Endocrine Pancreas Plasticity Under Physiological and Pathological Conditions

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Endocrine pancreas plasticity may be defined as the ability of the organ to adapt the β-cell mass to the variations in insulin demand. For example, during late pregnancy and obesity, the increase of the β-cell mass, in association with β-cell hyperactivity, contributes to insulin oversecretion in response to insulin resistance. There is increasing evidence that the ability of the β-cell mass to expand in adult mammals is much higher than previously thought. During pregnancy, placental hormones, especially placental lactogens, are mainly responsible for the changes in β-cell mass. The factors involved in β-cell growth in obesity are far from clear, although increased free fatty acids seem to be the main candidate. Many data suggest that the impairment of insulin secretion in type 2 diabetes is partly related to reduction of β-cell mass, at least relative to prevailing insulin demand. This defect may originate from genetic predisposition, but the situation is likely worsened by environmental factors such as hyperglycemia (glucotoxicity) and hyperlipidemia (lipotoxicity). Better understanding of β-cell growth and regeneration mechanisms may allow new strategies in the treatment of type 2 diabetes based on early limitation of β-cell damage and/or restoration of a functional β-cell mass. *Diabetes* 50 (Suppl. 1):S30–S35, 2001

Endocrine pancreas plasticity may be defined as the ability of the organ to adapt the β-cell mass to the variations in insulin demand to warrant optimal control of glucose homeostasis. Our current knowledge is that in adult mammals, the β-cell mass is governed by a permanent balance between β-cell growth (β-cell replication and neogenesis) and β-cell death (mainly apoptosis). Disruption of this balance may lead to rapid and marked changes in islet cell mass. For example, during late pregnancy and obesity, the increase of the β-cell mass contributes to insulin oversecretion in response to insulin resistance. There is now growing evidence that impaired insulin secretion in type 2 diabetes is in part related to the reduction of β-cell mass in relation to prevailing insulin demand; i.e., the ability of the β-cell mass to expand in response to insulin resistance is altered.

We review here the main factors involved in islet plasticity in adults and the possible causes of its deterioration in type 2 diabetes.

FACTORS INVOLVED IN β-CELL MASS CHANGES IN THE ADULT

During development, endocrine cells arise from undifferentiated stem cells located in pancreatic ducts, which migrate into the exocrine pancreas to form the islets of Langerhans (neogenesis). Then, differentiated β-cells proliferate within the islets (replication). These processes are essential during development of the endocrine pancreas, but they are also necessary for the further islet cell mass homeostasis.

In vitro and in vivo studies have shown that β-cells from fetuses and adults respond to the same stimuli: nutrients, hormones, or growth factors. In this review, we will focus only on the role of glucose and insulin.

Glucose appears to be a potent stimulus of pancreatic β-cell growth both in vitro and in vivo. In vitro, the proliferative rate of rodent β-cells increases with increasing glucose concentrations (1). The proliferative response of human islets to glucose is also important but is already maximal at low glucose concentrations (5.6 mmol/l) (2).

In adult humans and rats, the β-cell mass seems quite stable with a low β-cell replication rate (3% per day vs. 10% per day in the fetus) (3). For this reason, the role of glucose in adult islets was considered marginal. However, the potent effect of glucose on β-cell mass growth was clearly demonstrated by Bonner-Weir et al. (4) in nondiabetic rats infused with glucose for 96 h. This effect resulted from both β-cell hyperplasia and hypertrophy. Using a similar protocol of glucose infusion in unrestrained rats, we further stressed the impressive efficiency of glucose on β-cell growth because a 24-h glucose infusion was sufficient to maximally increase the β-cell mass in both nondiabetic and mildly diabetic rats (5) (Fig. 1). The potent and rapid effect of glucose infusion was even more impressive in mildly diabetic rats because glucose promoted complete regeneration of β-cell mass (mild diabetes was induced by low-dose streptozotocin, resulting in a 50% reduction of β-cell mass). The increase of β-cell mass was due mainly to rapid activation of neogenesis of new endocrine cells rather than to increase in β-cell proliferation (5) (Figs. 2–4). A role for glucose in β-cell regeneration was indirectly suggested by previous studies showing that the reduction of β-cell mass induced by a high dose of streptozotocin in the neonate rat (6), 90% pancreatectomy in young adult rats (7), or cellophane wrapping of the head of the

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FFA, free fatty acid; IRS, insulin receptor substrate; MODY, maturity-onset diabetes of the young.

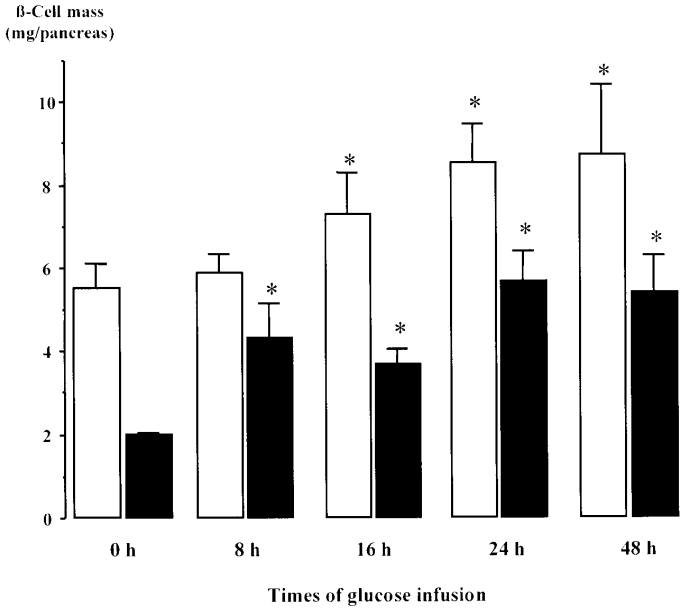

FIG. 1. Total pancreatic β-cell mass variations in nondiabetic (□) and diabetic (■) rats infused with glucose for 48 h. Values are means ± SE of three to five rats in each group. *$P < 0.05$.

pancreas in hamsters (8) was followed by sustained β-cell regeneration (50–75% of control). In all the above studies, partial restoration of the β-cell mass did not prevent hyperglycemia; therefore, it was inferred that the increase in β-cell mass was favored by high glucose levels (3). In addition to promoting β-cell neogenesis and/or replication, glucose may be involved in the control of apoptosis by inhibition of the β-cell "suicide" program (9). In short-term exposure to glucose, it seems that the β-cell apoptotic rate decreases and the number of viable β-cells increases when glucose concentration is raised (9).

Nevertheless, the precise role of changes in glucose concentration is difficult to appreciate in vivo because of the interference of concomitant variations in plasma insulin levels. Interplay between glucose and insulin seems to be important for the control of islet cell proliferation in vivo (10). Moreover, some studies have shown that insulin itself may stimulate pancreatic cell mitosis in vitro (11). In vivo studies also suggest that insulin may be involved in β-cell growth. Indeed, insulin treatment stimulates β-cell proliferation in fetal islets transplanted into diabetic rats (12,13). Moreover, insulin therapy improved β-cell regeneration in newborn rats injected with streptozotocin on the day of birth (14). It must be pointed out that in experiments showing the promoting effect of insulin

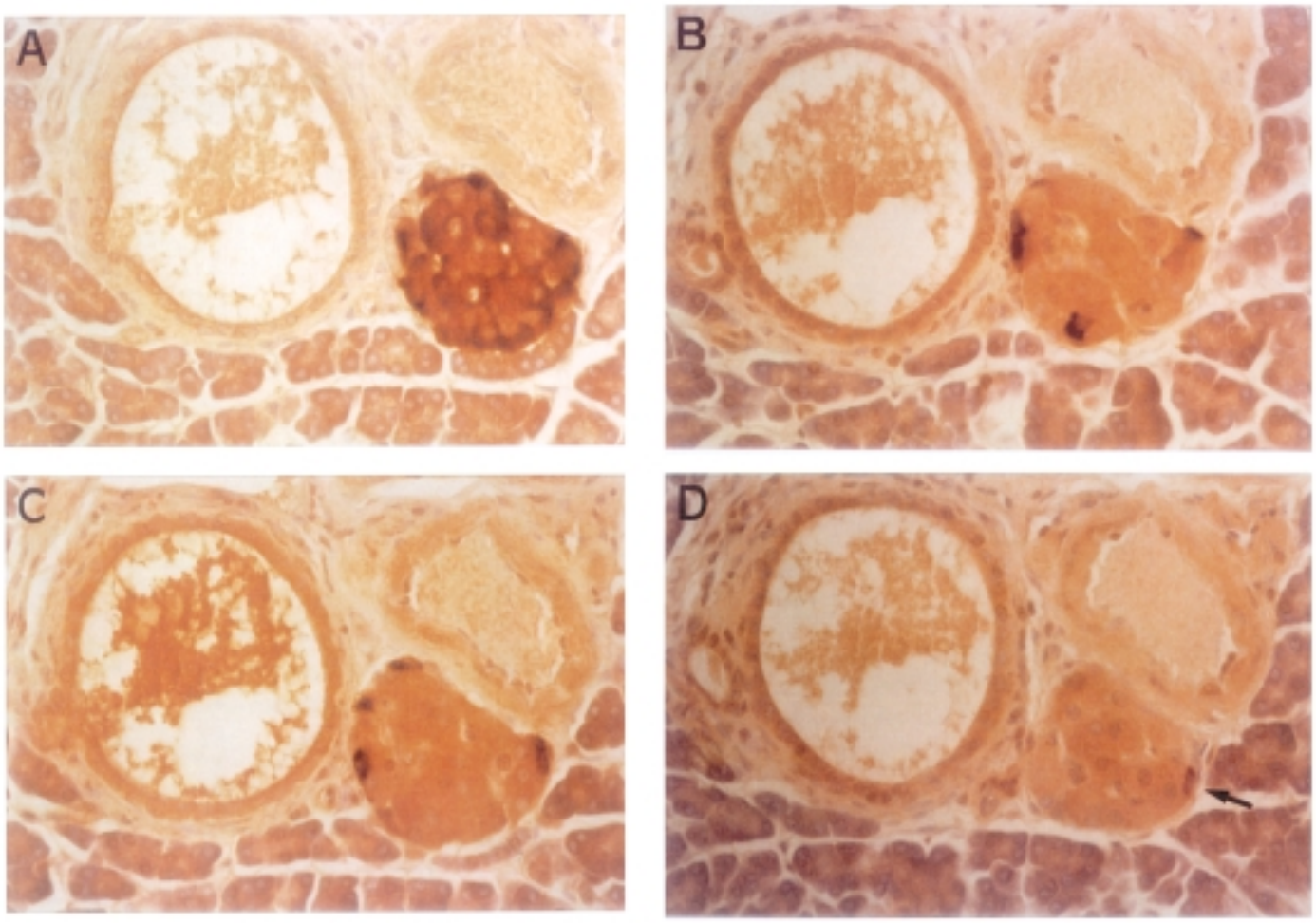

FIG. 2. Evidence of endocrine cell budding from pancreatic ducts in the pancreas of rats infused with glucose for 24 h. Endocrine cells were immunostained for insulin (*A*), glucagon (*B*), somatostatin (*C*), and pancreatic polypeptide (*D*) and revealed with peroxidase. Magnification ×400.

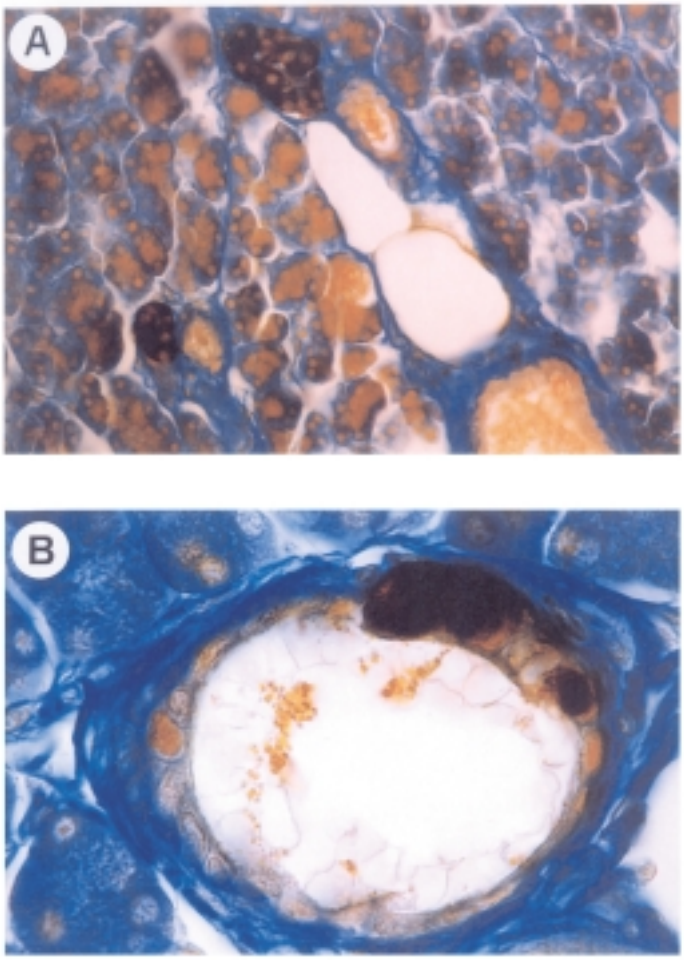

FIG. 3. Evidence of insulin-positive cell budding from pancreatic ducts in the pancreas of rats infused with glucose for 24 h. *A*: β-Cell cluster near a pancreatic duct to which it is attached by collagen fibers (magnification ×200). *B*: Presence of β-cells within the ductal epithelium (magnification ×1,000). In brown: insulin-positive cells revealed with peroxidase. In blue: collagen fibers revealed with aniline blue.

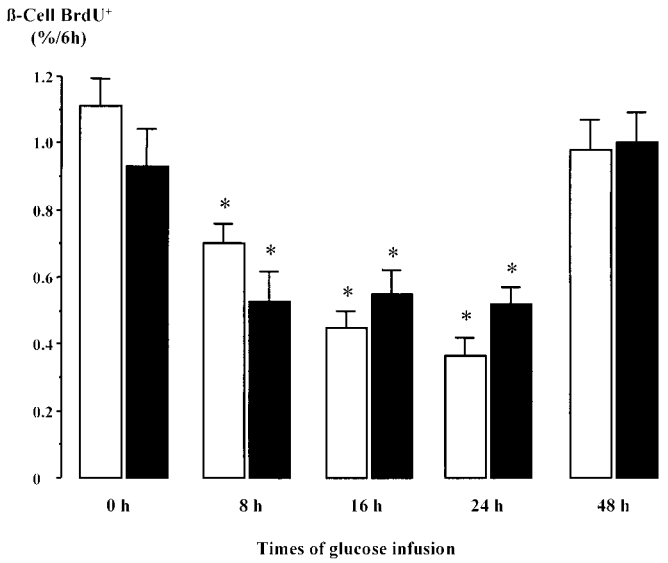

FIG. 4. β-Cell replication rates expressed as the percentage of 5-bromo-2′deoxyuridine (BrdU)-positive β-cells per 6 h in nondiabetic (□) and diabetic (■) rats infused with glucose for 48 h. Values are means ± SE of three to five rats in each group. *$P < 0.05$.

treatment on β-cell regeneration, plasma glucose remained higher than normal (12,13). The need for a critical plasma glucose level for β-cell growth was further emphasized by the study of Koiter et al. (10), who observed that hyperinsulinemia associated with hypoglycemia suppressed β-cell replication in rats. Taken together, these studies stress the difficulty in appreciating the specific role of insulin in vivo on the proliferating process. We attempted to discriminate between the effect of hyperglycemia and hyperinsulinemia by performing hyperinsulinemic-euglycemic clamps in unrestrained rats over 48 h (15). Plasma insulin concentrations were maintained throughout infusions at a level similar to that obtained during glucose infusions. We found that insulin infusion per se did not promote β-cell growth, thus excluding a major role for insulin. This conclusion is also supported by the fact that syngeneic islets transplanted from lean mice into obese hyperglycemic mice grew rapidly and intensively, whereas when they were implanted into hyperinsulinemic and euglycemic or slightly hypoglycemic mice, islet growth was not observed (16). Therefore, short-term hyperinsulinemia per se is probably not a determinant of β-cell mass increase in adult rats. However, this does not exclude long-term effects of insulin and/or the fact that both hyperglycemia and hyperinsulinemia are required for maximal islet growth. The interest in the role of insulin was highlighted by recent studies showing that endocrine pancreas plasticity was tightly dependent on the islet insulin receptor, insulin receptor substrate (IRS)-1 and IRS-2. In mice, knockout of IRS-1 provokes insulin resistance compensated by increased β-cell mass (17). On the other hand, mice deficient for IRS-2 rapidly developed diabetes after birth because of the reduction of β-cell mass and altered β-cell function (18). Four-month-old knockout mice for the insulin receptor presented altered insulin secretion in response to glucose and decreased β-cell mass (19). However, according to recent data, the role played by IRS-2 in endocrine pancreas plasticity could depend on IGF-I receptor activation (20). Thus, it is possible that the effect of insulin on β-cell growth could in fact be due to IGF-I. In any case, the pancreatic response to variations of the insulin demand seems to involve the key role of IRS-2.

ENDOCRINE PANCREAS PLASTICITY IN NONDIABETIC SUBJECTS

Pregnancy. In mammals, including humans, pregnancy results in profound changes in maternal metabolism and insulin secretion that allow optimal nutrient supply to the fetus. In particular, during the last third of pregnancy, there is marked insulin resistance accompanied by dramatic increase in the insulin response to glucose (21). In rats, high endocrine pancreas activity in late pregnancy results from three processes that have been well documented: *1*) higher β-cell sensitivity to glucose, *2*) increased insulin biosynthesis, and *3*) important modifications of the architecture of the islets, with β-cell hyperplasia and hypertrophy and increased β-cell interactions through gap junctions (22).

Placental hormones, i.e., placental lactogen hormone, prolactin, estrogen, and progesterone, play a key role in this situation (23). In women, the pattern of secretion of placental

hormones is well correlated with enhancement of the insulin response to glucose and β-cell growth (23). The chronology of the variations in β-cell mass has been studied in the pregnant rat by Scaglia et al. (24), who found that β-cell mass is almost doubled at the end of pregnancy. After parturition, it decreases progressively to reach normal values around 10 days postpartum. The involution of the islet cell mass results both from a decrease in cell replication and volume and from markedly increased apoptosis. The changes in β-cell mass during and after pregnancy and the mechanisms involved are a good illustration of the plasticity of the endocrine pancreas.

Obesity. Insulin resistance is a main feature in obesity. However, glucose homeostasis remains close to normal subjects in 75–80% of all obese subjects. As in pregnancy, euglycemia is maintained by increased insulin secretion to overcome insulin resistance. Insulin oversecretion is due not only to enhanced individual β-cell activity but also to β-cell growth. This is well documented in animal models of obesity and probably occurs also in humans. Klöppel et al. (25) clearly observed endocrine tissue hyperplasia in nondiabetic obese subjects. In the nondiabetic Zucker *fa/fa* rat, β-cell mass is fourfold higher than that in lean Fa rats as a result of β-cell hyperplasia and hypertrophy; neogenesis rather than replication of preexisting β-cells is the main mechanism (26).

The factors involved in β-cell mass increase in the obese are not fully understood. Free fatty acids (FFAs) may play a crucial role. When pancreatic islets from nondiabetic nonobese rats were cultured for 7 days with high amounts of long-chain FFAs, islet hyperplasia was observed; concomitantly, insulin secretion in response to glucose was markedly increased (26). Other studies ascribe a crucial role to leptin in β-cell growth in obesity. Plasma leptin levels are high in obese subjects, and the leptin receptor is present in islet cells (27). β-Cell lines like RINm5F and MIN6, which express the leptin receptor, are highly responsive to leptin, which stimulates cell proliferation at low concentrations (1–5 nmol/l), close to levels found in obese subjects (28,29). However, leptin is probably not the main factor of β-cell expansion in obesity because totally leptin-resistant *fa/fa* Zucker rats show dramatic increase in β-cell mass. Therefore, although FFAs are a main candidate, the additional factors involved in β-cell growth in obesity remain to be clarified.

ENDOCRINE PANCREAS PLASTICITY IN TYPE 2 DIABETIC SUBJECTS

β-Cell mass and type 2 diabetes. With some exceptions (30,31), human autopsy studies repeatedly show a 40–60% reduction of β-cell mass in patients with type 2 diabetes (25,32–34). The study of Klöppel et al. (25) is of particular interest because it compared weight-matched subjects. It was found that the highest β-cell mass was observed in nondiabetic obese individuals, and the lowest β-cell mass was observed in nonobese diabetic patients. In diabetic subjects, β-cell mass was 50% decreased compared with that in obese nondiabetic subjects and was only slightly lower than that in lean control subjects. These data stress that subjects with type 2 diabetes present low β-cell reserves in regard to insulin needs, thus pointing to altered endocrine pancreas plasticity. When detectable, the islet cell mass changes seem to be specific for the β-cells, because the non–β-cell mass (α, δ, and PP) was unchanged (35). This is also correlated to decreased pancreatic insulin content (36). Moreover, it is noteworthy that the increased incidence of glucose intolerance coincides with decreased capacity of β-cells to replicate (37,38). It may be questioned whether this deficiency corresponds to a genetic predisposition or is the result of an altered metabolic environment; these hypotheses are not mutually exclusive.

Role of genetic factors. Stöffers et al. (39) showed that a mutation in the gene coding for pancreatic duodenal homeobox transcription factor-1 is associated with maturity-onset diabetes of the young (MODY). Nevertheless, mutations in the genes coding for BETA-2/NeuroD and Nkx2.2 are not associated with MODY in Japanese populations (40), nor is there a relationship between mutations of BETA-2/NeuroD and PAX-4 genes and type 2 diabetes in French families (41).

Studies performed on animal models are more suggestive because in animal models of type 2 diabetes, the genetic background influences β-cell growth (3). In the adult Goto-Kakisaki (GK) rat, β-cell mass is 50% decreased, and it is reduced already in the fetus (42) and the newborn (43) at a time when glucose homeostasis is normal. This suggests that impaired development of the β-cell mass participates in the cascade of events that leads to diabetes. However, extrapolating to humans seems hazardous, and genetic studies remain poorly conclusive.

Role of environmental factors. Because hyperglycemia and hyperlipidemia are the main features of obese type 2 diabetes, the role of chronic hyperglycemia and high plasma FFA concentrations have been extensively explored.

Role of glucose: glucose toxicity and β-cell mass. Although glucose promotes β-cell growth (see above), it is suggested that the concept of glucose toxicity be extended to β-cell mass homeostasis when hyperglycemia is of long duration. When GK rats are fed a carbohydrate-rich diet for 6 weeks, hyperglycemia and glucose intolerance worsen. This further deterioration is accompanied by a 50% reduction of β-cell mass when compared with GK rats fed a normal diet. This drastic reduction in β-cell mass is mainly related to stimulation of apoptosis, whereas β-cell proliferation remains close to normal (44). Similar results have been obtained by Donath et al. (45) in *Psammomys obesus*. In the wild state, these animals are lean, active, and nondiabetic; when constrained under laboratory conditions with free access to food, they become mildly obese and diabetic. Concomitantly, β-cell apoptosis is stimulated in parallel with the deterioration of glucose homeostasis to reach 12- to 14-fold the normal value after 10 days of a hypercaloric diet. β-Cell proliferation, which is very low in prediabetic animals, is markedly increased at the beginning of the syndrome and then decreases progressively with the duration of diabetes. The combination of these events results in a sharp decrease in β-cell mass in diabetic *Psammomys obesus*.

The mechanisms of the toxic effect of chronic hyperglycemia are far from fully understood. A role of advanced glycosylation end products has been suggested but not confirmed (45). Amylin, whose synthesis is increased by elevated glucose concentrations (46), may be one possible mediator of glucose-induced apoptosis (47).

Role of FFAs: lipotoxicity and β-cell mass. The concept of β-cell lipotoxicity is more recent but is now supported by solid experimental data (48). The relationship between excess lipids and β-cell mass has been studied on a model of a spontaneously obese and diabetic rat (the Zucker diabetic fatty [ZDF] rat [*fa/fa*]), in which initial insular hyperplasia

allows compensation for insulin resistance. Later, β-cell mass remains static and finally decreases progressively with aging (49). Concomitantly, insulin secretion is impaired, leading to severe diabetes (49a). This deleterious process is the consequence of a sevenfold increase of β-cell apoptosis, whereas β-cell replication and neogenesis are normal (50). The stimulation of apoptosis seems to be due to a large accumulation of triglycerides within the islet (49). Islet lipid accumulation is also observed in the OLETF rat (51). Lipolytic substances such as leptin or troglitazone are able to protect ZDF rat β-cells from apoptosis (52,53). Moreover, in islets from ZDF rats cultured in the presence of high FFA levels, the apoptotic process is strongly stimulated (54). From these data, a crucial role may be ascribed to intra-islet fatty acid accumulation in the sequence of events leading to inadequate β-cell mass. Although caution is warranted before transposing to humans, these findings may help one understand the process that leads from obesity to type 2 diabetes in 20% of the obese subjects.

PERSPECTIVES

There is increasing evidence that the lack of pancreatic plasticity is of crucial importance for the development of type 2 diabetes. These new insights into the pathophysiology of the disease open novel therapeutic approaches in which the goal is to preserve β-cell mass. A first approach should aim at reducing β-cell apoptosis. Poor current knowledge in this field does not allow for the definition of potential targets for which to develop new drugs. However, the concepts of glucotoxicity and lipotoxicity may give us new insights; e.g., normalization of glycemia and triglyceridemia are likely to be of crucial importance. In rats, the residual β-cell mass was partially restored after intraportal islet transplantation (55). In these rats, the sixfold increase in β-cell mass was due to β-cell regeneration within the islets and β-cell neogenesis from pancreatic duct cells. The authors concluded that normalization of glycemia by the islet graft could be one of the main factors leading to this trophic effect. If hyperglycemia increases β-cell death, intensive antidiabetic treatment at the very beginning of the disease could stop the process. Obviously, such a preventive treatment may be successful only in patients in whom diabetes was diagnosed early, when sufficient insulin secretion remains to reduce hyperglycemia.

In a second strategy, we could consider the possibility of inducing β-cell regeneration in patients with type 2 diabetes by stimulating either the replication of preexisting β-cells or the differentiation of new β-cells from stem cells. In the adult rat with experimental diabetes similar to type 2 diabetes, complete β-cell regeneration could be induced after only 24 h of glucose infusion (15) (Fig. 1). Some studies in humans suggest that regeneration could occur also in islets of diabetic patients. Islet hyperplasia has been described in young patients with MODY or in the prediabetic adolescent (33). These observations suggest that at early stages of the disease, the pancreas of diabetic patients retains a regenerative potential that could be exploited. More generally, similar strategies could be used to restore the β-cell mass also in type 1 diabetes, i.e., to increase the volume of islets before or after transplantation.

These perspectives do not seem unrealistic. A recent study showed that culture of mouse pancreatic ductal epithelial cells was able to produce functioning islets containing α-, β-, and δ-cells (56). When these in vitro–generated islets were grafted into diabetic NOD mice, diabetes was reversed (56). An alternative approach was used very recently by Ferber et al. (57), who transferred the PDX-1 gene to the liver of mice through adenoviral vectors; this resulted in the expression of insulin 1 and 2 genes in the liver, with a dramatic increase in hepatic insulin content and plasma insulin concentration. When repeated in mice made severely diabetic by streptozotocin, hyperglycemia was largely corrected. Such studies are very promising, but whatever the strategy used, we obviously need a better understanding of β-cell growth and regeneration mechanisms. Provided their limits are well appreciated, animal models with diabetes-like syndromes could make important contributions.

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