I. INTRODUCTION

Blood glucose homeostasis is controlled by the endocrine cells of the pancreas, which are located in the islets of Langerhans. The islet cells monitor the concentration of glucose in the blood and secrete hormones with opposite effects. When after a meal the blood glucose concentration is increasing, the beta cells, which are the most numerous islet cells, secrete the hormone insulin to reduce blood glucose. Insulin stimulates the uptake of glucose by cells of the body and stimulates the conversion of glucose to glycogen in the liver. If the glucose level falls too far, islet alpha cells secrete the hormone glucagon, which stimulates the breakdown of glycogen to glucose in the liver and therefore increases blood glucose between meals. Optimal control of blood glucose levels depends on delicate changes in insulin production and secretion by the pancreatic beta cells and on their capacity for a large increase of secretion after meals, requiring large stores of insulin (68). Very important is the need for the
beta-cell mass to be closely regulated by glucose and hormonal effects on beta-cell replication, size, apoptotic elimination and, under certain conditions, neogenesis from progenitor cells. Failure to adapt to changes in body mass, pregnancy, insulin sensitivity of peripheral tissues, or tissue injury may lead to the development of chronically elevated blood glucose, or diabetes (68, 92, 126). The increasing global prevalence of diabetes (164) has stimulated efforts to develop new therapeutic strategies like beta-cell replacement or regenerative medicine. The existing therapies with exogenous insulin or hypoglycemic agents for type 1 and type 2 diabetes are unsatisfactory, since they do not offer a cure and are mostly insufficient for preventing the secondary complications associated with diabetes (99). Transplantation of a sufficient number of pancreatic beta-cells can normalize blood glucose levels and may prevent the devastating complications of diabetes (73, 143). However, beta-cells from cadaver pancreases are in such short supply that transplants can be provided only to a limited number of patients. This problem could be overcome by devising ways of generating more beta-cells from the available donors. An alternative approach to cell transplantation would be to induce an increase or regeneration of the endogenous beta-cell mass, pregnancy, insulin sensitivity of peripheral tissues, or tissue injury may lead to the development of chronically elevated blood glucose, or diabetes (68, 92, 126). The increasing global prevalence of diabetes (164) has stimulated efforts to develop new therapeutic strategies like beta-cell replacement or regenerative medicine. The existing therapies with exogenous insulin or hypoglycemic agents for type 1 and type 2 diabetes are unsatisfactory, since they do not offer a cure and are mostly insufficient for preventing the secondary complications associated with diabetes (99). Transplantation of a sufficient number of pancreatic beta-cells can normalize blood glucose levels and may prevent the devastating complications of diabetes (73, 143). However, beta-cells from cadaver pancreases are in such short supply that transplants can be provided only to a limited number of patients. This problem could be overcome by devising ways of generating more beta-cells from the available donors. An alternative approach to cell transplantation would be to induce an increase or regeneration of the endogenous beta-cell mass in the pancreas by growth factors. Both approaches require understanding of the mechanisms that regulate the pancreatic beta-cell mass.

II. PRENATAL DEVELOPMENT OF THE BETA-CELL MASS

During the gastrulation process of embryonic development, three germ layers are formed: ectoderm, endoderm, and mesoderm. Although old text books may still proclaim that pancreatic endocrine cells are derivatives of the neural crest (ectoderm) and exocrine cells of the endoderm, it is now generally accepted that they both derive from the endoderm. The first formal proof for this was provided by coculturing genetically tagged pancreatic epithelium with unlabeled mesenchyme. Endocrine and exocrine cells arise from the epithelium (106).

A. Embryogenesis

The embryonic endoderm develops into the primitive gut from which the digestive system originates. The pancreas derives from two parts of epithelium that bud dorsally and ventrally from the foregut/midgut around embryonic day 9 in mouse and which fuse later on (reviewed in Ref. 139). There has been much progress in recent years in our understanding of the major steps leading to the embryonic development of the endocrine pancreas. In general, the induction and patterning of organs are regulated by extracellular signals from neighboring cells. This leads to the expression of tissue- and cell type-specific patterns of transcription factors. In particular, the repertoire of transcription factors has been identified that drives the differentiation of endodermal cells to beta cells (reviewed in Refs. 38, 39, 66, 98, 165). Pancreatic development occurs in an endodermal region where the transcription factor Pdx1 (pancreas-duodenum homeobox) is expressed and where the extracellular signaling molecule sonic hedgehog is repressed. Ptf1a (pancreas transcription factor, a basic helix-loop-helix transcription factor, or bHLH) is required for specification of ventral pancreas development, whereas the homeobox transcription factor Hlxb9 is required for dorsal specification. It has been well established by genetic tracing experiments that both exocrine and endocrine cells of the pancreas derive from a pool of endodermal cells that express the transcription factors Pdx1 (55) and Ptf1a (72). The “protodifferentiated” pancreatic progenitor cells also express the bHLH transcription factor Hes1 (hairy and enhancer of split homolog), which is a transcriptional repressor keeping cells undifferentiated (4). Hes1 is a downstream target of the Delta-Notch signaling system that inhibits the endocrine cell fate, by a process called lateral inhibition. Knocking out Hes1 or other mediators of the Notch signaling pathway in transgenic mice leads to accelerated formation of endocrine cells, but abnormally few cells are formed as there is insufficient expansion of progenitors (64, 65). Proliferation of the progenitor cells is stimulated by fibroblast growth factors (FGFs) (40). Other factors produced by the surrounding mesenchyme are crucial for proper pancreatic development (reviewed in Ref. 130). The homeoprotein Isil (Islet-1) is required for development of the mesenchyme of the dorsal pancreatic bud and also for differentiation of the dorsal pancreatic epithelium to endocrine cells (1). Between E14 and E17 in embryonic mice, endocrine cells are derived from a subset of HNF1b-expressing duct cells (88), which transiently express the transcription factor Ngn3 (55). Among the Ngn3-expressing cells, transient Pax4 (paired homeobox gene) expression specifies the beta-cell phenotype, and differentiated beta cells eventually express high levels of Pdx1, Nkx6.1, Nkx2.2, and Pax6 transcription factors (reviewed in Ref. 66). When the first hormone-expressing cells appear, they stop dividing and are considered “postmitotic” cells (64). However, later in fetal growth, endocrine cells appear to be able to reenter the cell cycle (see below).

B. Fetal Growth

The fastest expansion of the beta-cell mass occurs in late fetal gestation, with an approximate doubling of the beta-cell population each day starting from the 16th day postconception in rats (93). In fetal rats, mitotic activity is restricted to a limited number of beta cells (±10%) that

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can engage into the cell cycle (58, 146). A daily cell birth rate of 10% was calculated from the observed beta-cell mitotic indexes. Thus, in the rat fetus where the beta-cell number increases at a rate of ~100% per day, beta-cell division can account for not more than 10–20% of the total growth (147). The remaining 80% or more has been attributed to the process of neogenesis from rapidly proliferating “undifferentiated” precursor cells. A much higher frequency of DNA-synthesizing cells has been observed in the immediate vicinity of the rapidly growing fetal islets than in the islets themselves. These proliferating islet precursors express the same cytokeratin proteins that are characteristic for the cells lining exocrine ducts in the adult pancreas (21). “Transitional” forms of differentiation, represented by cells coexpressing insulin and duct-type cytokeratin, have been taken as evidence for the conversion of ductlike precursors into beta cells (21). Similar conclusions were drawn from studies of human fetal pancreas, where it was shown that immature beta cells express cytokeratin-19, an intermediate filament protein characteristic for adult duct cells (23). In human fetal pancreas, rapid expansion of the beta-cell mass has been noted from ~20 wk (141). The fraction of endocrine cells undergoing replication in human fetal pancreas is extremely low (23, 70, 111), which is in favor of massive differentiation from nonendocrine progenitor cells as the major mechanism of beta-cell mass expansion during this phase.

It appears that fetal life represents a critical “window” in which the appropriate number of beta cells is set in place. If this goes wrong due to for example intrauterine growth retardation (IUGR), initially the number of beta cells may be sufficient but from a certain age on the beta-cell mass becomes unable to sufficiently compensate for the increased needs of the body. In a model of IUGR induced by reducing the blood flow to the fetus, no significant differences in beta-cell mass were found at 1 and 7 wk of age, but in 15-wk-old IUGR rats, the relative beta-cell mass was 50% that of controls, and by 26 wk of age, beta-cell mass was less than one-third that of controls (137). These animals developed glucose intolerance as seen in type 2 diabetes. Fetal malnutrition resulting from maternal food restriction in rats leads to a reduced beta-cell mass in the offspring (48). Beta-cell replication was not affected in this model, indicating that a deficient neogenesis of beta cells during fetal life caused the effects. Perinatal malnutrition also leads to the development of type 2 diabetes later in life, apparently by the inability of the beta-cell mass to adapt to additional demands placed by ageing (50). Similar observations were made in the Goto-Kakisaki (GK) rat, which is a genetic model of type 2 diabetes obtained by selective inbreeding of mildly glucose-intolerant Wistar rats (97). GK fetuses show a reduction of the beta-cell mass to 23% of control values. The adult pancreatic insulin content and beta-cell mass remain decreased to 32 and 47% of control values, respectively. Although neonates until 14 days of age display normal basal plasma glucose, the adult diabetic GK rats exhibit higher basal plasma glucose and become glucose intolerant. Although fetal development is impaired in the GK pancreas, cultures of explanted pancreatic rudiments did not reveal deficiencies in growth or differentiation compared with Wistar rats (95). This suggests that extrinsic factors regulating fetal growth, differentiation, and survival of beta cells and their precursors are deficient in this strain.

## III. POSTNATAL GROWTH OF THE BETA-CELL MASS

It is sometimes believed that we are born with a fixed number of beta cells that will not increase further during life. However, the available evidence shows that this is not true but that the beta-cell population still grows at least until adolescence, as most other tissues and organs. Furthermore, most tissues in our body are subjected to continuous turnover, since the life span of most cell types is shorter than that of the organism. The average life span of cells can differ greatly dependent on cell type. In the case of human beta cells, however, it remains unknown.

### A. Neonatal Growth

In neonatal rodents, growth of the beta-cell mass still occurs but at a reduced rate compared with late fetal growth (71, 93, 94, 158). Evidence has been reported for the existence of both beta-cell replication and neogenesis, the latter from ductlike cells that surround neonatal islets and exhibit a much higher replicative activity than the differentiated islet cells (20). These islet precursor cells disappear after the first week of life, and no further morphological evidence of neogenesis from such precursors is found thereafter. With a recent transgenic approach, it was confirmed that the beta-cell mass expands by self-replication and not by neogenesis after the first week of life. In knockout mice lacking the cyclin D2 positive regulator of the cell cycle, neonatal beta cells failed to replicate, whereas replicative activity of acinar and ductal cells remained unaffected (52). Although born with a normal beta-cell mass, these mice failed to further expand their beta-cell mass in the neonatal period between 7 and 14 days.

In a rat model of food-restricted mothers it was found that from birth to 3 mo of age, the offspring showed a reduced beta-cell mass while beta-cell replication was similar to or higher than in controls. Apparently, even increased beta-cell replication is insufficient to restore a functional beta-cell mass when neogenesis and beta-cell mass are already affected at birth (49).
A transient “wave” of beta-cell apoptosis has been reported around the time of weaning and is associated with a transiently decreased growth rate of islets in rodents (129), pigs (13), and humans (70). It may be that in this period, important changes take place at the level of beta-cell maturation.

There is no direct evidence of beta-cell neogenesis from stem/progenitor cells in the undisturbed adult rodent pancreas (42).

B. Compensation for Body Mass

Whereas in some rapidly renewing gastrointestinal tissues like the gut mucosa, there is a constant replacement of cells that are derived from a stem/progenitor pool, this has not been shown to be the case in the pancreas. However, the beta-cell population remains a dynamic mass, probably throughout life. After the growth of the beta-cell mass has considerably slowed down by the time of weaning in rats, it continues to expand at a relatively slow pace during adult life (96, 159). Islet mass appears to increase mainly by an increased size of individual islets rather than by the formation of new islets (138). Between 1 and 7 mo of age in rats, the beta-cell mass increases six- to sevenfold in females and males, respectively (94). Comparable figures were obtained by other investigators using tissue morphometry (96, 159).

On average, the postnatal beta-cell population grows at a rate between 2 and 3% per day in male rats (42, 96, 159). Growth is significantly less in females, which obtain a lower body weight than males (159). After 7 mo of age, growth of the beta-cell population slows down considerably but still continues until 20 mo, which is old age for rats (96). A linear correlation was found between beta-cell mass and body weight (96), and such a relationship was also found in larger mammals like pigs (13). However, with increasing age, the beta cell number per kilogram body weight decreases significantly in rats (159). A critical number seems to be ~10 million beta cells per kilogram body weight. When beta cell number decreases below this value, e.g., in streptozotocin-treated rats where part of the beta cells have been destroyed, animals become glucose intolerant (159).

Excessive increase in body weight leads to obesity. Obesity is correlated with insulin resistance and is associated with a compensatory physiological response at the level of beta-cell mass increase. In nondiabetic animal models of obesity, for example, the Zucker fa/fa rat, beta cell mass is increased fourfold compared with lean controls (109). Animals eventually become diabetic when they age and become more obese and glucose intolerant. There is also evidence for an increased beta-cell mass in nondiabetic obese humans (74). In hyperglycemic obese ob/ob mice, insulin resistance progressively develops up to 6 mo of age and is accompanied by marked islet hyperplasia in response to the sustained hyperglycemia (14, 151). Beta-cell hyperplasia is also found in db/db mutant mice (46, 162). The sand rat, Psammomys obesus, is an animal that is adapted to a low-calorie diet and shows insulin resistance. When the animal is fed a high-calorie diet, it is unable to compensate for the hyperglycemia. A transient increase in beta-cell replication is insufficient to cope with the observed increase in apoptotic cell death, and hence, the animals become diabetic (36).

Histologically, dividing beta cells can be recognized in adult pancreas, but they are very scarce. Using immunohistochemical demonstration of bromodeoxyuridine incorporation (a thymidine analog), the quantitative importance of beta-cell replication can be evaluated. Beta-cell replication is progressively reduced during postnatal life, with <0.25% of beta cells being in S-phase of the cell cycle after 3 mo of age (96, 159). Yet, when the beta-cell birth rate is calculated based on the observed frequency of cells in S-phase and an S-phase duration of 6.4 h (146), it was concluded that beta-cell replication can theoretically account for the observed net increase in cell number (3–20 wk of age) (159). A recent genetic tracing experiment in mice has demonstrated that this is indeed the case (37). In this study, total beta cell numbers were counted from enzymatically dissociated pancreata. A 6.5-fold increase was noted between 3 and 12 mo of age, which corresponds to an average daily growth rate in the range of 2–3%, as in rats. With the use of a tamoxifen-induced Cre/Lox system, a pulse of reporter gene expression in insulin-transcribing cells could be followed over time. This allowed the discrimination between beta cells generated from preexisting beta cells and beta cells derived from another source of cells which did not previously express insulin. The results demonstrated that new beta cells in the adult animals were formed exclusively by replication of preexisting beta cells (37). Thus these findings confirm the conclusion drawn from cytokinetic studies, i.e., that self-replication is sufficient for the slow expansion of the beta-cell mass as a compensation for normal growth in rats and mice.

The study of Dor et al. (37) also suggested that no new islets are formed during adult life but rather that islets present in old mice are all derived from islets that were present at ~2 mo of age. It is possible, however, that growing islets can generate smaller ones by fission as indicated by the study of Seymour et al. (134). In this study, the cell composition of pancreatic islets was analyzed in mosaic mice with a lacZ insertion on the X-chromosome. In female heterozygous mice, due to random silencing of one of the two X-chromosomes during development, tissues become a mosaic of cells, some of which express the lacZ-reporter (β-galactosidase) and others not. The authors of this study noted that islets are initially of mixed composition and hence must be formed by aggregation of several cells. Later
on in postnatal life, the heterogeneity of islets diminished. This suggests that following cell turnover, older islets contain descendants of only a small number of the preexisting islet cells that have replicated.

Species-related differences cannot be excluded, and it remains to be found whether the same applies to human pancreas. Differences do exist between human and rodent pancreas, for example, with respect to cellular composition, tissue organization, and beta-cell replicative activity. For instance, in human pancreas there exists a population of extra-islet beta cells that occur scattered over the exocrine tissue and represent 15% of all beta cells (24). Such cells are quite rare in normal rodent pancreas. Also, replicative activity of human beta cells is lower compared with rodents and decreases with age (154). In mice, an age-dependent decrease of beta-cell replicative capacity has been noted, and this capacity depends on the genetic background (148).

C. Compensation in Pregnancy

Besides adaptation to increased body weight and thus increased demand for insulin, the beta cell mass can also compensate for the increased demand during pregnancy. Failure to compensate is thought to lead to maternal or gestational diabetes. With an incidence of 3–5%, this represents one of the top health concerns related to pregnancy. Although gestational diabetes usually goes away after delivery, mothers are at risk for developing type 2 diabetes later in life. At the end of pregnancy in rats, the beta-cell mass is ~2.5-fold increased compared with non-pregnant females (10, 156). This results from both an increased beta cell number and cellular hypertrophy. Islet enlargement and beta-cell hyperplasia have also been observed in autopsies from pregnant humans (155). During experimental diabetes induced by streptozotocin in rats, however, the endocrine pancreas has an impaired capacity to compensate during pregnancy (156). After pregnancy, a rapid decrease of the beta-cell mass occurs in postpartum rats (89). This is accompanied by decreased beta-cell replication and beta-cell size, and by an increased frequency of apoptosis (128).

These studies demonstrate the capability of beta cells to up- and downregulate their mass using the mechanisms of beta-cell replication and apoptotic cell death, and changes in beta cell size, to achieve homeostasis in conditions of changing insulin demand. It is not clear whether neogenesis from precursor cells is contributing to beta-cell mass increase during pregnancy.

D. Regulation by Growth Factors

Which molecules regulate the beta cell mass? Insulin, several other hormones, and glucose have been reported to play an important role.

1. Insulin

Transgenic mice with a beta cell-specific knockout of the insulin receptor exhibit a decreased beta cell mass in adults and develop diabetes (102). Insulin resistance, leading to hyperinsulinemia, stimulates an increase in beta-cell mass. This was shown in mice that were made double heterozygous for null alleles in the insulin receptor and insulin receptor substrate-1 genes (IR/IRS mice). The animals showed an expected 50% reduction in expression of these two proteins and exhibited severe insulin resistance with 5- to 50-fold elevated plasma insulin levels. A compensatory beta-cell hyperplasia was observed with a significant increase in beta-cell mass (27). In insulin-resistant IR/IRS mice that were crossed with Pdx1-heterozygous mice, there was a high number of apoptotic beta cells, and the compensatory beta-cell growth response was completely abrogated (75). These data indicate that the Pdx1 transcription factor is a crucial regulator of the adaptive response of the beta-cell mass in insulin-resistant mice. This homeodomain protein seems to be important for beta-cell proliferation and apoptosis (75) as well as for neogenesis (135).

An increased beta-cell number was also observed in acutely euglycemic-hyperinsulinemic infused rats (103). On the other hand, hyperinsulinemia caused by ectopic transplantation of a rat insulinoma has been shown to lead to a significant reduction of the beta-cell mass in the pancreas (11). This reduction in beta-cell mass was non-immune-mediated and resulted from apoptosis of beta cells. These observations indicate that insulin can control beta cell population dynamics, although the mechanism is still unclear.

2. Glucose

Glucose itself has an effect on beta-cell population dynamics. With fetal islets, it is possible to increase the proportion of cycling beta cells in vitro by increasing the glucose concentration of the culture medium (146). Glucose infusion in rats during a period of only 24 h increases the beta cell number by ~50% (8). Seven days after stopping glucose infusion, the beta-cell mass returned to basal values, and this was accompanied by increased beta-cell apoptosis. By combining simultaneous infusion of either glucose and/or insulin or glucose and diazoxide, hyperglycemic-hyperinsulinemic rats, hyperglycemic-euinsulinemic rats, and euglycemic-hyperinsulinemic rats could be studied (103). In all groups, beta cell number was significantly increased, namely, with 50–70% compared with controls, within 48 h. The rate of beta-cell proliferation decreased, which indicates that neogenesis from precursor cells was responsible for the increased beta-cell mass (103). A longer glucose infusion time was reported to result in increased beta-cell replication and hypertrophy besides neogenesis, and leads to sustained effects on
beta-cell mass even after glucose infusion is stopped (16, 152). Glucose has been shown to promote beta-cell survival by suppressing a constitutive apoptotic program in vitro (62). However, high glucose concentrations have also been reported to stimulate beta-cell apoptosis in vitro and in the sand rat Psammomys obesus, an animal model of type 2 diabetes which becomes hyperglycemic upon feeding a high-calorie diet (36). This “glucotoxicity” may also involve cytokines like interleukin (IL)-1, which is secreted by human islets in the presence of high glucose and leads to beta-cell apoptosis (87). Chronic hyperglycemia may thus be detrimental to beta cells and plays a role in the development of diabetes by decreasing the beta-cell mass (reviewed in Ref. 69). Increased beta-cell apoptosis has indeed been observed in humans with type 2 diabetes (30). Several studies based on tissue morphometry have reported a diminished beta cell mass in type 2 diabetic patients compared with control subjects (30, 33, 74, 125, 168).

### 3. Placental hormones

During pregnancy, placental hormones, especially placental lactogens, are mainly responsible for the changes in beta-cell mass (reviewed in Refs. 100, 140). These hormones stimulate beta-cell proliferation in isolated islets, and beta-cell mass is reduced 26–42% in receptor-deficient mice (44).

### 4. Glucagon-like peptide-1

The gastrointestinal incretin hormone glucagon-like peptide-1 (Glp-1) has been repeatedly reported to affect beta-cell function, replication, apoptosis, and neogenesis (reviewed in Refs. 25, 28). In obese hyperglycemic ob/ob mice, a Glp-1 analog ameliorates glycemia and increases beta-cell mass and beta-cell replication (116). Glp-1 delays the onset of diabetes in db/db mice (162), and in diabetic mice it lowers glycemia and increases beta-cell mass through neogenesis from Pdx1-expressing ductal precursor cells (142). Similar results were obtained with glucose-intolerant aged Wistar rats (107). With the use of rat and human duct cell lines in vitro, it was found that Glp-1 can induce differentiation to a functional beta-cell phenotype in cells expressing Pdx1 (63, 170). Glp-1 therefore seems to be a differentiation factor that can play an important role in neogenesis, besides having stimulatory effects on already differentiated beta cells via increased replication and decreased apoptosis (84). Surprisingly, pancreatic beta-cell mass is normal in Glp-1-receptor knockout mice (85), and it can normally adapt to obesity (132). It is possible that other gastrointestinal/incretin hormones can compensate when Glp-1 is knocked out.

### 5. Other growth factors

Several growth factors have been reported to exert a stimulatory effect on beta-cell replication in vivo or in vitro. These include insulin-like growth factors (IGF-I and -II), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and others (reviewed in Refs. 47, 60). IGF-II exerts an islet protective role in late fetal gestation and immediately after birth and thereby is important in defining the adult beta-cell mass (61, 108). In general however, unless transgenic approaches are used to induce overexpression of these factors in islets, the mitogenic effect on beta-cell mass is rather modest, and their role under normal physiological conditions is not clear. When these growth factors are added to primary cultured adult beta cells, no significant expansion of cell number has so far been reported. An exception may be the stimulation of human islet cell expansion in vitro by hepatocyte growth factor (HGF) (57). However, it was subsequently reported that HGF stimulates replication of contaminating ductal cells but not of the insulin-positive beta cells (81). Ductal cells are known to contaminate human islet preparations (73, 143).

### 6. Cell cycle regulators

It seems that mature beta cells are highly refractory to mitotic stimulation. Cell cycling is regulated intracellularly by protooncoproteins like cyclins and cyclin-dependent kinases (cdks), which are positive regulators, and by tumor suppressors or cdk inhibitors as negative regulators. Little is known on the molecular mechanisms that regulate, and mainly suppress, beta-cell replication. Using cyclin D2 −/− mice, it was shown that this particular D-cyclin is dispensable during the embryonic-fetal development of the beta-cell mass but that it is indispensable for neonatal replication of beta cells (52). Neonatal beta cells apparently fail to efficiently upregulate other D-cyclins, in contrast to acinar and ductal cells which retained replicative activity in these mice. Thus the cyclin D2 positive regulator of the cell cycle plays an important role in the control of beta-cell replication. Growth hormone and prolactin increase the expression of cyclin D2 via the STAT5 pathway in beta cells (45). Overexpression of cdk-4, or certain mutated form of this kinase, leads to insensitivity to cell cycle suppressors of the INK4 type. This results in increased beta-cell proliferation in vitro and in transgenic mice, apparently without loss of beta-cell function (34, 90, 114). In contrast, forcing beta cells to reenter the cell cycle by overexpression of the protooncogene c-Myc leads to beta-cell apoptosis (79). Thus the growth-promoting and oncogenic potential of c-Myc in beta cells is normally strongly counterbalanced by apoptosis. However, upon transgenic coexpression of c-Myc and the antiapoptotic factor Bcl-xl, the beta cells in the transgenic mice proliferate and form aggressive tumors (105). A previous study had shown that overexpression of Bcl-xl in beta cells prevents their cell death but at the same time leads to mitochondrial defects leading to im-
paired insulin secretion and hence hyperglycemia (171). Interestingly, when the beta cell transcription factor Pax-4 is overexpressed by adenoviral transduction in vitro, an increase in beta cell proliferation was observed with a concomitant increase in Bcl-xl and c-Myc. Thus induction of Pax-4 activates c-myc and beta-cell proliferation, and at the same time activates Bcl-xl thereby suppressing beta-cell apoptosis (26). Bcl-xl activity, however, alters mitochondrial calcium levels and ATP production, which explains impaired insulin secretion. Thus cell cycle regulators might represent therapeutic targets to manipulate the size of the beta-cell mass, provided that the apoptotic program can be controlled and that functional defects can be circumvented.

IV. REGENERATION

In several organs of our body, tissue parts which have been damaged can be regenerated. The regenerative capacity of, for example, liver tissue is well-known. It appears that in the absence of major external stimuli, the beta-cell population has only a very limited potential for regeneration. This is probably due to the limited replication capacity of beta cells and to the fact that neogenesis from precursor cells is not readily reactivated. Yet, under certain conditions where major external stimuli are applied, there can be a quite vigorous regenerative expansion of the beta-cell mass. Such regenerative growth may result from activation of otherwise quiescent precursor/progenitor or stem cells.

Regeneration of the endocrine pancreas has been studied in different experimental models that differ in the extent and selectivity of the tissue injury that is inflicted. The two major types of injury are caused by toxic drugs and surgery, respectively.

A. Destruction of Beta Cells

Selective destruction of beta cells can be obtained by injecting streptozotocin or alloxan. Streptozotocin is a DNA-alkylating agent and alloxan a generator of oxygen free radicals, both causing extensive DNA damage (149). Their selectivity is thought to be due to a better uptake by beta cells, although at higher doses also liver and kidney cells become affected. Experimental models can be quite different depending on the dose and the route of administration; for example, intraperitoneal injection has less severe effects than intravenous injection at a given dose.

In rats, intravenous administration of 100 mg/kg streptozotocin on the day of birth reduces the total beta-cell mass by ~90% in 48 h. Twenty days later, <40% of the normal beta-cell mass is restored (158). Up to a certain body weight the animals can maintain normoglycemia, but at the age of ~6 wk they become glucose intolerant. This neonatal-streptozotocin model is considered as an experimental model of type 2 diabetes wherein beta-cell mass compensation is deficient (15, 32). In this model, replicative activity of beta cells is increased, but apparently insufficient to regenerate a functional mass (15).

The capacity to regenerate following a toxic insult like streptozotocin rapidly declines during the first 5 days of life in rats (159). This indicates that beta-cell regeneration beyond the critical perinatal time window is inefficient, possibly because there is no more neogenesis operating from precursor cells. Interestingly, when the hormone Glp-1 is administered to streptozotocin-treated newborn rats, beta-cell neogenesis is stimulated, and this results in improved glucose homeostasis persisting at adult age (153).

When subtotal beta-cell destruction is accomplished in adult rodents, there is no “spontaneous” regeneration, and it is well known that the animals remain diabetic or die (2, 83 121). The exception may be the selectively perfused rat where alloxan is perfused in part of the pancreas while another part is clamped off and spared from the toxic effects (157). Preexisting beta cells from the spared part proliferate, whereas neogenesis of beta cells from duct cells leads to regeneration in the perfused part.

After near-total destruction of the beta-cell mass, application of a major external stimulus has been found to induce regeneration. In a recently described experimental model of beta-cell destruction by alloxan, the combination of two factors, gastrin and EGF, were found to restore glycemic control in mice (121). In this model of beta-cell regeneration, a beta-cell growth rate of >30% per day was observed, leading to a beta-cell population doubling time of only 3 days. Although there was no complete regeneration of the original beta-cell number after subtotal destruction, regenerative growth induced by the gastrin and EGF treatment led to the restoration of 30–40% of the normal beta-cell mass within 7 days. The treatment had no effect on beta-cell replication, cell size, or apoptosis, and therefore, the regenerative effect could be attributed to neogenesis from precursor cells. Indeed, a pulse-chase labeling with the thymidine analog bromodeoxyuridine confirmed an influx of labeled cells from a replicating insulin-negative pool into the regenerating islets. The proliferating precursor cells express ductal-type cytokeratin markers. An “intermediate phenotype” characterized by cells coexpressing the beta-cell marker insulin and the ductal cytokeratin marker was taken as evidence for a transition from exocrine duct cells to endocrine beta cells (121). Gastrin hormone is expressed in fetal and in regenerating pancreas, and its high-affinity receptor cholecystokinin (CCK)-B is not expressed by beta cells themselves (118, 124, 161). Gastrin can be considered an important regulator of beta-cell neogenesis (see also sect. vB).
Betacellulin, a member of the EGF family, has also been reported to promote beta-cell neogenesis and to improve glucose metabolism in adult animals after beta-cell destruction (83, 167).

In NOD mice, beta cells are destroyed by a spontaneous autoimmune reaction leading to a type 1-like diabetes condition. Immune suppression in combination with Glp-1 (analog) treatment could restore normoglycemia and improve islet histology (101). The mechanism of this remission was not clear, however, and it may have involved regeneration as well as increased survival of beta cells. It should be pointed out that Glp-1 has been shown to protect beta cells from apoptosis (84).

**B. Surgical Injury**

Partial pancreatectomy represents another model of tissue injury wherein regeneration has been studied in rodents. In contrast to the robust liver regeneration following partial hepatectomy, surgical removal of part of the pancreas is followed only by a limited regenerative growth, and there is never a complete restoration of the original pancreatic volume. Furthermore, the regenerative response is proportional to the amount of pancreas removed. If half the pancreatic volume is left after hemipancreatectomy, this residual pancreas will grow only 20%. In the case of one-third of the volume being left after two-thirds pancreatectomy, the residual pancreas grows only 30%. When only one-tenth of the volume is left after subtotal pancreatectomy, the residual pancreatic volume grows 80% (104). Regenerative growth after partial pancreatectomy is thus not impressive and never complete. Except maybe for subtotal (90–95%) pancreatectomy, it cannot be considered as an important growth stimulus. The same is true for the endocrine part of the pancreas, with only a 30% expansion of the residual beta-cell mass being observed after two-thirds pancreatectomy in mice, and this over a period of 5 wk (35). The beta-cell growth rate following two-thirds pancreatectomy is thus rather modest. Dor et al. (37) using a transgenic cell labeling approach showed that after two-thirds pancreatectomy there is no evidence for the formation of new beta cells by differentiation from insulin-negative progenitor or stem cells. This conclusion seems at stake with the observations of others who reported neogenesis of beta cells from proliferating ducts after subtotal pancreatectomy (17). This apparent discrepancy may be explained by a difference in the extent of tissue damage and growth-stimulating capacity between the two models that were used, i.e., between 70 and 90% pancreatectomy. Another important difference is that a hyperglycemic condition is induced by 90% but not by 70% pancreatectomy. Thus it remains possible that in the latter case only beta-cell replication is activated, whereas in the case of 90% pancreatectomy, a more vigorous beta-cell growth is obtained by neogenesis in addition to beta-cell replication. Unfortunately, these reports (17, 37) do not mention the beta-cell number before and after the pancreatectomy insult so that it is impossible to compare the respective beta-cell growth rates that were induced by the different treatments. However, in other studies of 90% pancreatectomized rats, a doubling of the remnant beta-cell mass was obtained within 1 wk postsurgery (110), whereas only a 30–40% increase over 4-wk time was observed following 60% pancreatectomy (80). These data confirm that subtotal pancreatectomy is a much stronger growth stimulus for the beta-cell population than two-thirds pancreatectomy. In 90% pancreatectomized rats, depletion of beta cells by streptozotocin treatment before surgery does not impair the regenerative capacity of the pancreas and demonstrates that partial beta-cell regeneration can be accomplished by neogenesis rather than replication of beta cells (43).

Others have used 90–95% pancreatectomy as a rodent model of type 2 diabetes and showed that daily administration of the Glp-1 analog exendin-4 during 10 days postpancreatectomy attenuates the development of diabetes. It was reported that exendin-4 stimulates the regeneration of the pancreas and expansion of the beta-cell mass by the processes of neogenesis and replication of beta cells (166). After 70% partial pancreatectomy, Glp-1 was also found to act as a potent stimulator of beta-cell regeneration (35). Glp-1 receptor knockout mice showed worse glucose intolerance after partial pancreatectomy compared with wild-type mice, and this correlated with a significant defect in beta-cell mass regeneration (35). Another regeneration-promoting factor after subtotal pancreatectomy is betacellulin, a growth factor that belongs to the family of EGFs (82). Interestingly, Glp-1 has been shown to transactivate the EGF receptor and thereby stimulate beta-cell replication (29). On the other hand, in the GK-rat genetic model which spontaneously develops “type 2” diabetes, regeneration of the beta-cell mass following 90% pancreatectomy is impaired due to the decreased capacity for both replication and neogenesis (110).

An experimental model that leads to robust hyperplasia of beta cells is partial duct ligation. This consists in the closure, or ligation, with surgical thread of part of the main duct of the pancreas. As a consequence of this obstruction, exocrine secretory products will leak into the interstitial space and lead to tissue damage and inflammation. The part of the pancreas that lies downstream of the ligation (≈50% of the pancreatic volume) is not affected histologically and continues to function normally. During the first week postligation, a pronounced beta-cell hyperplasia occurs in the ligated part, although the animals remain normoglycemic (160). Beta-cell replication is only slightly elevated, which is strongly suggest-
ing that the increased beta-cell number results from neogenesis. This is also indirectly indicated by the observation of intermediate phenotypes between ductal and endocrine cells (160) or exocrine acinar and endocrine cells (9, 77). Whereas part of the affected acinar exocrine cells are eliminated by apoptosis, the remaining ones transdifferentiate into ductlike cells and thereby contribute to the formation of metaplastic ductal complexes. These cells start to express transforming growth factor-α (TGF-α), gastrin (161), and the high-affinity gastrin/CCK-B receptor, which is not expressed on beta cells (118). Cells in these ductal complexes proliferate and can differentiate into endocrine cells (160). The observed increase in beta-cell number is 80% in only 7 days time. In this model of stimulated expansion of the beta-cell mass, a further increase in the beta-cell growth rate is achieved by infusing the rats with gastrin hormone (119). This leads to a beta-cell number doubling in only 3 days time, or a growth rate which is ~10 times faster than in control conditions. Obviously, this robust hyperplasia cannot be accounted for by beta cell self-replication, as the fraction of beta cells being engaged in the cell cycle remains very low (119). Administration of a selective CCK-B gastrin receptor antagonist completely prevents the duct ligation-induced increase in beta-cell mass (120). This means that gastrin is a crucial factor in the regulation of beta-cell neogenesis during regeneration.

A protein extract from partially obstructed pancreas (by wrapping the organ with cellophane) has been reported to induce islet neogenesis from ducts, and it could reverse streptozotocin-induced diabetes when administered to hamsters (122, 123). From this regenerating pancreas, a novel pancreatic gene called INGAP has been identified whose protein product is capable of initiating neogenesis from ducts and has an antiapoptotic effect. Transgenic mice in which beta cells express insulin-like growth factor (IGF)-I can recover from immune attack of the islets following multiple low doses of streptozotocin (51). IGF-I is thought to stimulate beta-cell proliferation and neogenesis from ducts and has an antiapoptotic effect.

Transgenic mice that overexpress the inflammatory cytokine interferon-γ in pancreatic islets show features indicative of rapid beta-cell renewal by the process of neogenesis from ductal complexes (54). Beta-cell hyperplasia was also observed in transgenic mice with pancreatic overexpression of IL-6, another important inflammatory cytokine (31). These studies suggest that inflammatory cytokines may induce or regulate beta-cell neogenesis. It can be assumed that these cytokines are also increased in conditions like surgical or toxin-mediated tissue injury, where inflammation is obviously present. Indeed, it has been reported that in the absence of inflammation, subtotal pancreatectomy does not lead to beta-cell regeneration (76). Recently, it was found that the IL-6-related cytokine leukemia inhibitory factor (LIF) acts synergistically with EGF to induce beta-cell neogenesis in vitro (5).

In summary, whereas self-replication of beta cells leads to a slow expansion in physiological conditions, neogenesis from progenitor cells can lead to vigorous expansion and partial regeneration of the beta-cell mass in short time (see also Table 1). This process can be considerably enhanced by exogenous factors like the hormones gastrin and Glp-1, and possibly by inflammatory mediators or cytokines. The precise molecular regulation of neogenesis, however, remains to be unravelled.

V. STEM CELLS AND PHENOTYPIC PLASTICITY IN THE PANCREAS

According to a growing number of reports, beta cells or at least beta cell-like cells, can be generated ex vivo

### TABLE 1. List of some experimental models of beta-cell regeneration wherein the increase in beta cell number or mass was quantified

<table>
<thead>
<tr>
<th>Experimental Model</th>
<th>Beta-Cell Numerical Increase/Time</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse 70% pancreatectomy</td>
<td>30% in 35 days</td>
<td>35</td>
</tr>
<tr>
<td>Rat 60–70% pancreatectomy</td>
<td>40% in 28 days</td>
<td>80</td>
</tr>
<tr>
<td>Rat 90% pancreatectomy</td>
<td>100% in 7 days</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>200% in 14 days</td>
<td></td>
</tr>
<tr>
<td>Rat duct ligation</td>
<td>80% in 7 days</td>
<td>160</td>
</tr>
<tr>
<td>Rat duct ligation + gastrin</td>
<td>100% in 3 days</td>
<td>119</td>
</tr>
<tr>
<td>Mouse alloxan + gastrin/EGF</td>
<td>100% in 3 days</td>
<td>121</td>
</tr>
</tbody>
</table>

The regenerative growth rate of the beta-cell mass differs considerably between these models. Note that beta-cell replication was increased to some extent in the first four models but not in the latter two. The highest growth rates are seen in the latter four experimental models, wherein beta-cell neogenesis was shown to be active. EGF, epidermal growth factor.
from either undifferentiated stem cells or from transdifferentiating mature pancreatic cells (Fig. 1). Even if the physiological relevance of these findings remains unclear, it is clear that they may find applications in cell therapy by providing a source of cells to restore the beta-cell mass.

A. Stem Cells

Stem cells are defined as clonogenic, self-renewing, multipotent cells and are known to reside in a number of organs such as bone marrow, intestine, skin, brain, and others. In the liver, oval cells are resident stem cells that participate to tissue regeneration under certain conditions (3, 41). However, it is not yet clear whether such cells also reside in the postnatal pancreas. It has been claimed that exocrine ducts (18) and islets (56) would harbor islet progenitor cells. Although this possibility has already been raised for a long time (reviewed in Ref. 22), only recently a few reports have given support to the existence of true progenitor or stem cells in the adult pancreas. First, it was reported that cells can be isolated from normal rodent pancreas (ducts?), which can be subcultured over long periods of time. In differentiation conditions in vitro, these self-renewing cells generated insulin-producing cells that could reverse diabetes upon transplantation (113). Another report claimed that self-renewing cells expressing the neural stem cell marker nestin were able to generate cells with phenotypic characteristics of pancreatic endocrine and exocrine cells, and of hepatocytes (172). More recently, clonogenic cells were isolated from rodent pancreas that were able to give rise to cells with neural or pancreatic features, including expression and secretion of insulin, but these had only limited self-renewal capacity (133). Others found clonogenic cells that were able to generate insulin-expressing cells and that could be enriched by sorting for the c-met receptor (receptor for HGF) (145). It is not clear whether these progenitor or stem cells actually take part in renewal, expansion, or regeneration of beta cells or other pancreatic cells in vivo. It is hoped, however, that if such cells also reside in human pancreas, they could be used for ex vivo generation of beta cells. Besides stem cells that reside in the pancreas, extrapancreatic or embryonic stem cells (ES cells) are candidates for ex vivo beta-cell generation. There have been reports on the differentiation of beta cell-like cells from mouse and human ES cells, rat hepatic oval cells, mouse intestinal epithelium, and mouse bone marrow (reviewed in Refs. 12, 144).

B. Transdifferentiation

Transdifferentiation has been originally defined as a phenotypic switch from one differentiated state to another. In simpler vertebrates like the newt for example, regeneration of major body parts like the limbs is accomplished by differentiated cells rather than by stem cells. The differentiated cells at the site of tissue injury first dedifferentiate whereby they reacquire embryonic plasticity, then proliferate and finally redifferentiate. This system differs from stem cell-mediated regeneration in that the
VI. DIABETES IN HUMANS

A lot of information on beta-cell biology has been obtained in animal studies. However, we are mainly interested in human beta cells and human diabetes. There is no doubt that a critical reduction in beta-cell mass is responsible for the appearance of type 1 diabetes symptoms. Hence, transplantation studies have demonstrated that the diabetic metabolic defects can be restored in type 1 patients by restoring a functional beta-cell mass (73, 143). Animal data (see sect. II B) strongly suggest that the impairment of insulin secretion in type 2 diabetes is also partly related to reduction of beta-cell mass, at least relative to prevailing insulin demand. In fact, this idea is relatively new, and it is actually still a matter of debate whether humans with type 2 diabetes have a reduced or suboptimal beta-cell mass. In humans, such a defect in the beta-cell mass may originate from genetic predisposition, but the situation is likely worsened by environmental factors such as hyperglycemic glucotoxicity and hyperlipidemic lipotoxicity. An increase in beta-cell apoptosis may prevent the adaptive increase in beta-cell mass in type 2 diabetes (30). For obvious reasons, it is difficult to collect data on the size of the beta-cell mass in humans, and there is probably a large variation in the beta-cell volume density among individuals. Nevertheless, several studies have clearly documented that the beta-cell mass in type 2 diabetic patients is reduced at least 20% compared with control subjects (30, 33, 74, 125, 168). We have also to take into account that in insulin-resistant subjects, there is need for a larger beta-cell mass than in normal control subjects.

VII. SUMMARY

Based on the available evidence that we have discussed above, a general concept on beta-cell mass regulation can be proposed as follows. The number of beta cells in the endocrine pancreas seems to be determined during a critical window phase that is situated somewhere in the last quarter of fetal gestation and in the first few days after birth, at least in rodents. This critical beta-cell population size is developed mainly by the process of neogenesis. Neogenesis, which can be defined as the formation of new beta cells by the differentiation of previously insulin-negative precursor cells, is a process that normally stops shortly after birth. Postnatal adaptation of the beta-cell population size in response to changing metabolic demands is accomplished by an interplay of beta-cell replication and apoptosis. New beta cells are thus added to the original number the individual is born with, by replication of preexisting cells. It appears that if the perinatal beta-cell number is suboptimal, for example, as a result of deficient fetal nutrition, postnatal beta-cell replication does not suffice to compensate later in life. Nutritional overload, obesity, limited physical exercise, and ageing will further aggravate the situation and lead to the development of type 2 diabetes symptoms.
Neogenesis by the process of transdifferentiation and/or stem cells appears to be activated only after severe injury to endocrine or exocrine pancreatic tissue, or when strong additional stimuli like Glp-1 or gastrin are provided. Beta-cell self-replication can also be increased by certain stimuli like glucose and other nutrients, insulin and several growth factors, but it apparently cannot lead to an efficient regeneration. This can be explained by the small fraction of cells engaged in the cell cycle, by the observation that increased beta-cell replication is associated with increased apoptotic death, and possibly by a glucotoxic effect of chronic hyperglycemia.

Recently, some confusion has been raised as to the relative importance of neogenesis, or even its mere existence, following the paper by Dor et al. (37). This paper has shown that there is no neogenesis during normal physiological renewal and compensatory growth of the murine beta cell mass, confirming previous studies (see sect. iii). In addition, no evidence for neogenesis was found following two-thirds pancreatectomy. Care should be taken, however, to avoid over-interpretation of these data, since this is an experimental model causing only very limited regenerative growth (see sect. iv). In other experimental models with a much more important beta-cell growth (Table 1), it is clear that beta-cell neogenesis does operate. A comparable “duality” is known from liver regeneration where there has been a long debate concerning the relative importance of hepatocyte self-replication versus stem cell derivation. It is now known that under some conditions, like after two-thirds pancreatectomy, regeneration is caused by replication of residual hepatocytes, whereas under other conditions hepatic stem cells are called upon, namely when hepatocyte replication is compromised by certain drugs or when there is extensive inflammation (3, 41). Nevertheless, it would be interesting for future studies to apply a genetic tracing approach similar to the one introduced by Dor et al., to confirm the occurrence of neogenesis and to trace the beta cell precursors in the pancreas. It is to be expected that different types of precursors may be involved, again much depending on the experimental conditions used to cause injury to pancreatic tissue(s).

Combinations of agents that stimulate beta-cell replication, inhibit beta-cell apoptosis, and induce beta-cell neogenesis, are probably the best option for pharmacological restoration of a functional beta-cell mass in diabetics. It is very likely, however, that a pharmacological treatment resulting in regeneration of beta cells would be inefficient in type 1 patients due to the expected recurrence of autoimmune and inflammatory destruction of the newly formed beta cells. So, whether the knowledge on beta-cell mass control is to find application in cell therapy (ex vivo generation) or in regenerative therapy, controlling the immune system represents the major challenge. In the case of type 2 diabetes, the situation is different, since this is not an autoimmune disease. Pharmacological regulation of the beta-cell mass therefore represents an interesting target for the treatment of type 2 diabetes patients that benefit from insulin treatment or that have become insulin dependent.

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