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A Review on Recent Advances in the Pancreas Regeneration

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ABSTRACT:
The replacement of functional pancreatic β-cells is seen as an attractive potential therapy for diabetes, because diabetes results from an inadequate β-cell mass. Inducing replication of the remaining β-cells and new islet formation from progenitors within the pancreas (neogenesis) are the most direct ways to increase the β-cell mass. Stimulation of both replication and neogenesis have been reported in rodents, but their clinical significance must still be shown. Because human islet transplantation is limited by the scarcity of donors and graft failure within a few years, efforts have recently concentrated on the use of stem cells to replace the deficient β-cells. Currently, embryonic stem cells and induced pluripotent stem cells achieve high levels of β-cell differentiation, but their clinical use is still hampered by ethical issues and/or the risk of developing tumors after transplantation. Pancreatic epithelial cells (duct, acinar, or α-cells) represent an appealing alternative to stem cells because they demonstrate β-cell differentiation capacities. Yet translation of such capacity to human cells after significant in vitro expansion has yet to be achieved. Besides providing new β-cells, cell therapy also has to address the question on how to protect the transplanted cells from destruction by the immune system via either allo- or autoimmunity. Encouraging developments have been made in encapsulation and immunomodulation techniques, but many challenges still remain. Herein, we discuss recent advances in the search for β-cell replacement therapies, current strategies for circumventing the immune system, and mandatory steps for new techniques to be translated from bench to clinics.

KEY WORDS: Diabetes , Pancreas , Progenitor cells , Transplantation, Cellular therapy.

I. INTRODUCTION:

Diabetes results from an inadequate mass of func tional-cells. In type 1diabetes(T1D),theimmune system attacks and destroys the-cells by mecha nisms still incompletely understood.Type 2 dia betes (T2D) accounts for 95% of diabetes cases worldwideandisassociatedwithobesityleadingto insulin resistance and-cell dysfunction. Exoge nousinsulinororalagentscanprovidetightcontrol of the disease, but only the replacement of-cells allows physiological control of glycemia.

Human is let transplantation is successful in restoring normal glycemia but is limited by the need for toxic immu nosuppressive drugs, the scarcity of donors, and graft failure usually within a few years. Alterna tive ways for replacing-cells are needed and may theoretically be obtained by (a) replication of re maining-cells, (b) neogenesis, or the differentia tion of new islet cells from pancreatic progenitors or stem cells, and (c) transplantation of-cells de rived from stem or somatic precursor cells. These areas of research, still being mostly experimental, will be addressed in this review.

Regeneration of the endocrine pancreas:

The majority of endocrine islets, owing to their central importance in diabetes. Historically, studies of islet regeneration relied on rodent injury models, including pancreatectomy, pancreatic duct ligation, and chemical ablation of islet cells. In pancreatectomy, removal of up to 90% of the rat pancreas does not affect glucose homeostasis, suggesting a large reserve capacity, as 10% of the islet mass is sufficient to maintain blood glucose control. By contrast, resection of 50–60% of the pancreas in humans triggers insulin-dependent diabetes. Young rodents show tissue growth and sprouting from the cut surface after pancreatectomy. Observations of rare samples from children also suggest tissue growth after pancrea tectomy.

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The capacity for this type of regeneration, however, declines sharply in adult animals and is absent in adult humans. A second injury model used to study pancreas regeneration is duct ligation which mimics obstructive pancreatitis. Physical ligation of the pancreatic ducts causes widespread acinar cell death, but the endo crine islets are spared and no substantial endocrine regeneration is observed. In a third injury model, pancreatic β-cells can be specifi cally ablated using streptozotocin (STZ) or alloxan, chemical toxins that structurally mimic glucose and are selectively imported into β-cells. Depending on drug dosage, the entire β-cell mass can be partially or nearly completely ablated in a few days. Extensive studies have found no convincing evidence for β-cell regeneration in adult animals following chemical ablation.

Despite the lack of substantial islet regeneration in injury models, islet hyperplasia is observed during pregnancy, in obesity, or under insulin resistance conditions in animal models. For instance, mouse pancreatic β-cell mass increases by fold during pregnancy, stimulated at least partly by the pregnancy hormones placental lactogen and prolactin, and involving signalling through serotonin, Menin, and FoxM. High-fat diet-induced obesity in mice is also accompanied The adult endocrine pancreas (islets of Langerhans) is made up of four major endocrine cell types with each secreting a major hormone: insulin (β-cells), glucagon (α-cells), somatostatin (δ-cells), and pancreatic polypeptide (PP cells).

Animal studies have shown that β-cell replication is a major mode of regeneration and repair in homeostasis, injury, by impressive increases in islet cell mass24. Experimentally induced insulin resistance, such as liver-specific knockout of insulin receptors, induces up to a tenfold increase in β-cell mass. The molecular path ways that drive these increases in β-cell mass in obesity and insulin resistance have yet to be fully elucidated.

Evidence of In Vivo Regeneration Capacity of β-Cells

Much enthusiasm about regenerating pancreatic β-cells in situ has been driven by evidence of the impressive proliferation capacity of postnatal rodent β-cells in situations of increased metabolic demand. For example, in the mouse, pre-existing β-cells were shown to be responsible for the pancreas regeneration that occurs after 70% pancreatectomy, in a seminal work by Melton and coworkers. In the human, normal expansion of the β-cell mass occurs during the neonatal period but fades early in childhood. β-Cell regeneration has been suggested by the observation of residual β-cells in T1D patients after onset or even many years after diagnosis. Whether this represents a replication capacity, neogenesis, or resistance to apoptosis is unknown. Adult β-cells have some capacity to expand with obesity whereas β-cell replication is usually negligible when analyzed at autopsy.

However, an interesting equivalent of the "honeymoon period" has been described in T1D patients during pregnancy with measurable C-peptide levels and transient reduction of the insulin requirements. Replication was initially suggested as the mechanism of this phenomenon, but a recent autopsy study on pancreases during or after pregnancy suggested neogenesis by showing increased relative volume of β-cells, increased proportion of small islets, and increased number of insulin^{+} cells in the ducts but no change in β-cell replication, cell size, or apoptosis frequency. Thus, the question remains open as to whether β-cell regeneration could be exploited for therapy.

Neogenesis has recently been a source of intense debate, with many lineage tracing studies in mice showing contradictory results. Controversy reached its climax when two recent reports obtained distinctly different results using similar tracing methods for Sox9-expressing populations. Kopp et al. described derivation of non-β-endocrine cells from the ducts in early postnatal life but no endocrine or acinar cell neogenesis in adult mice either physiologically or after pancreatic duct ligation (PDL).

However, Furuyama et al. obtained massive X-gal staining of acinar cells 8 weeks after tamoxifen pulses were given during the postnatal period in Sox9$^{IRES-CreERT2}$; Rosa26R mice. They also showed that duct cells accounted for a mere 1% of pancreatic endocrine cells at 8 weeks when tracing was initiated on the first day of life. These results clearly underline technical limitations of current lineage tracing approaches.

In the human pancreas, indirect evidence of neogenesis has been provided by showing cells coexpressing cytokeratin and insulin. Many studies have demonstrated the presence of cells containing insulin within the ducts, either at autopsy or in biopsy from organ donors. Currently, the general concept is that, after birth, neogenesis from ducts occurs mostly in the neonatal period and, as shown in rodents, can be stimulated in the regeneration following injury.

Beta Cell Replacement Therapy
Embryonic Stem Cells:

Embryonic stem cells (ESCs) have ad vantages over other potential sources because they

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are now readily available, are highly expandable, and can be differenti ated to-cells. Many studies have demonstrated the deri vation of Pdx1 or endocrine cells from ESCs, and some groups generated insulin or C-peptide-secreting cells. Using a stepwise differentiation protocol mimicking pancreatic embry onic development, a team from Novocell (now Viacyte) drove ESCstowardanendocrinephenotypeinvitroandobtaine dupto 12%insulin cells. When early pancreatic progenitors were transplanted into diabetic SCID mice, over time these cells be cameglucose-responsive and secreted large amounts of insulin, comparable to amounts released from human islets, leading to near normalization of blood glucose levels in the transplanted animals.

In addition to ethical issues, clinical use of ESCs is still seri ously hamperedbytherisk ofin vivo teratoma formation. This risk is mainly associated with the transplantation of undifferentiated phenotypes, and efforts are now being con centratedontheselectionofdifferentiatedcells.Recents tud ies showedthepossibility of sorting for ESC-derived endoder mal cells using cell surface markers (CD49e CD141 CD238, EpCAM SSEA1 SSEA3, or CD24 selection) without detectable teratoma formation 160 days post-trans plantation. Investigations are now needed to determine how re liable the elimination of undifferentiated cells can be, as only a few cells are necessary for tumorogenesis. Also, differentiated cells may retain epigenetic traits of original cells, as has been described after reprogramming to pluripotency, after epithelial-mesenchymal transition (EMT) of-cells, and af ter transdifferentiation of hepatocytes into neurons Whether the differentiated products might revert to a less dif ferentiated and potentially dangerous state is unknown.

Induced Pluripotent Stem Cells.

Induced pluripotent stem cells (iPSCs) have the unique property of allowing the generation of autologous cells that might be useful for therapy. The β-cell differentiation potential of iPSCs has been shown in vitro with demonstration of partial glucose-responsive C-peptide release. Moreover, recent studies highlighted the potential of mouse and rhesus monkey iPSCs to reverse hyperglycemia after in vitro differentiation and transplantation in diabetic mouse models.

Although huge efforts are invested in iPSC research, hurdles concerning their clinical application are multiple: long, complex, and costly in vitro procedures; low reprogramming efficiency; risk of insertional mutagenesis and of permanent transgene genome integration; tumor formation; use of Klf4 and c-Myc oncogenic factors; and the need for animal feeder cells. Safety issues have recently been raised because coding mutations or epigenetic anomalies were observed after reprogramming, and undefined limitations also exist as to how to induce iPSC differentiation without generating large numbers of undifferentiated cells.

Human Pancreatic Epithelial Cells. (1) Human Islets.

Because islet donors are scarce, exploitation of human β-cells for therapy could be obtained by expanding the cells in vitro. Because epithelial cells have limited mitotic activity in vitro, an alternative way of forcing their expansion could be via a phenotype shift. Accordingly, human β-cells were shown to be able to proliferate in vitro after shifting toward a mesenchymal phenotype through EMT. These mesenchymal-like cells appear to have been directly derived from original β-cells, as confirmed by lineage tracing experiments with human cells; however, mouse β-cells were shown not to be able to undergo EMT. Moreover, it has not been convincingly shown that these mesenchymal-like cells can be differentiated into bona fide β-cells.

(2) Duct Cells.

Several studies showed the potential for differentiation of cells derived from the islet-depleted exocrine tissue. These studies all used relatively unselected populations, making identification of the starting material difficult and contamination of residual β-cells a possible explanation of the observed results. Yet clear demonstration of the β-cell differentiation of human duct cells has been provided on purified populations expressing CA19–9 antigen. Although these cells are numerous in the pancreas and are easily purified, they lack sustained proliferation and tend to lose their phenotype in vitro. New techniques are thus needed to derive proliferating cells from the ducts that are able to differentiate into islet cells.

(3) Acinar Cells.

Controversy exists concerning the in vivo potential for rodent acinar cells to differentiate into β-cells after injury since a report showing no acinar-to-β cell reprogramming after 70% pancreatectomy, PDL, or caerulein-induced pancreatitis. A recent study shed new light on the potential of exocrine cells by showing their reprogramming into β-cells after injection of viral vectors carrying Pdx1, Ngn3, and MafA transcription factors into the mouse pancreas. The differentiated products had many characteristics

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of bona fide β-cells with regard to expression profile and insulin content and could partially reverse the diabetic state. Although the in vitro expansion of human acinar cells has not yet been successful, these cells remain an attractive source for β-cell engineering, and their therapeutic potential will no doubt be fully explored.

Porcine Islets.

Pigs represent an unlimited supply of islets for transplantation, and the purity of islet preparations is usually higher in pigs than in humans. Proof-of-concept of unmodified adult porcine islet xenotransplantation has been made in nonhuman primates using immunosuppression , microencapsulation , or a subcutaneous macrodevice without immunosuppression. The team of Elliott reported only modest changes with transplantation into nonhuman primates of microencapsulated porcine neonatal pancreatic cell clusters.

Importantly, they also showed no evidence of porcine endogenous retrovirus infection after a similar transplant in a human subject. Preliminary data from phase I and IIa clinical trials showed a possible reduction of hypoglycemia unawareness after neonatal porcine islet xenotransplantation without immunosuppression in T1D patients, whereas daily insulin needs were only modestly reduced

.Although still experimental, porcine islet xenotransplantation is a promising area of investigation, and efforts currently focused on improvement of immunosuppressive regimens might help overcome current limitations. Genetic modification is another way of making pig islets more suitable for transplantation, and transgenic animals are being evaluated for their potential to avoid rejection or even retrovirus infections.

Other.

Multipotent mesenchymal stromal cells (MSCs) are easily isolated from many tissue sources, are highly expandable in vitro, are resistant to cryopreservation, and have the potential to differentiate into many different lineages.Reversal of diabetes has been reported with human MSCs that differentiated into insulin^{+} cells after transplantation into STZ-diabetic rats without immunosuppression ; however, these data need confirmation.

MSCs seem to be able to enhance results of experimental islet transplantation, perhaps via paracrine effects, including the secretion of angiogenic cytokines and antiapoptotic factors, that can regulate endothelial and epithelial permeability, decrease inflammation, and enhance tissue repair. This is corroborated by reports showing amelioration of hyperglycemia after intravenous or intracardiac infusion of MSCs in STZ-diabetic mice.

Following up on their previous work with very small embryonic-like stem cells, Ratajczak et al. recently published the isolation of these cells in mouse pancreas and their differentiation into β-cell-like derivatives.Although interesting, these results need corroboration by other teams and careful analysis of the in vivo behavior and potential of the cells after transplantation.

(B) How Can the Immune Destruction of the Transplanted Cells Be Circumvented?
Encapsulation.

Cell transplantation for treating diabetes is challenged by the host immune system via allo- and autoimmunity. Current immunosuppression protocols are still associated with numerous side effects that mitigate the benefits of cell therapy compared with conventional treatment. One alternative way of bypassing the immune system is to encapsulate the cells within a barrier allowing diffusion of glucose, other nutrients, and insulin but not of larger molecules, cells, or antibodies.

This system has the theoretical advantage of precluding the need for immunosuppression and allowing the use of various cell types including porcine islets or islet cells derived from stem/precursor cell sources. It also sequesters the cells, thus avoiding dissemination of potentially tumorogenic derivatives of genetically-modified cells.

Encapsulation can be configured using many different polymers, methods, and sizes.Microcapsules have some advantages such as being small and easily placed into the peritoneal cavity. Their smaller size (conformal to 1,000 μm) improves surface-to-volume ratios and exchange of nutrients and molecules.

Macroencapsulation is challenging because of the size of the devices that must be implanted, as well as the problems of slow nutrient delivery and delayed insulin secretory responses to stimuli. The first phase I trial with microencapsulated human islets in two T1D patients without immunosuppression showed a decrease in insulin daily needs and detectable C-peptide levels after 1 year of follow-up.

A more recent study demonstrated the safety of the technique in four T1D patients but detected only low levels of C-peptide secretion and no change in insulin requirements. Ongoing trials should provide more information about the feasibility of this technique. Preliminary results from the team of

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Calafiore using intraperitoneal injection protocols showed possible reduction of insulin requirements and improvement of hypoglycemia unawareness and HbA1c levels.

Immunomodulation.

Current aims of immunotherapy are to prevent the onset of T1D or reverse autoimmunity to preserve residual β-cell function and maintain endogenous insulin production. Reports have shown some preservation of insulin secretion in new-onset diabetes using vaccination with glutamic acid decarboxylase or anti-CD3 antibodies , for periods extending up to 5 years post-treatment.

However, two phase III clinical trials using anti-CD3 monoclonal antibodies (teplizumab and otelixizumab) failed to meet their primary endpoints. Other antibodies, such as anti-CD20 rituximab, or antigen-specific agents, such as GAD65 and DiaPep277, are currently being tested. Whether beneficial effects on autoimmunity can be obtained with similar procedures in patients with long-standing T1D is unknown, and near total β-cell depletion will likely require interventions to enhance β-cell regeneration.

(C) Where to Transplant?

The optimal site for transplantation has not been determined. Islets were first transplanted into the peritoneal cavity When injected into the peritoneal cavity, encapsulated syngeneic and allogenic islets reversed diabetes in Balb/c and NOD mice for up to 350 days after transplantation.

However, the peritoneum does not allow easy access to the grafts as islets can disperse throughout the cavity or form a sediment on the pelvic floor. Portal vein injection, which has been used for the initial clinical trials, is associated with the risk of portal hypertension and portal vein thrombosis and has been suggested to activate the innate immune system and instant blood-mediated inflammatory reaction that probably contributes to some degree of cell death. The kidney subcapsular space provides an alternative microenvironment and allows relatively easy graft retrieval. However, one study concluded that porcine islet xenotransplantation in this site in primates had poor graft survival.

The omental pouch, which is accessible and potentially reconstructed, appears to provide good blood supply. Survival of microencapsulated syngeneic islets in the omental pouch has been shown up to 400 days post-transplantation in NOD mice.

Furthermore, a report concluded that transplanted islets functioned better in the omentum than under the kidney capsule. The gastric submucosal space is another potential site because it provides good oxygenization of the grafts because of its vascularization and is accessible via endoscopy for transplantation and subsequent biopsy.

The subcutaneous site, although easy to access, is associated with mechanical strain, is thought to be immunologically hostile, and has poor vascularization; however, this might be ameliorated by prevascularization techniques. Even the pancreas, the natural home of islets, holds some theoretical attractions, but there are major concerns about the risk of pancreatitis.

(D) Requirements for Translational Studies

Before new sources of cells can be considered for therapy, they must fulfill some prerequisites. In vitro, these include being sufficient in number to provide a clinically relevant mass, cryoresistant to allow good manufacturing practice-compliant cell banking, and genetically stable with no signs of genomic alteration after expansion. Besides being able to engraft, survive, and function long-term, transplanted cells must have minimal neoplastic potential.

Cell localization at the time of infusion can be followed by tracing cells with a radioactive label, as was recently shown during infusion of MSCs in cirrhotic patients. This technique may be useful for in vivo procedures in preclinical studies to obtain reassurance about the noninvasiveness of the transplanted cells. A detailed checklist of desirable features will be helpful to evaluate whether a cell type can be an adequate candidate for β-cell replacement therapy.

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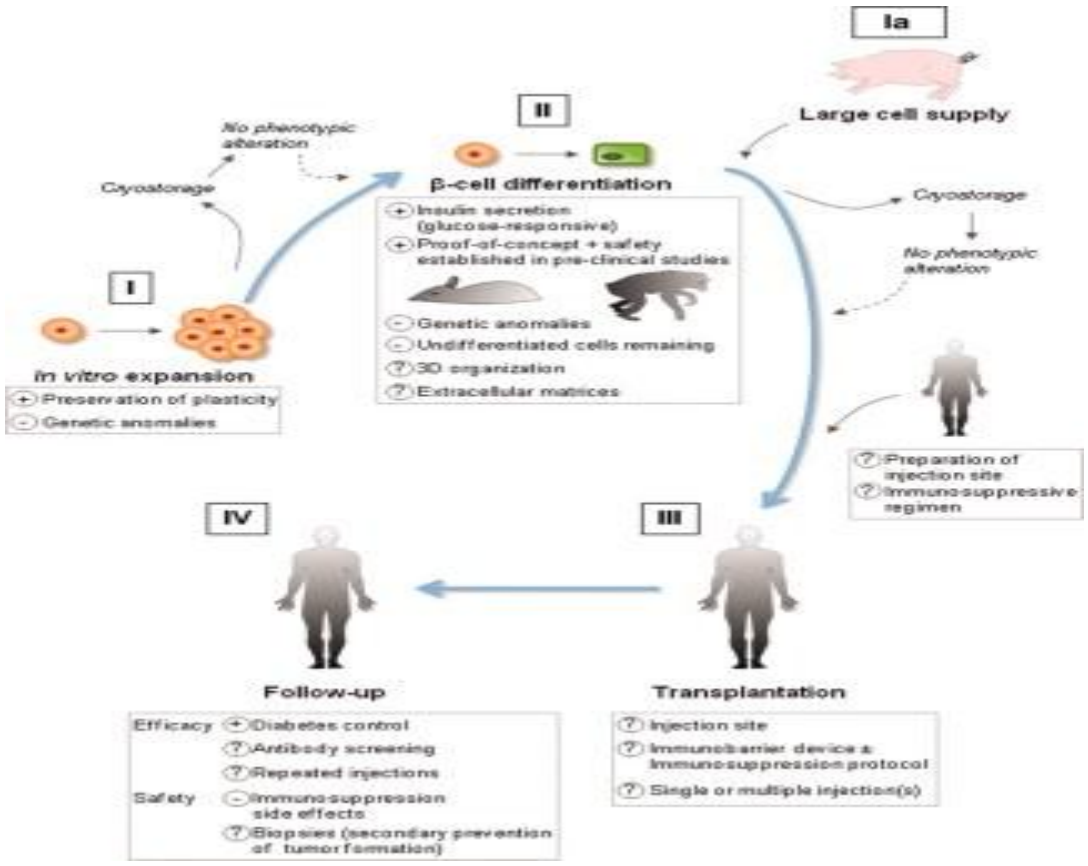

Figure 1

Requirements and remaining uncertainties concerning cell therapy for diabetes. Candidate cells for β-cell replacement therapy should be expandable in vitro as required for clinical application, with preservation of their plasticity and without generation of genetic anomalies (I). The cells might be usable when fresh or after cryostorage. Quality control after storage should allow selection of batches without phenotypic alteration. A reliable β-cell differentiation protocol should then generate glucose-responsive insulin-secreting cells in large amounts with no undifferentiated remaining cells (II).

Whether current protocols would benefit from 3D cell organization or inclusion of extracellular matrices needs evaluation. Some partially differentiated phenotypes could be tolerated at this point, whereas generation of genetic anomalies by the differentiation protocol must be excluded. Proof-of-concept and safety assurance should be provided in small and probably large animal models before cells can be considered for clinical use. Differentiated products should ideally also be storable for future use. Cell sources allowing large tissue supply (e.g., porcine islets) might be immediately available for patients, but storage with cryostorage or long-term culture might be warranted to facilitate clinical application (Ia).

Uncertainties remain concerning the preparation of the diabetic patient with regard to the infusion site or immunosuppressive regimen. Investigations are still mandatory to determine the ideal injection site, the immunoprotective protocol (immunobarrier device ± immunosuppression), and the amount of tissue needed (III). Besides the control of diabetes and monitoring of treatment side effects, clinical follow-up will be required to determine whether the patient is tolerating the graft, whether repeated injections are needed, or whether the graft contains cells with tumorogenic potential (IV). Desired, undesired, and unknown features are represented, respectively, by (+), (−), and (?). Abbreviation: 3D, three-dimensional.

II. Conclusion

For T1D, β-cell replacement therapy is a two-pronged problem that requires (a) replacing the β-cells and (b) controlling the autoimmunity and allorejection. There is reason to be optimistic that sufficient numbers of β-cells for transplantation will someday be available. Therapy with cells derived

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from stem cells has gained attention with high levels of differentiation obtained with ESCs and iPSCs. However, safety is a critical issue with these cell types and might delay their clinical applications. New insights on progenitor or somatic cell differentiation have opened the door for investigation, but in vitro evaluation is necessary to understand their potential for therapy. Stimulating replication or neoformation of β-cells within the pancreas could be a less invasive procedure and of high clinical value. New compounds being tested may be helpful in increasing β-cell mass. Progress is also being made with parallel developments of new immunosuppressive regimens, the quest to induce tolerance, and new approaches to encapsulation.

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