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REVIEW ARTICLE

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Islet cell neogenesis in the pancreas

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Abstract The beta cell population of the endocrine pancreas may expand by either of two processes, neogenesis or replication. While replication requires the existence of an already differentiatied beta cell, neogenesis depends on the presence of active stem cells. Since the replicative activity of highly specialized cells such as beta cells seems to be limited, it is interesting to study the potential of the endocrine pancreas for beta cell neogenesis. In this article we review both the current state of knowledge of beta cell neogenesis under natural conditions and in response to stimulation, and the significance of neogenesis for beta cell growth in health and disease.

Key words Beta cell · Duct cell · Pancreas · Islets of Langerhans · Diabetes

Introduction

The total number of insulin-producing β-cells in the pancreas is a critical factor in the regulation of glucose homeostasis. Insulin-dependent diabetes is known to result when the number of β-cells is severely reduced due to autoimmune destruction [2]. Transplantation of islet β-cells to diabetic rats has been shown to restore glucose homeostasis, provided a large enough quantity of cells are grafted [15, 30, 47]. The availability of sufficient donor material (an adequate number of β-cells) is also a major problem if islet cell transplantation is to become a treatment for human diabetes [46]. It is thus very important to understand the mechanisms regulating β-cell growth in the pancreas. Knowing more about β-cell growth and regeneration could lead us to new, clinically relevant strategies, such as the induction of β-cell regeneration in the diabetic pancreas or expansion of the islet graft mass before or after transplantation. New β-cells can be formed either by mitotic division of a pre-existing, differentiated β-cell, or by differentiation from an undifferentiated precursor or stem cell. For clarity, we will define the former mechanism as "replication" (β-cell mitosis) and the latter as "neogenesis" (stem cell differentiation).

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Replication versus neogenesis

Histomorphometric studies have revealed that during late fetal gestation [14, 29, 57] and early neonatal life [7, 60], most new β-cells are formed by the process of neogenesis. This process, which may also be called "nesidioblastosis", has been defined morphologically as the formation of endocrine cell buds originating from the duct epithelium [10, 45]. In adult life β-cells are highly differentiated cells with a low proliferative capacity [12, 21, 56]. Only a small percentage of the β-cells enter the proliferative cell cycle; most cells do not divide [56]. Nevertheless, in adult rodents the β-cell population continues to expand, albeit slowly [39]. It has been suggested, though not proven, that the observed replication of differentiated β-cells is sufficient to ensure their postnatal growth [16, 21]. Since the life span of β-cells has not been ascertained, the extent of physiologically determined cell renewal remains unknown. Thus, the possibility that neogenesis may also play a role in maintaining β-cell number during adult life cannot be excluded. Moreover, most differentiated cells only undergo a limited number of mitotic cycles implying that the β-cell population requires a continuous supply of undifferentiated cells with the potential for mitotic division after endocrine differentiation. In adult rats the occurrence of small islet cell buds attached to ducts has been interpreted as an indication of continuing neogenesis [4]. That duct cells have such a progenitor cell function is supported by the observation that the proliferative activity of islet (and acinar) cells decreases exponentially with age, whereas duct cells continue to proliferate at a relatively high rate [41].

Islet cell neogenesis can be reactivated later in life by applying external stimuli, such as ligation of the exocrine ducts [25, 62] or partial (90%) pancreatectomy [6]. Neogenesis has also been reported to occur in transgenic mice that overexpress certain growth factors or cytokines in the exocrine pancreas or in the β-cells, such as interferon-γ [19], tumour necrosis factor-α [24], interleukin 6 [9], or a combination of transforming growth factor-α and gastrin [64]. These observations further indicate that stem cells capable of differentiating into β-cells are still present in the adult rodent pancreas. This was confirmed by a study in which transplanted epithelium from the adult rat pancreas was found to develop hormone-expressing islets, provided that fetal mesenchymal tissue was co-transplanted [13]. Other studies have revealed that the adult rodent pancreas contains multipotential stem cells that can, under certain experimental conditions, produce hepatocyte-like cells in the pancreas [38, 48]. The multipotential stem cells were located in or associated with the ductules and resembled the bile ductular and oval cells that represent the putative liver stem cells.

In summary, the normal rodent pancreas harbours islet stem cells, but it is not known whether these stem cells participate in the normal growth and renewal of β-cells in the adult. If this were the case, it would imply that in clinical islet transplantation, stem cells should be transplanted together with islet cells in order to maintain a functioning graft long term. Another application could be the controlled stimulation of stem cell activity to regenerate the endogenous β-cell population in diabetics.

Phenotype of pancreatic stem cells

As discussed above, there is evidence that the pancreas contains stem cells, or cells with stem cell potential, throughout life. This raises the question whether these stem cells could be recognized by means of specific differentiation markers. The phenotypic features of islet stem cells are largely unknown, but a few recent studies have identified some cell markers that are transiently expressed during embryonic ontogenesis and that could therefore represent markers of active islet stem cells.

Neuroendocrine and other peptide markers

The ontogenesis of the pancreas starts with the appearance of "protodifferentiated" epithelial cells, which form ductules from which both acinar (exocrine) and islet (endocrine) cells originate [45]. A recent study has shown that in the fetal rat pancreas, which begins to develop around day 9 postconception, the protodifferentiated duct cells transiently express the glucose transporter protein GLUT2, which is normally only expressed on mature β-cells [44]. After day 16 of gestation, when the histogenesis of the endocrine pancreas is in its quantitatively most important phase, GLUT2 disappears from the ductal cells and appears in the newly formed β-cells. Interestingly, we recently found that GLUT2 expression also appears transiently in proliferating ductules of the pancreas of duct-ligated adult rats. GLUT2 expression in these ductal cells preceded the neogenesis of islet cells [62]. Another potential marker of islet stem cells in the rat is the high affinity nerve growth factor receptor Trk-A, which is transiently expressed in ductular cells around newly formed islets [27]. In the 18 to 24-week-old human fetal pancreas, high acid β-galactosidase enzymatic activity has been reported in ducts and newly formed islets, whereas little or no activity was found in the adult pancreas [3]. The cells expressing this enzyme also expressed the neural marker tyrosine hydroxylase, which had previously been reported to occur in murine islet cell precursors [58, 59]. Therefore, both enzymes may be considered to be islet stem cell markers.

Other potential markers of islet cell precursors expressed in the murine embryonic pancreas during endocrine differentiation are neuropeptide Y (NPY) [59], peptide YY [60] and STF-1 [20]. Pancreatic polypeptide (PP) has also been proposed as an islet stem cell marker [22, 23], but the question has been raised whether this could represent NPY, which is structurally closely related to PP [59].

Cytoskeletal proteins

Although it is well recognized that the pancreatic stem cells are located in the exocrine ducts and ductules in both fetal and postnatal life, no phenotypic markers of rat ductal cells were available until recently. We have demonstrated that the cytoskeletal intermediate filament protein, cytokeratin 20 (CK20), represents a specific marker for adult rat pancreatic ductal cells [7, 8] and this cytokeratin protein is not found in normal adult islet cells. During islet cell neogenesis in the fetal (Bouwens, submitted for publication) and neonatal [7] pancreas and when neogenesis is induced by duct ligation in adult rats [62], transitional cytodifferentiation forms were observed that could be identified by their co-expression of CK20 and insulin or glucagon. These observations clearly demonstrated the transition from ductal cell to β-cell or α-cell. In the fetal pancreas from day 17 of gestation onwards, large ductal cell clusters that strongly express CK20 can be observed (Bouwens, submitted for publication) (Fig. 1). These cellular aggregates are larger than exocrine acini but have the size of more mature islets. In the middle of these structures, clusters of insulin- and glucagon-expressing cells are found. Whereas the hormone-expressing cell mass within these fetal islets gradually increases in size, the CK20 reactivity decreases, resulting in islets that only weakly express CK20 in the centre but still have a mantle of intensely CK20-positive cells in the periphery (Bouwens, submitted for publication) (Fig. 1). In the neonatal period the islets completely lose their CK20 reactivity [7], whereas the duct cells retain theirs. CK20 is therefore a good marker of fetal duc-

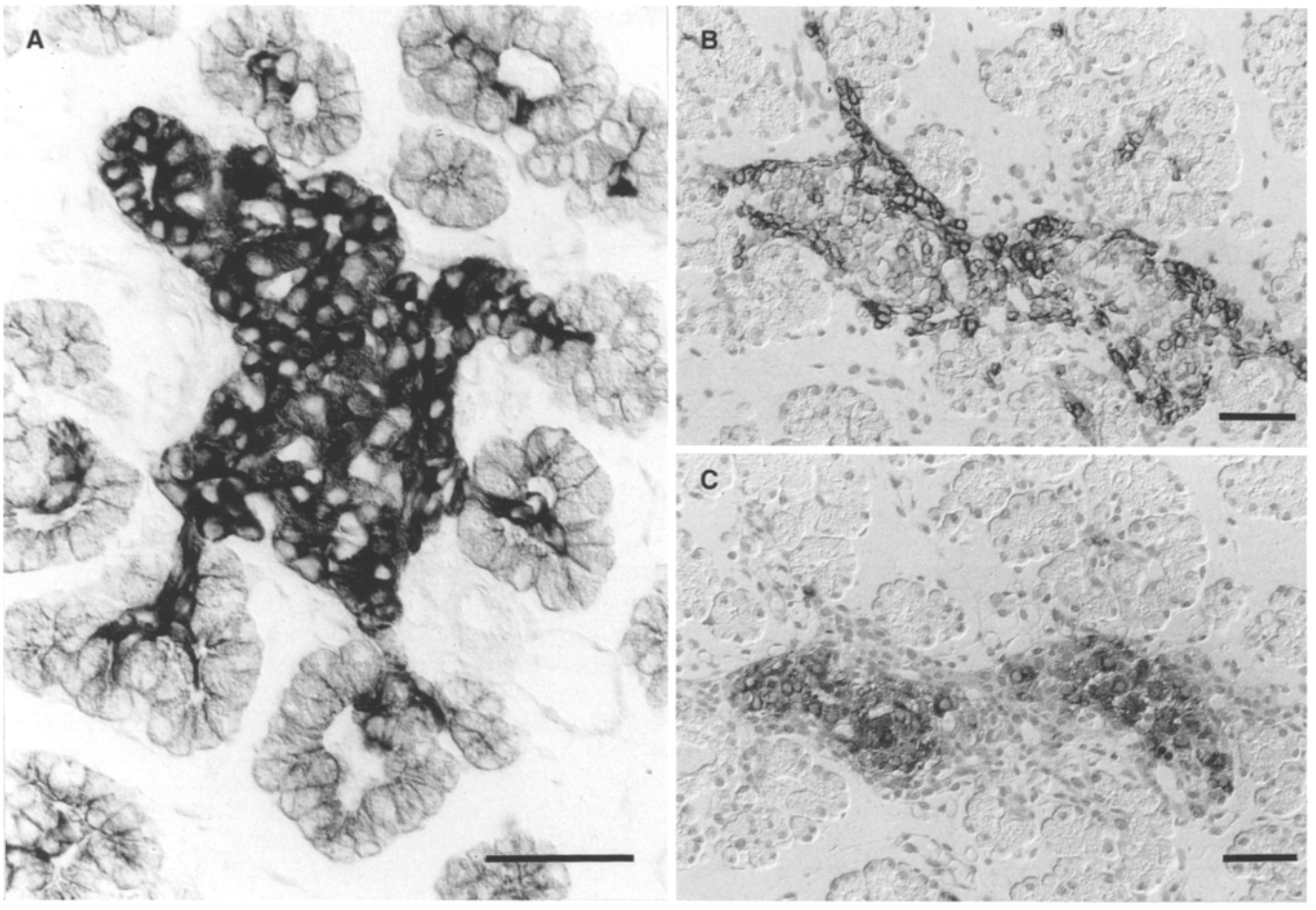

Fig. 1A–C Illustration of the successive stages in fetal rat islet development (phase contrast). **A** Pancreas on day 17 of gestation, immunoperoxidase stained for CK20. Note the intensely stained islet-forming unit of ductal origin, surrounded by exocrine acini. **B** Pancreas on day 19 of gestation, stained for CK20. Note that the islet mantle of ductal origin is intensely stained, whereas the differentiated endocrine cells in the center are weakly stained. **C** Consecutive section adjacent to the one in **B** but stained for insulin immunoreactivity to demonstrate the differentiated β-cells. *Bars*=40 μm

tal stem cells. It is also expressed in normal ductal cells (which may represent "resting" or "dormant" stem cells). We found, however, that another cytoskeletal marker, namely vimentin, was present in the fetal ductal cells during late gestation, i.e. when islet cell neogenesis is most pronounced (Bouwens, submitted for publication). Vimentin and cytokeratin co-expression might be a marker of epithelial stem cells [5, 55].

Single islet cells

Endocrine "islet" cells can also be found as single cells, which are apparently dispersed in the pancreatic (exocrine) tissue or close to the lining of an excretory duct [4]. Single β-cells are more common in neonatal than in adult rats, although their incidence again increases after duct ligation, which induces neogenesis [61, 62]. These cells are smaller than islet β-cells and most probably represent recently formed cells resulting from the differentiation of precursors located in the ducts or ductules by the process of budding [10]. An increased incidence of single endocrine cells, which were found close to centroacinar and ductular cells, has also been associated with neogenesis in the human pancreas [18]. It is not clear, however, whether such single endocrine cells will ultimately develop into islets and nothing is known about their functional importance.

Heterogeneity of precursor cells

The co-expression of different hormones and neuroendocrine markers by embryonic pancreatic cells has been taken as evidence for the existence of a common endocrine progenitor cell [20, 59, 60], which may derive from a stem cell common to all pancreatic epithelial cells [45]. As the endocrine progenitor cell develops, the expression of one hormone increases and that of another is extinguished, leading to the final differentiation that is characteristic of a mature islet cell type. However, the existence of multihormone-expressing cells in the early embryonic pancreas [11, 37] has led to some confusion. For instance, glucagon-insulin double-positive cells have been discussed as progenitors of all islet cell types [59, 60].

This hypothesis was recently refuted by a study showing that different islet cell types could be selectively ablated at their first appearance by targeted expression of hormone-promoter-driven toxigenes [23]. It was found that glucagon cells and insulin cells are not necessary for the development of the other islet cell types. In another recent paper the existence of two β-cell lineages was discussed [44]. One lineage would arise from GLUT2-negative (glucagon-positive?) precursors in an early phase of development and another from GLUT2-positive precursors later in development. However, the appearance of glucagon-insulin co-expressing cells early in gestation is probably related to the presence of immature α-cells rather than to β-cells, which arise later in gestation [45]. GLUT2 expression in the stem cells may then be related specifically to the neogenesis of all β-cells. The fact that in rats α-cells arise earlier than β-cells does not necessarily mean that the latter originate from the former. The undifferentiated, multipotential stem cells remain present throughout fetal development and can give rise to the different islet cell types in a certain order. In addition, it is clear that during late gestation many new α-cells (and other non-β-cells) are generated at the same time as the β-cells are being formed [29, 40].

So far there are no indications that ductal cells are heterogeneous with only some duct cells acting as β-stem cells. When "stem cell markers" GLUT2, acid β-galactosidase, vimentin, etc.) are expressed during periods of very active islet neogenesis (in the fetal pancreas or ductligated pancreas), all ductal cells appear to express the marker. This suggests that all ductal cells are capable of stem cell activity. In the normal adult it is possible, however, that they should be considered to be "dormant" or "facultative" stem cells, as has been proposed for bile ductal cells in the liver [5, 46, 55]. We must conclude, however, that our knowledge of stem cell markers in the adult is very limited at present. Finding more or better phenotypic markers of pancreatic stem cells, or stem cell activity, will help us to determine whether these stem cells constitute a subpopulation of duct cells or whether all duct cells have stem cell potential.

Growth and differentiation-inducing factors

The ultimate goal of the study of islet cell development is to be able to characterize the factors that regulate specific differentiation. What drives a ductal stem cell to divide and form a β-cell? Very little is known about this question, but there are a few interesting hypotheses (presented schematically in Fig. 2):

Fetal mesenchymal factors

According to the oldest hypothesis, fetal mesenchymal cells can release factors that regulate the differentiation of pancreatic epithelial cells [51]. More recently this hypothesis was confirmed when it was observed that co-transplantation of ductal epithelium with fetal mesenchyme led to endocrine differentiation in the graft, whereas this was not the case when either the epithelium or the mesenchyme was grafted alone [13]. So far, however, the long sought mesenchymal factors have not been identified. A recent study proposed that transforming growth factor-β1 (TGF-β1) may be one candidate for a mesenchymal factor that promotes the development of β-cells in tissue-cultured pancreatic rudiments [54]. TGF-β1 alone could not, however, account for the effects of "fetal mesenchymal factor". Other "mesenchymal factor" candidates may be hepatocyte growth factor [43], vascular endothelial growth factor [42] and nerve growth factor [28].

Cytokines

A number of studies of transgenic mice whose pancreas or β-cells overexpressed various cytokines have indicat-

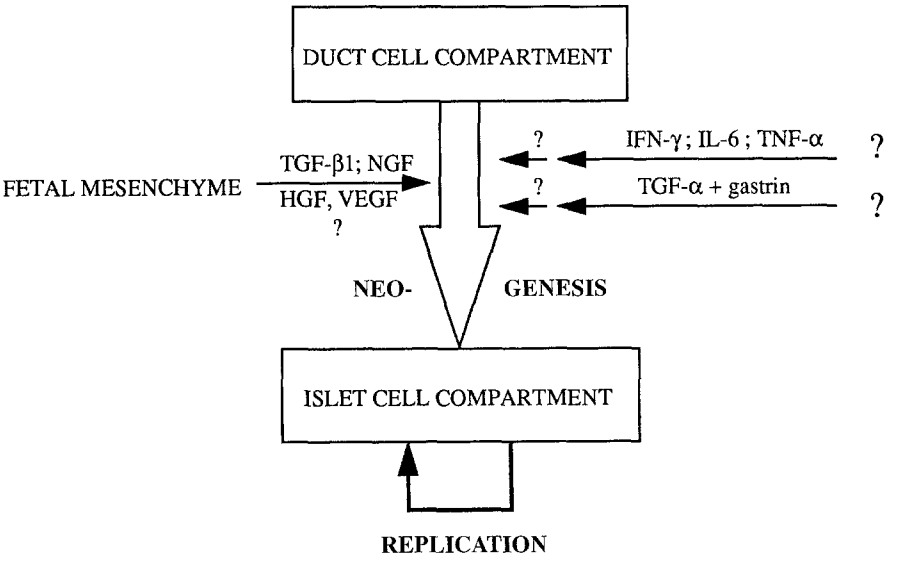

Fig. 2 Schematic representation of islet cell neogenesis and its regulation. The duct cell compartment contains stem cells that can feed new endocrine cells into the islet compartment (neogenesis). Known growth and differentiation factors that have been proposed as being involved in this differentiation axis are: fetal mesenchymal factors, cytokines and a combination of transforming growth factor-α and gastrin (cf. text)

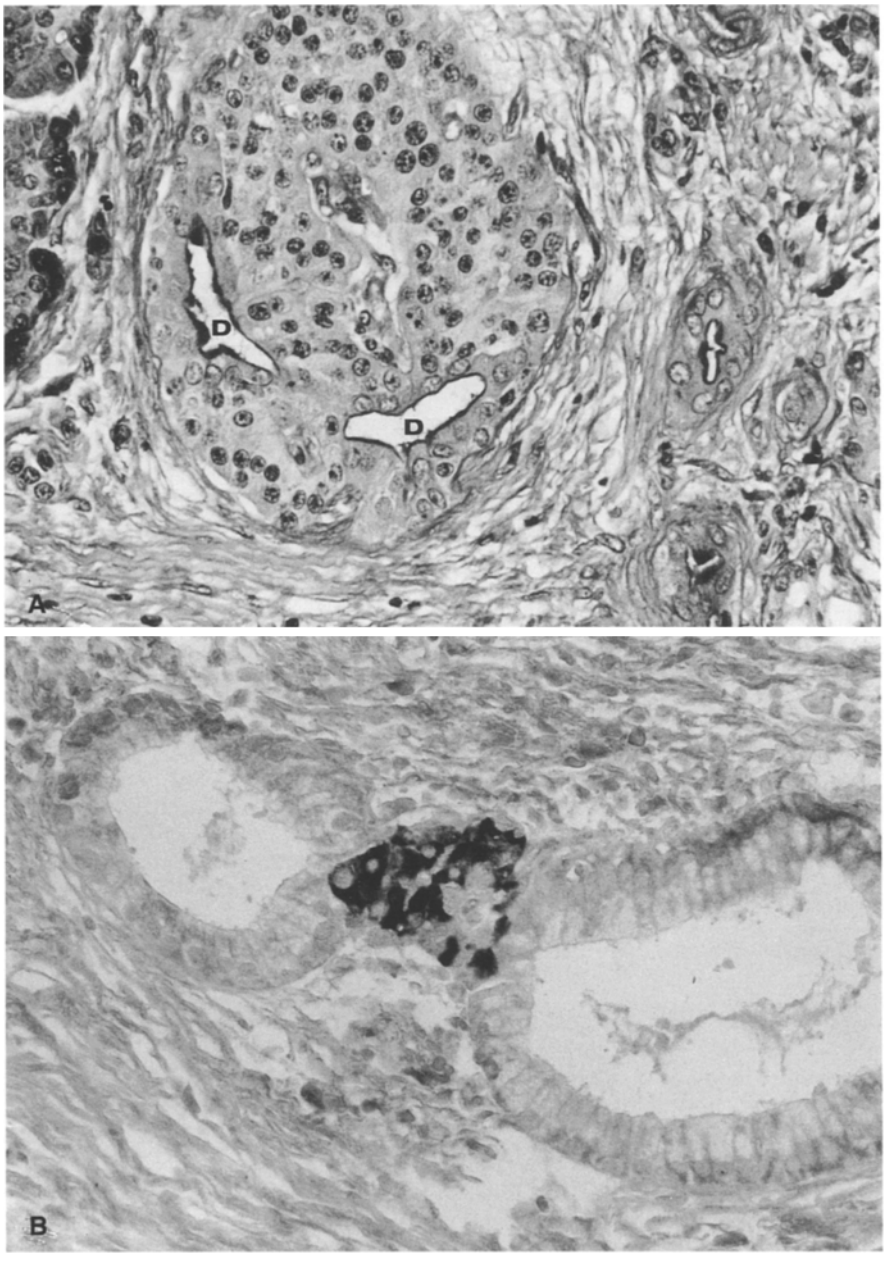

Fig. 3A, B Islet changes in chronic pancreatitis suggesting islet cell neogenesis from duct cells. **A** Inside an islet two small ducts (*D*) with mucin-producing cells. Periodic acid-Schiff, ×300. **B** Budding of islet cells staining mainly for insulin from pancreatic cells. ×300 (reprinted from [31], with permission)

ed that β-cell neogenesis occurs postnatally. The cytokine transgenes studied include interferon-γ [19], interleukin-6 [9] and tumour necrosis factor-α [24] transgenes. It is not clear from these studies whether the cytokines had a direct influence on stem cell differentiation or whether they induced the over- or underexpression of other factors, eventually leading to the observed effect. Inflammatory macrophages and other mesenchymal cells may play an important role.

TGF-α and gastrin

TGF-α-overexpressing transgenic mice were previously shown to contain metaplastic ducts in the pancreas [26, 53]. Gastrin is transiently produced in perinatal islets [33, 36] and hypergastrinaemia may have an effect on islet cell neogenesis [52]. Based on these observations, double transgenic mice were generated that overexpress TGF-α in their exocrine pancreas and gastrin in their β-cells [64]. These animals contained an increased islet mass (approximately double that of control mice), which was not noted in single transgenes that expressed either TGF-α or gastrin alone. As is the case with other transgenic studies, however, it is not clear whether the reported effect is directly due to the combination of the two peptides or whether it involves inducible secondary factors.

Ilotropin

Islet neogenesis can be induced in adult rodents by total [25, 62] or partial [50] obstruction of the pancreatic ducts. It has been reported that a cytosolic extract from partially ligated ("cellophane wrapped") hamster pancreas induced islet neogenesis when the extract was injected into other hamsters. The same extract even reversed diabetes in streptozotocin-treated hamsters [50]. However, the active factor(s), tentatively termed "ilotropin", has not been characterized so far [49, 50]. This observation and the finding that ductal proliferation and neogenesis occur in the ligated tail part of the pancreas but not in the non-ligated head part [62] suggest the existence of autocrine or paracrine pathways for the regulation of cell differentiation in the pancreas.

Neogenesis in diabetes

In adult rats complete β-cell destruction by the toxic agent streptozotocin (50–100 mg/kg) is not followed by β-cell regeneration and consequently leads to permanent diabetes [1]. In contrast, neonatal rats treated with streptozotocin on the day of birth show partial regeneration of the β-cell population and become normoglycaemic [61]. However, when streptozotocin is administered a few days after birth, the regenerative capacity is lost [63]. The possibility should be explored whether streptozotocin, given in the postnatal period, also affects the stem cells, or whether stem cell differentiation later in life requires the presence of β-cell products, such as insulin. In all other models of β-cell "regeneration" (90% pancreatectomy, duct ligation) a significant proportion of the β-cells remain present and are functional, and there are species differences in the sensitivity to this diabetogenic agent. For instance, in hamsters, recovery from streptozotocin damage can occur and involves neogenesis [50].
In human diabetes indications of neogenesis of β-cells are scarce or absent [32]. In non-insulin-dependent diabetics there is no evidence of β-cell neogenesis. In insulin-dependent diabetics of recent onset, neogenesis of β-cells from ductal cells has been described [18]. Other more recent studies, however, were unable to confirm this observation [17, 34]. In long-term type 1 diabetes the pancreas may show islets with increased numbers of cells that are immunoreactive for PP [18, 35]. This is interesting, since PP as well as the structurally closely related NPY have been proposed as markers of islet precursor cells [22, 23, 59]. Islet neogenesis seems to also occur in chronic pancreatitis, since in this condition islets are commonly found in close association with small ducts [31] (Fig. 3). It is not yet known, however, whether such islet alterations do indeed represent newly formed islet tissue.

Perspectives

Islet cell neogenesis from ductal stem cells may represent an important mechanism for β-cell growth or renewal. Activation of this process is desirable in conditions in which an increase in the existing β-cell numbers, either in the pancreas or in a graft, is functionally important. The available evidence indicates that stem cells remain present in the pancreas throughout life. It is conceivable that all ductal cells retain stem cell potential, but our current knowledge of these cells remains very limited. The main problem at present is to identify the factors regulating the endocrine differentiation of these stem cells. We are now waiting for the decisive experimental model in which islet cell neogenesis can be induced from adult pancreatic duct cells in culture.

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