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Islet growth and development in the adult

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Diabetes mellitus, whether type 1 (insulin-dependent diabetes mellitus; IDDM) or type 2 (non-insulin-dependent diabetes mellitus; NIDDM) results from an inadequate mass of functional pancreatic β -cells. In the first case, the β -cell mass is reduced by the autoimmune destruction of the β -cells, while, in the latter, there is incomplete compensation to meet the demand often imposed by insulin resistance. This latter point is not always appreciated. However, only 20% of those people with severe insulin resistance due to obesity, Cushing's disease, or acromegaly become diabetic, with the other 80% being able to compensate to maintain near-normal glycemic levels.

While data for the human are limited, a careful morphometric study of autopsied pancreases from NIDDM and non-diabetic patients (Kloppel *et al.* 1985) showed that, in both the diabetic and non-diabetic pancreas, the β -cell mass was about 40% increased in the obese subjects as compared with lean subjects, suggesting that there is compensatory growth of β -cell mass with the increasing insulin resistance of obesity. Experimental evidence from rodents showed that there is substantial compensatory effort by the β -cells to maintain normal glycemic levels in the face of obesity and insulin resistance. Two types of compensation can occur: a functional one in which each β -cell secretes more insulin and a second one in which there is a change in β -cell mass. Functional adaptations, such as the changes in threshold for glucose-induced insulin secretion that occur during pregnancy (Sorenson *et al.* 1987) and glucose-induced increases in glucokinase activity (Chen *et al.* 1994), are also involved in the maintenance of glucose homeostasis. Nonetheless, the β -cell mass itself is the major factor in the amount of insulin that can be secreted, and experimental evidence clearly shows that β -cell mass can increase or decrease. Thus, a better understanding of the regulation of the islet growth and development after

birth may provide important new strategies for the therapy of diabetes. The following discussion will highlight examples of our studies that address the question of regulation of islet growth in the adult.

Our underlying premise is that the functional β -cell mass is dynamic and is regulated to maintain euglycemia (Fig. 1). At any one time the number of β -cells is determined by the balance of cell renewal and cell loss (Finegood *et al.* 1995). Inherent in the regulation of β -cell mass is the question of β -cell turnover. Are β -cells slowly renewed or constantly, albeit slowly, expanding in mass? The replication rate after the neonatal period is low, and thus many have assumed that the β -cell mass does not turn over significantly and that one is born with all the β -cells one will ever have. Such reasoning ignores the fact that the β -cell mass continues to grow well into adulthood. As shown with morphometric data from wild-type mice of the same mixed background (C57Bl/6J/129J) (Withers *et al.* 1998, Bruning *et al.* 1997, Kopin *et al.* 1999, Westphal *et al.* 1999),

FIGURE 1. A schema for the dynamic state of β -cells. The β -cell population has a slow turnover, with cell renewal coming from replication of pre-existing β -cells and from differentiation of cells that act as precursors in the adult ductal epithelium. Cell deletion is by apoptosis. Hypertrophy of individual cells can increase the β -cell mass but not the number.

FIGURE 2. The β -cell mass of wild-type control mice of a mixed strain background (C57Bl/6J/129J) and body weight were found to be linearly related ($y = -0.27 + 0.054x$; $r = 0.831$). Each point represents one male mouse; the different symbols are from different aged mice as used in each experiment and represent the spread in body weight at any age; here, the youngest animals were studied at 4 weeks of age. These data were reported as the meaned controls in the following publications: Withers *et al.* 1998, Bruning *et al.* 1997, Kopin *et al.* 1999, Westphal *et al.* 1999. We showed previously (Finegood *et al.* 1995) that in the neonatal period of rats there is a remodeling of the endocrine pancreas and the β -cell mass does not increase for several days since there is increased apoptosis.

β -cell mass and body weight are linearly related (Fig. 2). We have previously published similar data on the relation of β -cell mass and age in the Sprague–Dawley rat (Finegood *et al.* 1995) and discussed a simple mass model equation that represents the normal expansion of β -cell mass. Hellerstrom *et al.* (1988) pointed out that with a β -cell birth rate of just under 3% new cells per day, the rodent β -cell mass would double in 1 month if there were negligible cell death. However, since the β -cell mass does not continue to double on a monthly basis throughout life, the β -cell, like most other cell types, must have a finite life span (Finegood *et al.* 1995). Physiological examples of apoptosis of β -cells have been shown *in vitro* (Rabinovitch *et al.* 1994) and *in vivo* during the involution of the β -cell mass in the post-partum pancreas (Scaglia *et al.* 1995) and in the normal neonatal rat (Scaglia *et al.* 1997). If the rate of β -cell death approaches the replication rate, then complete replacement of the β -cell population could occur. Thus, the endocrine pancreas should be considered to be a slowly renewed tissue (Finegood *et al.* 1995).

The mechanisms involved in this compensatory regulation are: (1) changes in replication rate, (2) changes in cell death rate, and (3) changes in rates of

differentiation from ‘precursor’ cells remaining in the adult pancreatic ducts. An additional parameter, that of cell size/volume, hypertrophy vs atrophy, is involved in the regulation of mass of β -cells, but not their number. Hypertrophy of the cells allows for sustained amplification of gene expression without cell division (Franch *et al.* 1995, Preisig 1999). While both apoptotic or neogeneic β -cells can be found, we do not have an estimate of the time involved for these processes and so it is not possible to give rates. However, with the simple mass equation, one can estimate the contribution of neogenesis and of apoptosis. In any given situation more than one of these mechanisms may be involved.

One particularly compelling case for β -cell compensation to meet the increased demand of insulin resistance was that of the mice that had known causes of insulin resistance, due to the ablation of one allele of the insulin receptor, of the insulin substrate-1 or both of these genes (Bruning *et al.* 1997). Up to 6 months of age, these mice were of comparable body weight to their wild-type littermates and maintained normoglycemia in spite of marked insulin resistance. Some had 400-fold increased plasma insulin levels compared with wild-type. Those mice with double heterozygosity of the null genes had up to 40-fold increased β -cell mass, while the mass of the non- β endocrine cells (that is the α , δ and pancreatic polypeptide cells) was not different among the groups. These double heterozygotes had very large islets in which the non- β -cells no longer formed a clear mantle around the β -cell core. While the increase in mass can be due to several of the mechanisms of islet growth, the massively enlarged islets support a continued stimulation of replication of the pre-existing β -cells.

Another example of the varied mechanisms of compensation and expansion of the β -cell mass in the adult rodent is the Zucker diabetic fatty (ZDF) rat. There is one colony of Zucker fatty (*fa/fa*) rats in which the male rats become diabetic at about 9 weeks of age but the females do not become diabetic at any age. As shown in the prediabetic 6-week-old ZDF male rats, there is an intrinsic defect in the β -cells of this colony as compared with either their lean littermates or the other non-diabetic Zucker fatty rats (Pick *et al.* 1998a). We examined the female ZDF rats and their lean littermates (*fa⁺/fa⁻* or *fa⁺/fa⁺*) at 12 weeks of age (Pick *et al.* 1998b). As expected based on the male data, the female ZDF rats had increased body weight and increased β -cell mass compared with the lean littermates; however, the female rats were compensated and had only slightly impaired glucose tolerance. In addition, both the β -cell size and replication were increased

FIGURE 3. β -cells regulate glucose homeostasis with great precision. As demand becomes greater, as with insulin resistance, the β -cells compensate. With insulin resistance, glucose uptake by peripheral tissues would be diminished, with resulting transient post-prandial hyperglycemic excursions. The mild hyperglycemia could signal the β -cells that more insulin is needed, so both functional and mass compensation could occur, including both enhanced β -cell replication and cell size. The increased β -cell mass would secrete more insulin that would overcome the insulin resistance and drive glucose uptake peripherally and a return to normoglycemia. One can envision such a scenario being repeated numerous times, maintaining compensation as long as the β -cell mass can increase. If, however, the β -cell mass no longer increases as in the ZDF female rats treated with dexamethasone, diabetes ensues.

compared with the lean animals. Thus hypertrophy plays an adaptive role in compensation of these animals. An additional part of these experiments was a 2-day treatment with dexamethasone. Dexamethasone has been shown to have both peripheral effects and direct effects on the β -cell itself. After 48-h treatment the lean Zucker females compensated with increased β -cell mass, from β -cell hypertrophy and increased replication. In contrast, the ZDF females that had been adequately compensated before treatment became frankly diabetic. The already hypertrophied β -cells did not increase any further in size; their replication was enhanced but, due to increased frequency of apoptosis, the β -cell mass did not increase. In muscle and renal tissue, hypertrophy has been seen as a rapid response to increased demand, with an arrest in G1 of the cell cycle (Franch *et al.* 1995, Preisig 1999). This arrest allows a sustained amplification of gene expression without completing the cell cycle. However, the ZDF data illustrate one of the potential drawbacks to hypertrophy as a

solution to increased demand; that is, that the hypertrophied cell is more vulnerable to apoptosis since the cell is arrested in the cell cycle.

In both these cases, it is unclear if the stimulus for β -cell expansion is something other than mild transient hyperglycemia. Glucose is one of the best stimuli for β -cell replication *in vitro* and *in vivo* (Bonner-Weir *et al.* 1989, Bonner-Weir & Smith 1994). One can envision glucose as the driving force in both the insulin-resistant mice and the ZDF rats (Fig. 3). As insulin resistance increases, glucose uptake by peripheral tissues would be diminished, with resulting transient post-prandial hyperglycemic excursions. The mild hyperglycemia could signal the β -cells that more insulin is needed, so compensation could occur, including both enhanced β -cell replication and cell size. The increased β -cell mass would secrete more insulin that would overcome the insulin resistance and drive glucose uptake peripherally. One can envision such a scenario being repeated numerous times, maintaining a compensation as long as the β -cell mass can

Neogenesis

FIGURE 4. Neogenesis of islet tissue occurs from mature adult pancreatic ducts. In the normal adult pancreas, the ducts are quiescent, being restrained by local factors. If the ducts are isolated from their stroma and put into primary culture, the ducts proliferate extensively showing considerable potential for expansion. Such findings suggest that local effects of matrix components and soluble factors, e.g. TGFβ, restrict the ductal expansion *in vivo*. Separate sets of stimuli lead to expansion of the ductal population by proliferation and to differentiation to mature phenotypes of endocrine, acinar and duct cells. An important question for neogenesis is what are the cells in the adult pancreatic ducts that have the multipotency to become any type of pancreatic phenotype. We hypothesize that a mature duct cell with rapid replication can transiently regain a more pluripotent, and less differentiated, phenotype. External stimuli, whether soluble factors or matrix components, can then direct differentiation of these multipotent cells to endocrine, acinar or mature duct phenotypes. EGF, epidermal growth factor; KGF, keratinocyte growth factor; HGF, human growth factor; GLP-1, glucagon-like peptide-1).

increase. If, however, the β-cell mass no longer increases as in the ZDF female rats treated with dexamethasone, diabetes ensues.

The third major mechanism for increasing β-cell number/mass is neogenesis or differentiation of new β-cells from cells within the adult pancreatic ducts that act as precursor cells. Evidence for neogenesis, that is, multiple insulin-positive cells budding from the ducts, is found in pancreases from obese humans and a number of animal models. We have been able to study the regulation of this process in a rat model of pancreatic regeneration, the partial pancreatectomized rat (Bonner-Weir *et al.* 1993, 1997, Sharma *et al.* 1999). In this model, removing all but 10% of the pancreas of young adult Sprague-Dawley rats, we found that within 4 weeks of surgery the β-cell mass was 45% and the pancreatic weight (reflecting the exocrine mass) was 27% that of sham-treated animals. There had been rapid regeneration of both endocrine and exocrine

tissues in a discordant fashion, due to both enhanced replication of pre-existing differentiated cells and expansion of duct tissue and its subsequent differentiation of both new acinar and islet cells. In fact, whole new lobes of pancreas were formed in patterns seeming to recapitulate embryonic development. In the normal adult pancreas, the ducts are quiescent, being restrained by local factors (Fig. 4). If the ducts are isolated from their stroma and put into primary culture, the duct cells proliferate extensively, showing considerable potential for expansion. Such findings suggest that local effects of matrix components and soluble factors, e.g. transforming growth factor-β (TGFβ), restrict the ductal expansion *in vivo*. These data suggest that there are separate sets of stimuli to expand the ductal population by proliferation and to drive differentiation to mature phenotypes of endocrine, acinar and duct cells. Duct proliferation appears to be glucose independent, since *in vivo* in

the partial pancreatectomy model, it occurs prior to any increased blood glucose (Bonner-Weir *et al.* 1993).

An important question for neogenesis is what are the cells in the adult pancreatic ducts that have the multipotency to become any type of pancreatic phenotype. These cells could be 'true' stem cells that divide asynchronously, precursor cells that are intermediates, or 'facultative' stem cells (Block *et al.* 1996). We favor the latter for several reasons. First, in the rat, the common pancreatic ducts, in which the first 5 bromodeoxyuridine (BrdU) incorporation after partial pancreatectomy is seen, are ultrastructurally homogenous: all columnar cells with lateral interdigitations; there are no 'undifferentiated' stem cells. Secondly, only a few cells incorporate BrdU before 24 h after surgery and all the cells that are arrested in mitosis by 4-h treatment with colchicine (from 20 h to 24 h after surgery) are these same columnar epithelial cells. Thirdly, there is a transient re-expression of the transcription factor, *pdx-1/idx-1/stf-1*, in the duct cells after replication; at 2–3 days after surgery all the cells in the common pancreatic duct express this protein, even after the cells are again quiescent (Sharma *et al.* 1999). Based on these findings, we hypothesize that a mature duct cell can transiently regain a more pluripotent, and less differentiated, phenotype after replication. External stimuli, whether soluble factors or matrix components, can then direct differentiation to endocrine, acinar or mature duct phenotypes.

Using this hypothesis, we have added to cultures of primary rat, pig, and human ducts those factors that we empirically defined in our *in vivo* pancreatectomized model. We obtained expanded tissue and then differentiation of islet tissue from these duct cells, as seen both by immunostaining of hormone-positive cells and by hormone content. These experimental conditions have not yet been optimized, but the data allow speculation on the potential of extensive expansion of the ductal tissue and its subsequent differentiation *in vitro* to form new islets.

The complex regulation of pancreatic growth involves an orchestration of numerous stimuli and growth factors. Characterization of the duct precursor cells in the adult pancreas and how their differentiation is regulated will advance our long-term goal of being able to expand β -cells.

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