Experimental models of β-cell regeneration

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Abstract
The control of glucose metabolism by pancreatic endocrine cells throughout life relies on a tight regulation of the mass of insulin-producing β-cells. How this homeostasis is achieved is not well understood. Over the last few years, experimental rodent models with altered β-cell mass, and, more recently, new transgenic approaches designed to tackle this problem, have provided abundant information. Processes such as β-cell proliferation and apoptosis, or even β-cell differentiation from poorly characterized progenitor cells, whether immature or differentiated, appear to be implicated. A complex picture is thus emerging in which the nature of the pancreatic lesion appears to determine the kind of regenerative response. The environment formed by acinar and ductal cells, and also by vascular and neuronal structures, which surround islets and penetrate into their β-cell core, might play crucial roles so far unsuspected, which should be explored in the near future.

Introduction
Pancreatic β-cells constitute approx. 80% of the endocrine pancreas, within the so-called islets of Langerhans. The β-cell mass assumes the production and release of insulin in order to regulate blood glucose homeostasis. As for any given organ or tissue, the islet final size results from the balance between cell number (proliferation/neogenesis against apoptosis/necrosis) and cell size (hypertrophy/hypotrophy). The β-cell mass is generated during prenatal development through consecutive steps of increasing commitment into pancreatic, endocrine and β-cell progenitors, followed by differentiation/maturation and proliferation of specified cells. Maintenance of the functional β-cell mass occurs during postnatal life in response to ever-changing physiological demands, such as during disease, aging or pregnancy. Variations in the β-cell mass have been reported in Type 1 and Type 2 diabetic patients [1–5]. The molecular basis of this adaptive process involves other cell types in addition to β-cells, probably through the activation of multiple players in a complex transcriptional network. Therefore, in order to unravel the cellular and molecular mechanisms involved in β-cell renewal and expansion throughout life, different experimental models have been devised. However, there is much room for improvement in our understanding. The most relevant studies are summarized in this short review.

β-Cell generation
Normal cell renewal and cell regeneration after injury are events probably supported by different mechanisms. In the pancreas, experimental evidence points towards three different processes involved in β-cell regeneration: (i) proliferation of pre-existing β-cells, (ii) neogenesis from undefined adult progenitor or stem cells, and (iii) transdifferentiation from terminally differentiated cells. The cellular ‘niche’, defined by the presence of different elements of the extracellular matrix and different cell types, such as endothelial cells, fibroblasts or inflammatory cells, may affect β-cell generation through cell–cell interactions and diffusible molecules (‘extrinsic’ signals). Characterizing regenerated β-cells may help in our understanding of the underlying regeneration mechanism. For example, if formation of new β-cells recapitulates embryogenesis, this would involve the expression of embryonic markers, such as pdx1, ptf1a or ngn3 [6]. Alternatively, co-expression in single cells of initial and terminal differentiation markers would, in contrast, suggest direct transdifferentiation, whereas loss of differentiation markers would be indicative of a passage through a de-differentiation stage. More recently, the use of genetic tools allowing the targeted labelling of putative progenitors or the targeted misexpression of genes in selected cell types permits the undertaking of the precise dissection of the involvement of these different processes.

Models triggering β-cell mass variations
In various pathological circumstances, such as in rodent models of Type 1 or Type 2 diabetes, β-cell resurgence may occur to a small extent, but very inefficiently. This has been explored under conditions characterized by the absence of autoimmunity or peripheral insulin resistance. Some experimental approaches partly mimic diabetes because they provoke either a reduction of the functional β-cell mass (conditional dysfunction of β-cells or conditional β-cell apoptosis) or pancreatitis (partial pancreatectomy, ductal ligation or cellophane wrapping). In addition, these models may be combined with the use of biomolecules known to stimulate cell differentiation and cell proliferation.

Key words: β-cell regeneration, diabetes, glucose metabolism, pancreas, transgenic model.
Abbreviations used: Ngn3, Neurogenin 3; PDL, pancreatic duct ligation; PDX-1, pancreatic duodenal homeobox-1; PX, pancreatectomy; STZ, streptozotocin.

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β-Cell dysfunction after genetic injury

β-Cell malfunction can be induced genetically. For instance, the conditional suppression of pdx1 expression in adult β-cells leads to glucose intolerance within 1 week and to diabetes within 2 weeks by shutting off insulin expression without compromising β-cell survival [7,8]. After this, small proliferating ductules appear concurrently with the expression of reg genes, initially identified in duct-ligated regenerating pancreas [9]. Similarly, the expression of a dominant-negative form of the Kir6.2G132S subunit of the K⁺ channel in β-cells of mice leads to deregulated insulin oversecretion and progressive β-cell apoptosis [10]. Neverthless, at 6 months of age, the mice regain the control over their glycaemia, probably because new β-cells appear adjacent to proliferating ductal (DBA⁺) cells within islets [11]. In a recent study, β-cell replication has been shown to be the main mechanism leading to diabetes recovery after partial β-cell ablation through the conditional expression of diphtheria toxin A in β-cells of transgenic mice [11a].

β-Cell-specific toxic agents: chemical injury

STZ (streptozotocin) and alloxan induce insulin deficiency (reviewed in [12]). STZ is a glucose-conjugated nitrosourea that enters into β-cells through the GLUT2-type glucose transporter, and induces DNA damage by generating free radicals (alkylating agent) [13]. Alloxan also activates GLUT2 and is toxic for rodent β-cells by inhibiting glucokinase and through the induction of reactive oxygen species. In both cases, the protocol of administration determines the nature and extent of β-cell mass reduction. A single high dose of STZ induces acute hyperglycaemia within 24 h due to massive β-cell necrosis, whereas multiple low-dose injections produce gradual hyperglycaemia associated with insulitis [14]. It is interesting to note the regenerative capacity of the rat neonatal pancreas when challenged with a single dose of STZ: this triggers a transient hyperglycaemia within 48 h, which is normalized within 2 weeks, when small clusters of highly proliferative β-cells are visible near ducts [15,16]. Surprisingly, at this stage, a significant proportion of proliferating insulin-expressing cells also express somatostatin [15,16]. In adult STZ-treated animals, cells co-expressing insulin, somatostatin and PDX-1 (pancreatic duodenal homeobox-1) are also observed located in the ductal epithelium, but they are not proliferating actively [16,17].

Intriguingly, many different cell types in the pancreas are affected by the loss of β-cells: some have reported the appearance of acinar cells expressing insulin and low levels of PDX-1 [17–19]. Similarly, others have described the expression of insulin in exocrine pancreatic cells (replicating ductal cells) in adult rats after a single diabetogenic dose of alloxan [20].

Surgical injury

Partial pancreatectomy

Partial pancreatectomy (PX) is the resection of a fraction of the pancreatic tissue containing both endocrine and exocrine cells. The extent of tissue removal is variable in rodents, from non-diabetogenic (60–70%) to diabetogenic (90%). The degree of regeneration efficiency that follows this injury depends on the level of glycaemia and the extent of resection [21]. This is particularly interesting since young patients (up to 2 years of age) with hyperinsulinaemia [the so-called permanent hyperinsulinaemic hypoglycaemia in infancy (PHHI)] have to undergo 95% PX. Interestingly, in many cases, pancreatectomized children display regeneration and restoration of a normal pancreatic mass [1].

50–70% subdiabetogenic PX

After 50–70% PX, rodents remain normoglycaemic and normoinsulinaemic [22]. However, the β-cell mass increases up to 68% of that of sham-treated controls 2 weeks after PX, with abundant small islets and an increased average size for larger islets [22]. During regeneration after non-diabetogenic PX, β-cell mass expansion is mainly due to replication of pre-existing β-cells [23–26]. Interestingly, however, β-cells located in small clusters rarely proliferate [22].

Involved with progression is the lack of neogenesis in this regeneration model. The presence of putative ductal precursors is not observed in main pancreatic ducts, and there is no induction of Ngn3 (Neurogenin 3) expression [22,27,28].

90% diabetogenic PX

At 3 weeks after 90% PX, the pancreatic insulin content of the remnant pancreas piece is doubled [29]. Here, β-cell mass expansion has been attributed to neogenesis, since there is a wave of proliferation initiated in main ducts, which is followed in smaller ducts and finally in islet β-cells [29–31]. In various of this view is the transient up-regulation of PDX-1 expression in ductal cells [32], and the fact that β-cell regeneration is unaffected in animals initially β-cell-depleted with STZ treatment before 90% PX [33].

Pancreatic duct ligation (PDL)

Rather than a pancreatic regeneration model, the PDL approach should be considered to be a very efficient model of pancreatic tissue remodelling. PDL directly affects the acinar compartment of the ligated part of the pancreas tail. This surgery results in acute pancreatitis with the subsequent development of ductal complexes originating from the remaining metaplastic acinar cells. During this process, specific markers appear in acinar cells, such as the embryonic ductal marker PGP9.5 (protein gene product 9.5) or LIF (leukaemia inhibitory factor) receptor components, which are involved in the transdifferentiation of acinar cells into cells expressing insulin in vitro [34–36]. In a recent elegant report, Heimberg and co-workers show that these ducts accumulate cells expressing the islet progenitor marker Ngn3; in addition, these Ngn3⁺ cells can be isolated and transplanted into Ngn3⁻/⁻ pancreatic primordia grown in vitro, where they give rise to all islet cell types [37].

Cellophane wrapping

The cellophane wrapping technique, initially established in hamsters, consists of the wrapping of the head of the pancreas,
leaving the gastric lobe unaffected [38]. As previously, this manoeuvre results in a partial duct obstruction. At 2 weeks after the surgery, small aggregates of proliferating endocrine cells appear budding from pancreatic ducts. This leads to a 2-fold increase in the β-cell mass 6 weeks after the wrapping [39]. Similarly to 90% partial PX, the transient induced wave of cell proliferation is first elicited in ductal epithelium and follows in the endocrine compartment [38].

Conclusions

The increasing global prevalence of diabetes has stimulated a big effort worldwide to develop new therapeutic strategies, including the so-called β-cell replacement therapy or regenerative medicine, to restore the β-cell mass. Therefore a better definition and understanding of the intrinsic capacity of the adult pancreas to generate new β-cells has become a real challenge. For Type 1 diabetes, the study of the mechanisms involved in β-cell regeneration will have to be accompanied by the development of strategies aimed at hampering β-cell autoimmunity.

Any disease resulting from massive or inappropriate cell death is amenable to cell replacement treatments. Several organs have the capacity of generating new cells after damage, such as the liver, bone marrow and skin. In contrast, the pancreas has a very limited spontaneous potential for regenerating new β-cells. This is probably linked to the limited replication ability of β-cells and to the fact that neogenesis from precursor cells is not readily reactivated. Several lines of evidence suggest that the nature and extent of the pancreatic lesion somewhat determine the extent of β-cell regeneration. Future research will focus on improving the intrinsic capability of the adult pancreas of restoring an insufficient β-cell mass.

References

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