a recent statement from U.S.

Figure 2. The role of islet transcription factors in endocrine differentiation in the developing pancreas. Figure adapted from Wilson *et al.*, Mech Dev 120:65–80, Copyright © 2003 by Elsevier Science Ireland Ltd., with permission.

President George W. Bush saying that, “There is no such thing as a spare embryo” (New York Times, May 26, 2005). A central question relating to this discussion is, when an embryo is to be considered dead. Thus, Landry and Zucker recently pointed out that depending on the definition of death before the onset of neural development, a significant fraction of these human embryos will be found to be “organismically” dead (56). Based on this standpoint, using such embryos to generate human stem cell lines would not contradict the ethics of the Catholic Church and other religious authorities. Just recently, two papers have been published describing novel techniques to derive mouse stem cells without affecting the subsequent development of the embryo (57,58). If similar techniques can be applied to human embryos, this may resolve some of the ethical concerns regarding the generation of ES cell line.

Establish and expand cell lines. The first step before ES cells can be used as therapy is to obtain ES cell lines from human embryos and to expand these without loss of their pluripotential properties, or being contaminated in ways that would preclude use as a therapy. ES cell lines have been established from rodents, rabbits, pigs, primates and humans (48,50,51,59–62). Human ES cells have typically been expanded as undifferentiated colonies on feeding layers of mouse embryonic fibroblasts. ES cells differentiate into ectodermal, mesodermal and endodermal structures after removal from this layer (50). The use of mouse fibroblasts as feeding layers has contaminated some of the limited supply of human ES cells available with mouse genes, precluding their therapeutic potential (50,51,63,64). However, it is possible to expand human ES cells without mouse feeder layer cells so that in future human ES cells can be expanded without this problem (65), although obtaining new ES cell lines is limited by the unresolved religious and ethical concerns raised earlier. Thus successful culture of human ES cells over 30 passages has recently been described in the absence of mouse embryonic fibroblasts by using only serum replacement media and high concentrations of basic fibroblast growth factor (66).

Development. The second step before ES cells can be considered as a therapeutic option for diabetes is to establish the means to drive development of the ES cell lines to differentiated and functioning beta-cells. In normal human

development ES cells are exposed to numerous complex and as yet minimally understood signals to generate fully differentiated beta-cells within the organelle, the islet of Langerhans. These include signals arising from both within the future developing beta-cells to activate transcriptional programs, but perhaps more importantly, neighboring cells that are not destined to form beta-cells can signal to influence progenitor cell differentiation into beta-cells (67–73). In the human embryo this process by definition begins at conception and is ongoing during the first year of life (74,75). Beta-cells develop from progenitor cells arising along with exocrine and ductal tissue in the evolving three-dimensional architecture of the primordial pancreatic bud from the foregut.

During embryonic development the pancreas forms from a ventral and a dorsal bud, protrusions of the primitive gut endoderm that fuse to form the pancreas. Subsequently, a cascade of transcription factors are activated which initiate further development and differentiation toward the specific cell types (Fig. 2). The specific hierarchy of these factors has previously been summarized in an elegant review by Wilson *et al* (68). A key player in this system is the transcription factor *PDX-1* (also referred to as *ipf1*), which is commonly expressed in all pancreatic progenitor cells (73,76,77). Also, *PDX-1* knock-out mice are characterized by an apancreatic phenotype (76). The cell fates choices of pancreatic progenitors are regulated by Notch signaling through HES1 activation (76,78,79). The transcription factor neurogenin3 (*NGN3*) plays an essential role in the differentiation of endocrine cells from the pool of pancreatic progenitor cells (80,81). Once *NGN3* is activated and the cell is determined toward an endocrine phenotype, a number of different factors control the ultimate fate as an alpha-, beta-, delta- or PP-cell. Specifically, activation of *Nkx2.2*, *Pax4* and *Nkx6.1* appears to be crucial for the differentiation of a beta-cell phenotype (68,82).

While there has been considerable progress in establishing some of the transcriptional programs required to develop adult beta-cells, most of the signals that initiate the transcriptional program remain unknown. As yet, little is known about the three-dimensional origins of signals that direct beta-cell development to develop in relation to one another and other cell types in the islet of Langerhans. In embryogenesis the developing beta-cells are organized in relation to developing vascular and neural input. It is likely that signals arising from these orchestrate aspects of islet development and yet inevitably these are not included in *ex vivo* culture (83,84). To emphasize the importance of extra-cellular signals, it is clear that extra-cellular matrix proteins play a major role in islet cell differentiation (85–87).

Since it takes more than 18 months to establish functional beta-cell mass in developing humans (75,88), it is not yet clear if it will be possible to drive ES cells to a useful mass of beta-cells or beta and other cell type aggregates, *ex vivo*. A concern is the fact that human ES cells tend to undergo senescence and differentiation within days in culture thereby losing their pluripotency (89).

Rejection. Another obstacle that will have to be overcome before ES cells can be used as therapy is to protect the resulting beta-cells from rejection, to avoid the need for

immunosuppression. Theoretically, ES cells can be manipulated *ex vivo* to induce immune-compatibility with the intended recipient (90,91) avoiding the need for immunosuppression and its attendant side effects (44,45).

Progress to date. Several *in vitro* studies have demonstrated that it is possible to obtain cells that express insulin from human ES cells (63,92,93). Given the discussion of the challenges that must be overcome above, it is not surprising that functionally useful beta-cells have not yet been obtained from human ES cells (63,92,93). Cells that have been generated from human ES cells that express insulin have been minimally glucose responsive (63). Expression of typical beta-cell markers, such as insulin, GLUT-2 and glucokinase, has been detected in some of these cells (94,95). However, insulin staining in ES cell-derived tissue preparations should be interpreted with caution, because insulin staining can overestimate the proportion of beta-cells, when insulin is present in the culture medium (96). Perhaps not surprisingly, preparations of human ES cells usually consist of a mixture of different cell types and purification of insulin-secreting cells from such cell clusters has proven to be technically difficult (64). Thus, even using various strategies to direct human ES cell differentiation toward a beta-cell-like phenotype, the overall yield of insulin-expressing cells is typically less than 1–3% of the total cell number. Current attempts to purify those cell populations include fluorescence-activated cell sorting (FACS) as well as the use of magnetic tagged antibodies against specific beta-cell surface markers (64). Alternatively, ES cells can be transfected with genetic constructs that couple the human insulin gene to drug resistance genes (*e.g.* the neomycin resistance gene). Through these means, beta-cells can be selected according to their resistance to neomycin treatment (64). However, none of these methods has yet yielded a 100% pure preparation of insulin-secreting cells.

Islets or beta-cells? An unresolved question: Exactly what is the target form that beta-cells should take if successfully developed from human ES cells? Adult pancreatic islets have a complex architecture, with the beta-cells being more preferentially located in the islet core and the other cell types, such as alpha-, delta- and PP-cells, being more abundant in the islet periphery (4,8). The main vascular supply of the islet comes from an arteriole that enters the islet from its beta-cell enriched core, from where the blood is being passed to the islet periphery (8). Also, beta-cells are tightly connected to each other *via* gap junctions, thereby allowing direct electrical coupling between neighboring cells (97). This specific structure of the islets appears to be important for the coordinated discharge of the different islet hormones (4,98,99). Thus single beta-cells obtained from islets are much less responsive than beta-cells electrically coupled (100). Insulin in health is secreted in discrete secretory bursts, the primary mechanism through which insulin secreted is regulated being the modulation of burst mass (101–105). Moreover insulin secreted per islet at a given stimulus of glucose is substantially increased once the islets are interconnected by a neural network (106). While it is already a considerable challenge to derive functional beta-cells from human ES cells, it would be altogether a more daunting challenge to recapitulate functional islets

Figure 3. Section of pancreas ($\times 20$ magnification) of a non-diabetic human subject stained for insulin (brown). Numerous pancreatic ducts are shown, with insulin-positive cells present in the duct walls. This finding has often been interpreted as an indicator of new islet formation. Figure reprinted from Butler *et al.*, Diabetes 52:102–110, Copyright © 2003 by the American Diabetes Association, Inc., with permission.

from human ES cells. Should beta-cells derived from human ES cells be established in micro cell clusters, or as a single organ? Would such an aggregate of beta-cells be functional, and most importantly not lead to life threatening hypoglycemia?

Where shall we put them? Assuming the limitations outlined above could be overcome, the next question arising would be where to best transplant such human ES-cell derived insulin-secreting cells, cell clusters or islets. Several considerations arise. Human ES cells have the potential to develop teratomas and possibly other cancers (107). Should these cells or cell clusters therefore be encapsulated so that they cannot escape? If so the issues of how large should the capsules be, where should they be implanted, how many would be required, and how long would the cells or cell clusters last in the capsules would have to be addressed.

If the newly formed beta-cells, cell clusters or islets could be transplanted without concern of cancer, then the optimal tissue bed to transplant them would presumably have a relatively high oxygen tension since beta-cells are sensitive to anoxia (108). Also it would need to be established if the cells, cells clusters or islets should direct their secretion into the hepatic portal circulation or the systemic circulation. Given the pivotal role of the liver as the major insulin-responsive organ (109), it seems most desirable to mimic the physiologic route of intra-portal insulin delivery. The fact that intra-portal transplanted islets deliver their insulin directly to the liver rather than into the systemic circulation (110) may therefore provide an argument in favor of intra-portal islet transplantation. Alternatively, the intestinal mucosa may be an attractive transplantation site because of its rich vascularisation and the high local concentrations of potential growth factors such as glucagon-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP), GLP-2, gastrin, etc. (111,112), but the surgical implantation of beta-cell clusters into the gut may be technically more demanding.

How long will they last? Based on the recent experiences with islet transplantation in humans, the life span of an islet

Figure 4. Beta-cell turnover in adult humans. Beta-cell mass is regulated by the new formation of cells *via* replication of existing beta-cells or new islet formation from adult stem cells as well as by the rate of beta-cell apoptosis. Deficient beta-cell mass in patients with diabetes may therefore be restored by either enhancing new beta-cell formation or by inhibiting beta-cell apoptosis.

appears to be limited and most patients who had received islet transplantations have to revert to insulin treatment within less than five years (46). Thus, to provide an ultimate cure for diabetes, human ES cell-derived islets or beta-cells would either need to maintain their ability to proliferate, or the transplantation procedure would have to be repeated at regular intervals. Assuming the former possibility, one major challenge would be how to control the proliferative activity of these cells to maintain a physiologic balance between apoptosis and replication. Adult human beta-cells apparently exhibit some plasticity which allows them to respond to increasing secretory demands, *e.g.* caused by insulin resistance or obesity, by increasing both in number and size (9,113,114). It is unlikely that this complex regulation of beta-cell mass could resemble insulin-secreting cells derived from human ES-cells. Moreover, potential risks imposed by continued replication of ES-cell derived transplants include loss of cell cycle control and the induction of neoplastic cell growth. Consistent with this consideration, the formation of teratomas has been observed in insulin-producing cell lines derived from ES-cells (107). Therefore, maintaining of a physiologic balance between replication and cell death appears to be a major challenge for beta-cell replacement therapies based on ES-cells.

NEW BETA-CELL FORMATION FROM ADULT STEM CELLS

There is an ongoing controversy regarding the existence of stem cells for new beta-cells in adult individuals (115–118). Such adult stem cells have been suggested to reside in exocrine pancreatic parenchyma (119), pancreatic ducts (113,120,121), pancreatic islets (122,123), liver (124), spleen (125), and in bone marrow (126). However, there is no direct evidence that directly supports the existence of any of these stem cell candidates for beta-cells in humans.

Stem cells in exocrine ducts. Some studies support the idea of new islet formation from exocrine duct cells (113). This

concept first evolved from the budding of the endocrine pancreas observed during embryonic development (73), and has gained support from different lines of evidence: Thus, endocrine cells can frequently be detected in exocrine ducts in both rodents and humans, and islets adjacent to exocrine ducts are commonly found throughout the pancreas (Fig. 3) (9,10,14,113,121). Moreover, in rodents the number of these ducto-insular complexes is increased during conditions of high secretory demands, *e.g.* during chronic glucose infusion or after partial pancreatectomy (121,127), implying compensatory new islet formation. In the adult human pancreas, exocrine ducts are often surrounded by areas of fibrous tissue, and the extent of this fibrosis is markedly increased in patients with type 1 diabetes (12,13,25,128). These findings seem to be consistent with the concept of attempted new islet formation and subsequent cell death leading to chronic inflammation in the peri-ductal area. Moreover, the presence of single beta-cells in the adult human pancreas has been interpreted as an early stage of differentiation of endocrine progenitor cells arising from the ductal epithelium (113). Recently, high levels of PDX-1 (IPF-1) expression were reported in human pancreatic ducts, but not in acinar tissue, suggesting additional similarities between islets and ductal cells (129). However, while all these findings suggest that the ductal epithelium does indeed harbor endocrine progenitor cells, there is no direct evidence, *e.g.* from lineage tracing studies, for this mechanism. It therefore cannot be excluded with certainty that the co-localization of endocrine cells with ductal cells in the adult pancreas is a coincidence rather than a consequence of ductal cell-derived new beta-cell formation.

Transdifferentiation of exocrine cells. Transdifferentiation of exocrine acinar cells into beta-cells has been proposed as an alternative mechanism for new beta-cell formation in the adult human pancreas (122,123). This concept has been supported by the observation of single beta-cells scattered throughout the exocrine parenchyma in a patient with type 1 diabetes after immunosuppressive treatment (130). Moreover, a number of recent studies have shown that under *in vitro* conditions acinar tissue can be directed toward a beta-cell like phenotype (120,131,132). However, it is important to note that the appearance of scattered single beta-cells in exocrine parenchyma is a common finding in non-diabetic pancreas as well and that the mere presence of these cells cannot directly prove that they were indeed originated from acinar cells (9,14). Also, directed transdifferentiation into insulin-secreting cells under culture conditions has been described for a number of different cell types and therefore cannot be taken as evidence for *in vivo* transdifferentiation (133–135).

Epithelial-to-mesenchymal transition. Recently, the concept of epithelial-to-mesenchymal transition has been proposed to account for new beta-cell formation in adult individuals (136). Based on this theory, adult beta-cells de-differentiate into fibroblast-like cells, which are capable of migrating and proliferating, and subsequently re-differentiate into hormone-expressing islet cells (136). However, given the obvious difficulties of carrying out longitudinal studies on human pancreatic tissue, it is difficult to judge whether this

mechanism plays a role for beta-cell regeneration in adult humans.

Bone marrow-derived stem cells. A highly controversial question is, whether new beta-cells could originate from bone-marrow derived cells (126,137–139). Ianus *et al* performed experiments, in which bone marrow from male donor mice that expressed GFP as well as CRE under the INS2 promotor, was transplanted into female mice that were depleted of their own bone marrow by irradiation. They reported that after 4–6 wk 1.7–3.0% of the islet beta-cells were derived from bone marrow cells (126). However, other investigators using similar experimental approaches failed to confirm these results (138–140).

REPLICATION AS THE PRIMARY MECHANISM OF NEW BETA-CELL FORMATION?

Recently, the existence of stem-cells for beta-cells in adult individuals has been challenged by the demonstration that in adult mice beta-cell replication almost exclusively accounts for the formation of new beta-cells (141). Using lineage tracing experiments, Dor *et al* showed that the vast majority of adult beta-cells were derived from pre-existing beta-cells, thereby ruling out new-beta-cell formation from adult stem cells (141). Moreover, recent studies in cyclin D2 knockout mice demonstrated that replication is the primary mechanism for maintaining postnatal beta-cell mass (71). However, the frequency of β -cell replication (Ki67 and insulin labeling) in adult human beta-cells is much lower than compared with that in rodents (9,25). It is therefore possible that β -cell turnover in humans is accomplished from a different source than in rodents.

STRATEGIES TO REPLENISH BETA-CELL MASS FROM ENDOGENOUS SOURCES

While the sources of new beta-cell formation remain to be elucidated, there is indirect evidence that ongoing beta-cell turnover may be present in adult humans. Beta-cell mass adaptively increases under conditions of high insulin resistance, *e.g.* in response to obesity or during pregnancy (9,142,143). The invariable presence of some beta-cell apoptosis in adult human pancreas, even in from non-diabetic individuals strongly suggests that some concomitant new beta-cell formation must be occurring to maintain beta-cell mass (9,25).

Assuming that adult beta-cells do turnover throughout life, beta-cell mass in patients with diabetes could theoretically be replenished by two different approaches: First, by enhancing new beta-cell formation, and second by inhibition of beta-cell apoptosis (Fig. 4). A number of approaches have been suggested to enhance beta-cell mass by increased beta-cell formation. Those include gut hormones, such as gastrin (144–146), GLP-1 (147,148), or GIP (149), growth factors, such as hepatocyte growth factors (150), epidermal growth factor (144), IGF-1 or -2 (IGF-1, IGF-2) (151–153), TGF- α (154,155), and growth hormone (153), or other factors such as betacellulin (154,156). Since most of these approaches have been examined either *in vitro* or in rodent models, it is not yet

clear if they are capable of enhancing beta-cell formation in humans. Also, newly forming beta-cells have increased vulnerability to apoptosis (157,158). Therefore, enhancing beta-cell replication and/or new islet formation in patients with type 1 or type 2 diabetes may lead to increased beta-cell apoptosis without a net gain in beta-cell mass.

The alternative approach is to target inhibition of excess beta-cell apoptosis in diabetes. Thus, given the putative ongoing beta-cell formation in adult life and the well documented increased beta-cell apoptosis in both type 1 and type 2 diabetes (9,25), reducing the rate of β -cell apoptosis may allow for beta-cell regeneration in these patients. In rodent models of type 1 diabetes, inhibition of beta-cell apoptosis leading to partial or total recovery of beta-cell mass has been achieved using many different approaches. In a recent review article, Roep and Atkinson were able to list 192 different approaches to cure diabetes in NOD mice (159). In humans, however, no intervention has accomplished comparable reversal of diabetes (160). To date the best outcome has been prevention or delay of the loss of β -cell function in patients with new-onset type 1 diabetes treated with CD-3 antibodies (161). In type 2 diabetes, beta-cell apoptosis has been suggested to be caused by the formation of toxic oligomers of hIAPP (9,29,162–164), free radicals, $\text{I}\beta$ and lipotoxicity (30–34;165). Although no specific strategies to prevent these putative toxic actions in humans, nonspecific inhibitors of beta-cell apoptosis have been described, many of which exhibit mitogenic properties as well (*e.g.*, GLP-1, gastrin, IGF-1 (146,148,153)). Other interesting candidates may be the potassium-channel openers (166,167), adiponectin (168), Inhibition of protein kinase C delta (169), inhibitors of $\text{NF}\kappa\text{B}$ (170), or the X-linked inhibitor of apoptosis protein (XIAP) (171), antioxidants (Vitamin C, E, NAC) (172), inhibitors of iNOS (173) or anti-inflammatory drugs, such as COX inhibitors or aspirin (174).

Rates of beta-cell turnover in humans. Successful use of the approach of suppression of beta-cell apoptosis to increase beta-cell mass from endogenous β -cell regeneration requires there to be sufficient new β -cell formation. Finegood *et al* attempted to quantitatively assess beta-cell turnover by using the frequencies of BrdU or thymidine incorporation in β -cells in rats (175). Based on these data, a turnover rate of $\sim 2\%$ beta-cells per day was calculated in adult rats (175). However, using continuous long-term BrdU labeling in adult mice, only 1/1,400 beta-cells underwent replication per day (176). Assuming no additional input from new islet formation, transdifferentiation or other potential sources, this would correspond to a proliferation rate of 0.0701% per day. Thus, even assuming a 0% rate of beta-cell death, recovery of a 50% deficit in beta-cell mass would be expected to occur after $\sim 1,429$ d, a time period that far exceeds the typical life span of a mouse. In humans, similar calculations are difficult to perform, since BrdU labeling cannot be used for obvious reasons, but based on the reported frequencies of Ki67 labeling, the turnover rate of beta-cells seems to be even slower (9,25,113,142). On the other hand, the increase in beta-cell mass observed in humans during pregnancy (143) implies that

this turnover rate can be increased by several-fold under certain conditions even in adult humans.

SUMMARY AND CONCLUSIONS

Major achievements in the isolation, culture and targeted differentiation of ES cells prompt hopes that it will one day be possible to replenish beta-cell mass in patients with diabetes using ES cell-derived engineered insulin-producing cells. However, in light of ethical concerns and technical obstacles still to be overcome, this approach is unlikely to be available as a therapy in the near future. Therefore, alternative methods to increase the number of insulin-secreting beta-cells in patients with diabetes should continue to be explored. The role of adult stem cells in the formation of new beta-cells is controversial, and the origin of such cells is unclear. In rodents the plasticity of the endocrine pancreas in adults and its ability to compensate for an experimental reduction of beta-cell mass suggests that there is ongoing regulated β -cell turnover in adults which can be targeted to reverse diabetes. The potential for this approach in humans is much less clear but is the basis of active investigation at present.

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