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Diabetes and stem cells according to:
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Islet replacement vs. regeneration: Hope or hype?

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Abstract: Type 1 diabetes is caused by autoimmune destruction of pancreatic islet β-cells. Management of this disease is burdensome both to the individual and society, costing over $100 billion annually. Shortage of pancreatic tissue, together with a lifetime requirement of immunosuppressive drugs to prevent rejection and recurrent disease, remain as major hurdles yet to be overcome prior to widespread applicability. Stem cells, with their potential of developing into pancreatic β-cells, appear to be the best prospect for overcoming the islet shortage. Current investigation, however (both embryonic and adult stem cells), is still in the preliminary stage and several more years remain before they can potentially be used in the clinical setting. Procedures that reduce *in vitro* manipulation of cells and allow stem cells to develop into islets *in vivo* are crucial. Furthermore, the regeneration of existing islets is a distinct possibility. Simplistically, it might be hypothesized that down-regulation of autoimmunity may give the pancreas the breathing space to regenerate islets. Supplementation with factors known to induce β-cell replication and neogenesis might further augment the regenerative processes. Clearly, islet-regeneration research will soon match the level of interest currently focused on *in vitro* stem cell-based approaches.

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The need to cure type 1 diabetes

Type 1 diabetes is an autoimmune disease accounting for more than 80% of all cases of diabetes in children and adolescents (1). In the United States, over 1 million individuals are currently diagnosed with type 1 diabetes and an additional approximately 25–30 000 new cases are diagnosed each year. The incidence of type 1 diabetes is rising worldwide, and the cost of diabetes and its complications exceed $100 billion annually. Although great strides have been made in treatment, current options often fall short. With inadequate control, chronic hyperglycemia leads to microvascular (retinopathy, blindness, nephropathy, and neuropathy) and macrovascular complications (cardiovascular, cerebro- and peripheral vascular disease). Unfortunately, optimal glycemic control cannot be easily achieved by most patients, and, even in some patients, attempts at maintaining euglycemia through intensive insulin treatment often leads to increased hypoglycemia. As a consequence, the morbidity and mortality rates are disproportionately high and the life span may be shortened by one-third for patients with type 1 diabetes. Three major diabetes-prevention trials initiated in high-risk individuals, in the mid 1990s, were not successful (2, 3). The need to cure diabetes is obvious. Promising results have been obtained with both whole organ and islet transplantation; yet, the paucity of available organs and tissues limits such approaches. Recurrent autoimmunity and prevention of rejection (alloimmunity) remain major hurdles yet to be overcome.

Experimental approaches to cure type 1 diabetes

The ecto-pancreatic transplantation of donor pancreas has proven fairly efficient in normalizing fasting and

postprandial blood glucose levels, hemoglobin A1c, with partial, if not always, complete restoration of insulin and C-peptide production. Recently, Shapiro et al. (4–9) have reversed type 1 diabetes with islet implantations in patients with brittle diabetes. The success of this Edmonton protocol has led to widespread adoption of this procedure. It remains to be determined whether the protocol will be universally successful. However, the initial success of this protocol underscores an already existing acute shortage of implantable islets and has provided a greater incentive for investigations into alternate sources of islets, such as stem cells. Currently, multiple approaches are being undertaken to satisfy the demand for islets for implantation. They include: xenogeneic porcine islets, transformed insulin-producing cell lines, transfection of different cell types to render them insulin-producing, differentiation of pancreatic ductal cells (a potential source of progenitor cells), bone marrow stem cells (*in vitro* and *in vivo*), *in vivo* trans-differentiation of liver cells, and *in vivo* regeneration of pancreatic islets. In this review, we will focus on the status of stem cell-based approaches, as well as the potential for regeneration of islet cells (Fig. 1).

Pancreatic embryogenesis

Both stem cell- and regeneration-based approaches can be developed successfully, in theory, with a thorough knowledge of molecular interactions, participating growth factors, and sequential induction of transcription factors that specify islet and pancreas formation. Once the key steps of embryonic development are understood, pancreatic tissues may be created from undifferentiated precursor cells by providing proper signals. Great strides have been made in fostering this understanding (Fig. 2).

Immediately following gastrulation, the mammalian endoderm consists of a sheet of undifferentiated cells that eventually forms the epithelial components of the gastrointestinal tract and its associated glands. Initially, this primitive endodermal sheet is transformed into a primitive gut tube surrounded by mesenchyme and the notochord on the dorsal side. It is during this embryonic stage when directed differentiation of the foregut endoderm to pro-pancreatic epithelium occurs. It is believed that the notochord, by secreting factors of the transforming growth factor (TGF)-β family (most notably activin-β B and fibroblast growth factor-2),

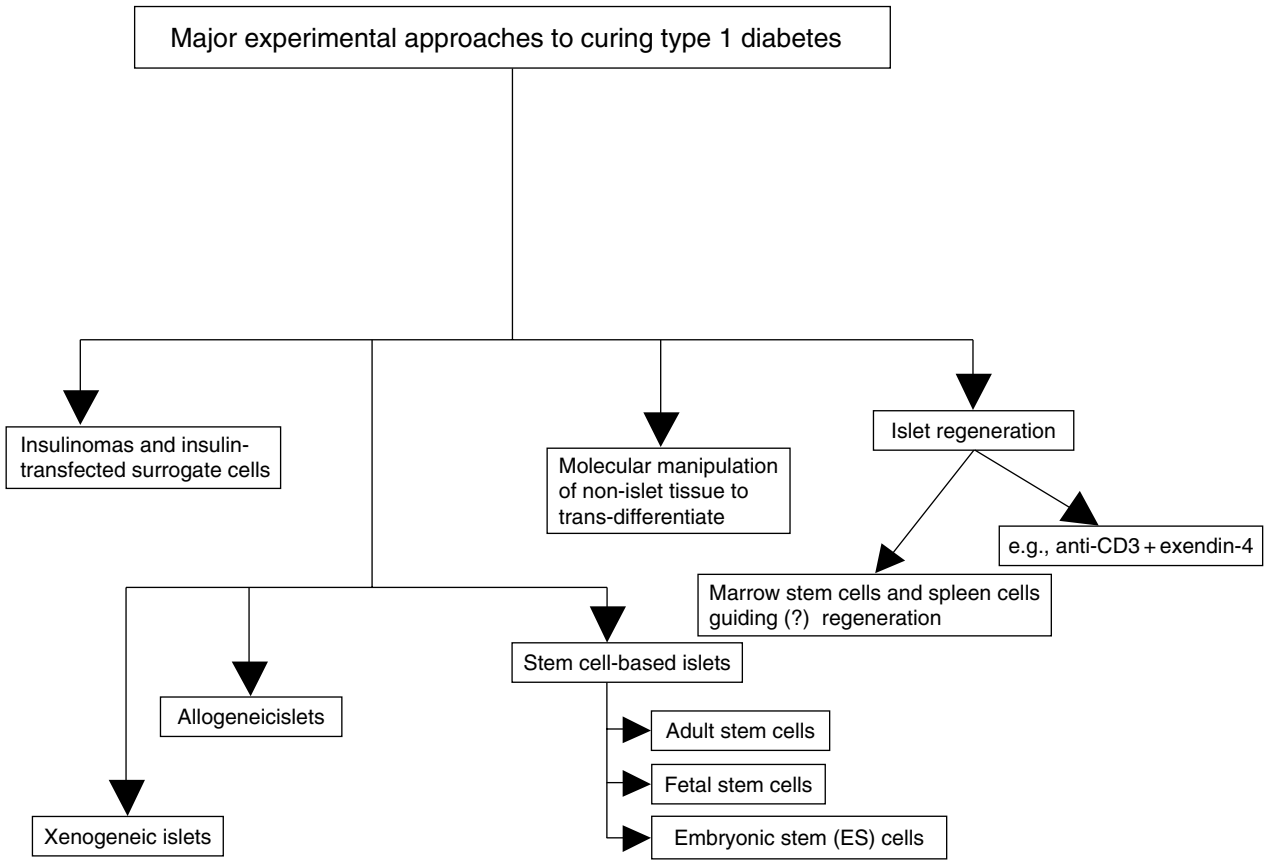


Fig. 1. Major experimental approaches to curing type 1 diabetes. In general, the approaches fall into two categories: those that require prolonged *in vitro* culture and manipulations (stem cells and surrogate cells), and those that require less or no *in vitro* manipulations (regeneration and *in vivo trans*-differentiation). When these approaches are optimized, the respective risks and benefits of each approach will determine its clinical usage.

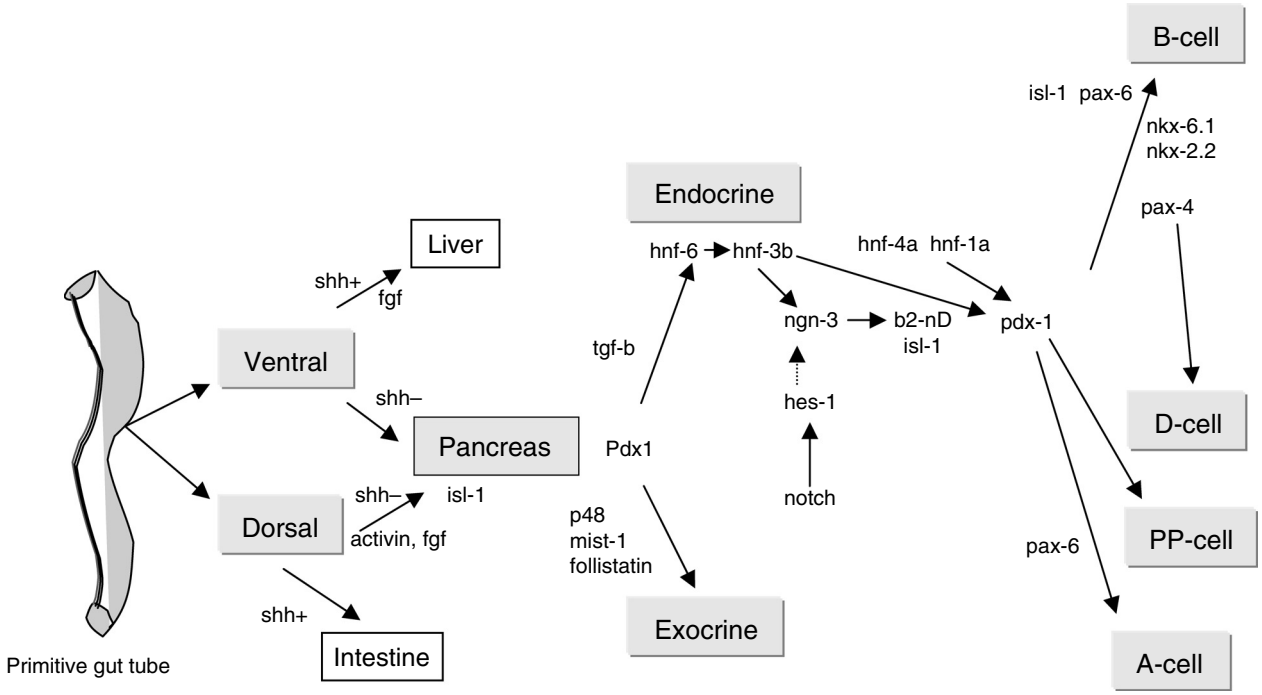


Fig. 2. Critical steps in the development and differentiation of the pancreas. Repression of the *shh* gene by dorsal mesenchymal factors (activin β-B and fgf) appears to lead to the development of the pancreas in the primitive gut tube. Notch signaling controls the choice between differentiation and progenitor cell fates. Accordingly, notch-receptor activation results in high *ngn-3* expression, which promotes early endocrine cell differentiation at the expense of cell proliferation. A concerted action by *pax-4*, *nkx 2.2*, ▶2/NeuroD (b2-nD), and *nkx-6.1* along with *pdx-1* is essential for normal β-cell differentiation and maturity.

represses the sonic hedgehog (*shh*) gene. This exclusion seems crucial for the induction of programmed pancreatic development under the control of the duodenal homeobox-1 (*pdx-1*) gene (10). Expression of *pdx-1* is essential for pancreas and β-cell development in both mice and humans (11, 12). Although the default fate of the ventral foregut endoderm appears to be the activation of the pancreatic pathway, if this tissue receives appropriate signals from adjacent cardiac mesoderm, the default pathway gives way to an activation of a liver-development pathway.

In addition to the natural absence of *shh* expression within the ventral bud, the ventral bud differs from the dorsal pancreatic bud by lacking Lim-homeodomain protein [islet factor 1 (Isl-1)] expression and by a delay in endocrine cell appearance (13, 14). At embryonic day 9 (e9 d) in rodents, *pdx-1* expression marks both the dorsal and ventral domains of the developing pancreas and defines where the buds will appear by embryonic day 10 (e10 d). As pancreatic buds expand and branch, signals from adjacent mesenchyme direct cells toward a pancreatic fate (15). At this developmental stage, the endocrine progenitor cells express the basic helix-loop-helix (bHLH) transcription factor, neurogenin-3 (Ngn-3). Ngn-3 is considered to be a pro-endocrine factor expressed in all endocrine islet-cell progenitors (16). Ngn-3 expression leads to the expression of extracellular notch ligands (such as delta, serrate, and jagged) in precursor cells that activate notch receptors on adjacent cells. Activation of notch receptors in adjacent cells results in the expression of *hes* genes. The *hes* genes not only encode bHLH factors that function to repress the expression of *ngn-3*, but also appear to be implicated in the pre-patterning of the endoderm. Finally, interdependency among the hedgehog, TGF-β and notch-signaling pathways has been implicated in the initiation of the pancreatic program due to accelerated endocrine expansion in *shh* and *hes-1* knock-out mice (17, 18) and the regulation by TGF-β of Shh, delta, serrate, and fringe ligands (19).

The crucial transcription factor genes in the development of pancreas include *hlxb9, Isl-1, hnf3*-β and *pdx-1* (12, 13, 20). In *hlxb9* null mice, the dorsal pancreatic lobe fails to develop while the ventral lobe develops normally with both exocrine and endocrine cells (20), shedding light on another differential factor in the regulation of dorsal vs. ventral pancreatic bud development. The *hlxb9* gene encodes for the homeobox factor HB9, which is expressed in the embryonic notochord and the gut endoderm, as well as in mature islet β-cells. Similar *to hlxb9, Isl-1* gene knock-out mice also fail to develop dorsal pancreatic buds (13). Like HB9, Isl-1 and Pdx-1 have been shown to play crucial roles in mature islets. HNF3-β, a member of the winged-helix family of transcription factors, appears to regulate *pdx-1* gene expression, suggesting that it functions upstream of *pdx-1* (21). Inactivation of the *hnf3*-β gene affects embryonic development at the level of foregut

morphogenesis (22). Ectopic expression of *ngn-3* under the regulatory control of the *pdx-1* promotor in transgenic mice led to poorly developed pancreatic buds at embryonic day 12 (e12 d), followed by the premature and precocious differentiation of pancreatic progenitor cells into endocrine cells, with a simultaneous depletion of exocrine cells (23). The *ngn-3* promotor has binding sites for HNF1-alpha, HNF3-β and HNF6, and the Hes-1 factor has been shown to repress expression of Ngn-3 by binding to several silencer sites located close to the transcription site (24). Thus, *ngn-3* is necessary and sufficient to drive endocrine pancreatic development. β2/NeuroD is another transcription factor apparently required to complete the differentiation of the various endocrine cell types. β2 functions downstream of the Ngn-3 factor, and its expression is dependent on that of *ngn-3* gene (25). Two members of the NK class of homeodomain proteins, Nkx2.2 and Nkx6.1, also appear to play significant roles in the development of pancreatic β-cells (26, 27). There are also paired domain homeobox genes *pax-4* and *pax-6*. Unlike *pax-4* expression which is restricted to β-cells, *pax-6* expression is present in all of the endocrine cell types (28, 29). Finally, two different operational views exist with respect to the lineage of islet cells. Experimental evidence suggests that α and β-cells differentiate independently from precursor cells without passing through a multihormonal transitional stage (30). However, repression of *pdx-1* gene expression apparently triggers trans-differentiation of β-cells into α-cells (31), implying that lineage specification has greater plasticity than previously suspected. It is believed that when stem/progenitor cells differentiate *in vitro*, a hierarchy of similar transcriptional regulators is operational.

Islet replacement with stem cells

Stem cells are defined as cells that self-renew and differentiate into other mature cell types. There are at least three types of stem cells being explored for clinical applications: embryonic stem (ES) cells, fetal stem cells, and adult stem cells. We will focus only on embryonic and adult stem cells (Fig. 3).

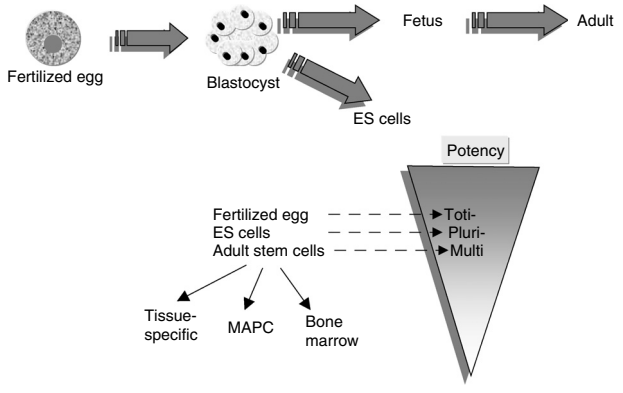


Fig. 3. Different types of stem cells. Embryonic stem (ES) and adult stem cells are being explored for their potential to be unlimited islet sources. Among adult stem cells, tissue-specific stem cells have restricted lineage potential, while multipotent adult pluripotent cells (MAPC) and bone marrow stem cells have a broader lineage potential.

ES cells

ES cells, derived from the inner cell mass of blastocysts, are pluripotent cells capable of giving rise to all of the tissues found in adults under the right conditions (32). ES cells can be adapted to grow *in vitro* for long periods while retaining chromosomal stability and pleuripotency. While the pleuripotency of ES cells is attractive for investigators working on different diseases, the pleuripotency also poses a serious problem in attempting to direct the cells to differentiate to a desired cell type. Induction of ES cells into hematopoietic (33), skeletal muscle (34), adipocyte (35), cardiac muscle (36), neuronal (37), and pancreatic islets (see below) has been reported. However, inherent problems with ES-cell differentiation include an inability to differentiate into a given cell type without contaminating cells, absence of definitive endodermal markers to select islet progenitor lineage cells, and presence of teratogenic undifferentiated ES cells.

With mouse ES cells, Soria et al. (38) enriched insulin+ cells using gene trapping methodology in which the neomycin resistance gene was placed under the control of the insulin promoter. In the presence of the antibiotic G418, only insulin+ cells survived. Such cells were very efficient in reversing hyperglycemia in all transplanted mice and normoglycemia was sustained for >11 wk. Lumelsky et al. (39) enriched cells expressing the neural stem-cell marker, nestin, and then forced those cells to differentiate into insulin-producing cells in the presence of nicotinamide. While the islet-like clusters derived from this procedure contained insulin and other hormone-expressing cells, they did not express the *pdx-1* gene, and the insulin+ cells expressed only about 2% of normal β-cell insulin content. These glucose-responsive cells were not effective in reversing hyperglycemia in mice. Following Lumelsky's procedure, another group demonstrated both *pdx-1* expression and the *in vivo* capability of differentiated cells to reduce hyperglycemia in streptozotocin (STZ)-treated mice (40). In the midst of these observations of islet-like cells from ES cells, a shocking revelation of insulin absorption by ES cells was reported (41), which highlighted a serious problem with the cytochemical analyses of insulin expression. Hori et al. (42) used almost the same protocol with supplementation of an inhibitor of phosphoinositide kinase (PI3K), LY294002. This inhibitor previously has been shown to promote increased insulin content of human fetal pancreas *in vitro* (43). With this modification, the secreted insulin levels of differentiated cells reached 13% of pancreatic islets, slightly reduced hyperglycemia upon implantation

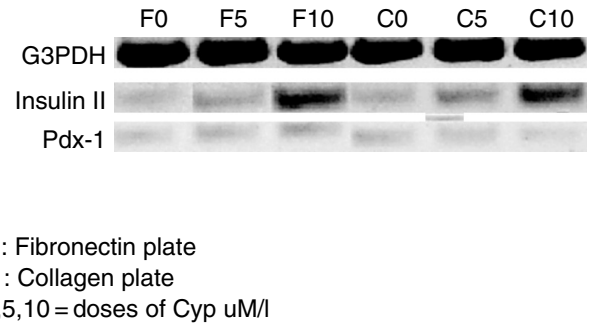


Fig. 4. Induction of insulin gene expression by cyclopamine (Cyp). During embryonic pancreas specification, repression of the *shh* gene results in induction of the *Pdx-1* gene which is crucial for insulin expression. Cyclopamine (Cyp), a steroidal alkaloid, is known to specifically antagonizes shh signaling through direct interaction with smo (smoothened), a protein that is related to G-protein-coupled receptors. Application of Cyp in mouse embryonic stem (ES) cell line ES-D3 at 10 μM prior to forming embryoid bodies induces insulin expression, both in collagen and fibronectin-coated plates as shown using reverse transcription-polymerase chain reaction (RT-PCR).

into diabetic mice, and there was no teratoma formation. In contrast, a more recent report pronounced that β-cells derived from mouse ES cells were not actually β-cells because, although they expressed insulin, they were not glucose responsive and exhibited an increased apoptosis upon differentiation (44). In Figure 4, the effect of cyclopamine (Cyp, an inhibitor of *shh*) on insulin expression in ES-D3 cell line, is presented. Because repression of the *shh* gene is a prerequisite for development of the pancreas, this observation of insulin induction by Cyp suggests the potential of this drug to augment differentiation towards a pancreatic lineage. With human ES cells, the effects of several growth factors have been studied (45). Although none of the growth factors tested to date is able to direct differentiation towards a single tissue type, nerve growth factor, and hepatocyte growth factor-induced differentiation of ES cells into all three germ layers, including the endoderm. Later, Assady et al. (46) demonstrated insulin-expressing cells appearing in human ES cultures. It is clear that substantial improvements need to be made in differentiation procedures to induce functional islets. There is also the need for additional newer human cell lines that can be adapted *in vitro* and made clinically suitable [to comply with Good Laboratory Practice/ Good Manufacturing Practice (GLP/GMP) standards] for human use.

Adult stem cells

In recent years, several adult stem cells have been described. Some tissue-specific stem cells from neuronal tissue, bone marrow, and muscles have also been suggested to have plasticity in that they are able to give rise to multiple lineages of cell types.

Pancreatic stem cells

Unlike ES cells, it is generally believed that adult stem cells are tissue specific (i.e. can give rise to only cell types found in that particular tissue) and are functionally rare except in tissues that constantly renew (e.g. blood, skin, gut, respiratory tract, and testis). However, multiple organs, including the brain, heart, liver, and neuronal system, have been shown to contain progenitor cells (47). We have shown that adult pancreatic progenitor cells can be derived from ducts (Fig. 5). By modifying culture conditions, we demonstrated the induction of islet-like clusters from an adherent layer of progenitor cells (48). Although the level of insulin produced was very low, upon culturing with nicotinamide, glucose-responsive insulin secretion was demonstrated. Upon implantation, either under the kidney capsule or under the skin, these clusters were able to normalize hyperglycemia. Similar work performed using human ductal tissue was reported by Bonner-Weir et al. (49). In this procedure, ductal epithelial layer was overlaid with matrigel, and islet-like clusters were shown to bud-off the gel. While there appeared to be significant insulin present, no *in vivo* functionality was reported. A more recent study demonstrated that a subpopulation of ductal progenitor cells were islet progenitors and were not nestin+. The differentiated clusters produced insulin almost to the extent of native islets. However, they were ineffective *in vivo* (50). One possible explanation is that cultured cells which have adapted to the *in vitro* environment do not survive *in vivo*. Besides pancreatic ducts, although disputed, islets have been reported to contain nestin+ progenitor cells (51–53). While *in vitro* derivation of progenitor cells has been established by many, it is not certain whether these cells are specialized stem cells that coexist with ductal epithelial cells, or are dedifferentiated duct cells expressing progenitor phenotypes. More recently, the role of pancreatic progenitor

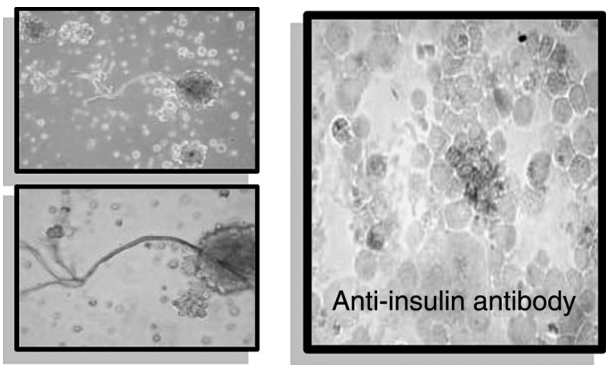


Fig. 5. Expression of insulin-expressing cells from adult human pancreatic duct-derived progenitor cells. When adult progenitor cells are allowed to grow as clusters in serum-free Dulbecco's modified Eagle's medium (DMEM) mixture for 1 wk, insulin expression appears within clusters, as shown by histochemistry after seeding clusters as adherent cells in regular culture plates.

cells, *in vivo*, in the maintenance and replication of mature islets, has been challenged (54).

Bone marrow stem cells

Bone marrow hematopoietic stem cells normally generate all the lineages of blood cells with bone marrow mesenchymal stem cells giving rise to fat cells, bone cells, and cartilage. This concept of limited tissue differentiation has been challenged by the observations of multiple non-hematopoietic tissues following transfer into animal models. Thus, unfractionated bone marrow stem cells have been reported to give rise to skin, lung epithelium, kidney epithelium, gut epithelium, liver cells, endothelium, myocardium, and neuronal cell types (55). Most recently, our colleagues and others have demonstrated the feasibility of deriving insulin+ islet-like clusters from mouse and rat bone marrow stem cells *in vitro* (56–58). Such insulin+ clusters, upon implantation into STZ-induced diabetic mice, could reverse hyperglycemia and also were capable of glucose-responsive insulin secretion *in vitro*. This has tremendous implications because there is relatively easy access to bone marrow stem cells for clinical manipulations. It is not clear, however, as to which bone marrow cells are capable of differentiating into islets, how much bone marrow cells are required to produce clinically meaningful number of islets, the applicability and scalability of this approach in humans, whether any cell fusion is involved, and to what extent these cells are actually related to native pancreatic islets. Because there has been little/no evidence that bone marrow stem cells directly give rise to islet cells *in vivo*, these *in vitro* observations may be due to cellular alterations induced in culture conditions.

Thus, whether bone marrow stem cells are actually capable of giving rise to any non-hematopoietic tissues is controversial (59, 60). Lack of application of stringent methodologies, over-interpretation of the data and poor reproducibility of the observations are not uncommon. Clearly, more rigid criteria need to be applied to justify the use of terms such as 'trans-differentiation' and 'plasticity' because mechanisms such as cell fusion (61) and contaminating stem cells (62) may clearly complicate the picture (63). Even if proven, whether such an approach has any *in vivo* applicability in humans remains to be determined.

Further issues to be addressed in a stem cell-derived islet implantation approach

Stem cell-based therapies as a cure for type 1 diabetes offer hope to overcoming the acute islet shortage. One of the major obstacles to islet transplantation therapy is the life-long requirement for immunosuppressive regimen. This is clearly not desirable for young patients with type 1 diabetes because of the long-term potential complications. Besides immunosuppressive drugs, these cells have undergone multiple manipulations in culture which may potentially transform cells and generate fusion hybrids. It is also not known how feasible it is to generate large numbers of islets from stem cells *in vitro* and how functional they will be. These issues are critical for human islet transplantations.

Pancreatic islets are delicate structures that can alter their functionality even in short term, *in vitro* cultures. Before these *in vitro* derived (manipulated) islets can be considered for use in clinical setting, the following information is vital: 1) reproducibility of the various *in vitro* differentiation protocols; 2) proliferative potential (which may vary from native islets); 3) functional and structural stability of differentiated islets; 4) consequences (if any) of undifferentiated cells present in the islets; 5) viability and longevity of these cells *in vivo*; 6) glucose responsiveness (*in vitro* and *in vivo*); 7) alterations (if any) of glucokinase and hexokinase ratio during *in vitro* cultures (which will influence glucose responsiveness); 8) sensitivity to multiple stimuli, including the counter-regulatory hormones to which normal islets are capable of responding; and 9) *in vivo* migratory pattern after implantation. It is imperative that these issues be addressed. This will take a substantial amount of time and effort, not to mention the regulatory hurdles that have to be carefully addressed.

Islet regeneration

Regeneration refers to the *in vivo* re-creation of islets within the diabetic pancreas. Several observations directly or indirectly support the notion that the pancreas has appreciable regenerative capacity. In the mouse, these include: 1) the derivation of islet cells *in vitro* from progenitor cells within pancreatic ducts (48, 49); 2) experimental manipulation of islets *in vitro* leading to ductal hyperplasia and islet neogenesis (64); 3) manipulations such as ductal ligation, cellophane wrapping, and partial pancreatectomy (Px), leading to either existing β-cell replication, or neogenesis from ducts in mice (65); 4) the expression of Pdx-1 on mouse duct cells via vectors inducing islet neogenesis in non-diabetic animals (66); 5) Pdx-1 is an antennapedia-like protein with inherent capability to transduce non–β- cells (progenitor or mature cells) to either differentiate or trans-differentiate into β-cells (67); 6) the sustained replicative capability of mature β-cells (54); 7) the transfer of adult spleen cells into diabetic mice leading to an attenuation of autoimmunity and the promotion of islet neogenesis (68); 8) the transfer of bone marrow-derived HSCs into diabetic mice, which reversed hyperglycemia by stimulating new endothelial cells within the pancreas, that either guided endogenous pancreatic progenitor cells or induced β-cell replication (69); 9) establishment of allogeneic chimerism leading to

endogenous β-cell recovery (70); 10) implantation of an insulinoma (in rats) demonstrating the insulin-sensing capabilities of endogenous β-cell mass (71); 11) pregnancy, which is a natural situation for β-cell proliferation (72); 12) treatment with the β-cell toxin, STZ-induced extra-pancreatic formation of insulin-producing cells in the bone marrow, spleen, thymus, and adipose tissue. A similar effect was seen with hyperglycemia, suggesting that hyperglycemia is a critical factor in β-cell regeneration (73); 13) recovery of endogenous β-cell function following islet transplantation (rats) (74); and 14) treatment of diabetic mice with exendin-4 along with anti-CD3, gastrin, or epidermal growth factor (EGF)-reversing overt diabetes (75, 76).

In humans, the following points are noted: 1) postmortem examination of the pancreas in non-diabetic subjects has demonstrated up to 18% of β-cells to be closely aligned to ducts, suggesting potential neogenesis (77); 2) persistence of C-peptide in diabetic patients with varying duration of diabetes (78–81); 3) persistence of anti-islet antibodies in some patients with longstanding type 1 diabetes (indirectly indicating the presence or generation of islet cells); 4) induction of endogenous C-peptide in recipients of intraportal allo-islet transplantations (82), the observation of regeneration following Px (83); 5) human diabetic pancreatic sections evidenced co-staining of cytokeratin with vimentin and massive ductal proliferation, suggesting neogenesis is a natural process in the diabetic (hyperglycemic) environment (84); and 6) in type 2 diabetic pancreas an increased apoptosis over neogenesis was observed, implying regenerative processes and counteractive cell-death programs are at play in the pathogenesis (85).

Collectively, all the aforementioned observations are suggestive of the existence of residual β-cells and neogenetic capabilities in diabetic animals and patients.

Rationale behind the regeneration approach

Residual islet β-cells exist in most patients with type 1 diabetes with varying duration of diabetes (86, 87). While about 10% of insulin content has been demonstrated in young patients (81), histological analyses found 5–20% of insulin+ cells (88). Therefore, residual islet β-cells are most likely to exist in most of the patients with type 1 diabetes with varying duration of diabetes. Hyperglycemia and other unknown stress factors may induce existing β-cell replication and/or neogenesis of islets from ductal tissue. The rate of β-cell production may be influenced by the diabetic environment, e.g. infiltrating cells (lymphocytes and macrophages) and locally produced cytokines. One likely outcome of insulitis on islet regeneration is increased death of mature β-cells by apoptosis, which might further accentuate the neogenesis process. However, continued autoimmune destruction of β-cells and poor glycemic control could mask the benefits of the β-cell regenerative process. Hence, it is postulated that by down-regulating autoimmunity, the pancreatic regenerative processes might be supported and augmented if β-cell differentiating/growth factors were introduced. Theoretically, therefore, a potential opportunity could exist for islet regeneration in the diabetic state with: 1) down-regulation of the autoimmune response with treatment with e.g. anti-CD3 antibody; 2) augmentation of tolerance; 3) enhancement of neogenesis or β-cell replication of residual cells by growth factors, including glucagon-like peptide (GLP)-1 or its long-lasting analogue, exendin-4; and 4) additional improvements by bone marrow, spleen cell infusions. Figure 6 illustrates a simplified version of the regeneration concept.

Potential regenerating agents

GLP-1 is a potent intestinal insulinotropic hormone that augments insulin secretion (89) and lowers glucose levels in rodents and in patients with both type 1 and type 2 diabetes (90). Its analogue, exendin-4, has been shown to enhance transcription factor Pdx-1 expression in the pancreatic ducts, thus enhancing its islet neogenic potential. It has been shown to stimulate both β-cell replication and neogenesis, resulting in increased β-cell

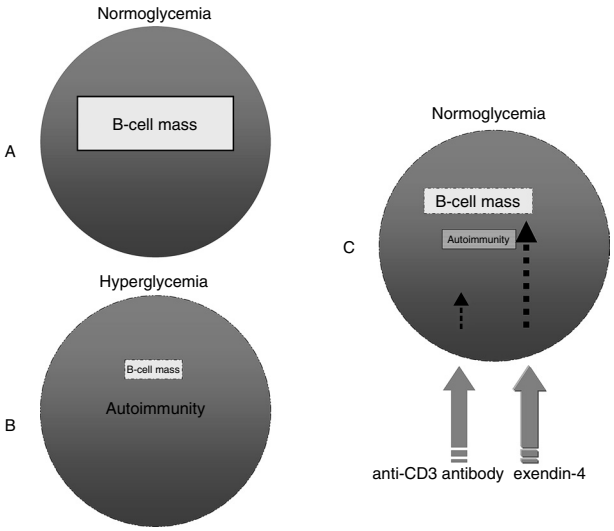


Fig. 6. Rationale behind islet regeneration approaches. β-cell mass is reduced substantially as autoimmune response evolves, resulting in hyperglycemia. Immunosuppression might allow for the attenuation of autoimmunity and limited regeneration of islets. It is conceivable, therefore, that regeneration might be promoted by agents or genes that can promote β-cell proliferation and differentiation [e.g. glucagons-like peptide-1 (GLP-1) or exendin-4]. In the Figure, A shows healthy β-cell mass, B shows the autoimmune process leading β-cell mass and hyperglycemia, and C illustrates anti-CD3 antibody attenuating autoimmunity with limited natural β-cell regeneration (thin arrow), while supplementation with exendin-4 supplement leads to stronger regeneration (thick arrow).

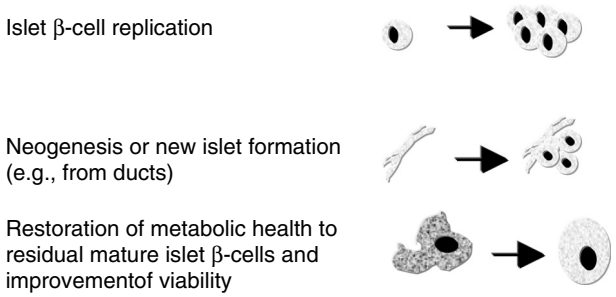


Fig. 7. Mechanisms of regeneration. There are at least three potential mechanisms that may orchestrate islet regeneration.

mass, reduced apoptosis, and improved glucose tolerance in diabetic rats and mice (91–93) and in glucose-intolerant rats (94). In the type 2 diabetic db/db mouse model, GLP-1 treatment delayed the onset of diabetes (95). Gastrin, another gut hormone, has also been shown to augment neogenesis of islets (96, 97). The gastrin receptor is expressed in the pancreas during embryonic development and in regenerating ductal epithelium. Gastrin is currently being used in conjunction with exendin-4 in mouse models of diabetes to further enhance islet neogenesis. Figure 7 depicts various mechanisms that might result in islet regeneration.

Most recent regeneration studies in animals

A recent and provocative study by Faustman et al. has kindled hope for the potential of islet regeneration to reverse diabetes (68). Established diabetes in non-obese diabetic (NOD) mice was reversed by the infusion of major histocompatibility complex class-I matched spleen cells. The autoimmune process was attenuated while at the same time, islets were regenerated. There was no evidence of engraftment, fusion, or trans-differentiation of brain, liver, or kidney cells. Up to 80% of donor cells were incorporated into the islets. These observations suggest that either splenocytes might have carried stem cells, or (because irradiated cells also regenerated islets) they produced growth factors that perhaps enabled endogenous generation of islets. Prior to *in vitro* bone marrow differentiation studies, Ianus et al. (98) demonstrated that up to 3% of insulin+ cells could come from donor bone marrow using green fluorescent protein (GFP)+ mice bone marrow. This was not reproduced by others using highly purified bone marrow stem cells (99). Hess et al. (69) reported reversal of hyperglycemia within a week after intravenous infusion of allogeneic bone marrow into STZ-induced diabetic mice. Cells were tracked with GFP+ marker. While only 2.5% of donor-derived insulin+ cells were found, islets were found to be replenished with endogenous cells. The authors suggested that donor GFP+ bone marrow stem cells guided endogenous growth of islets. Sadly, other groups have not observed such an antidiabetic effect of bone marrow infusion (100–102). Although these approaches are more promising due to their power to regenerate islets in the diabetic pancreas through a simple procedure, more information is needed on the mechanisms of splenocyte/bone marrow-mediated regeneration, besides reproducibility by others.

It is already known that treatment of overtly diabetic NOD mice with antilymphocyte serum (ALS) abrogates autoimmunity and can achieve partial clinical remission. Ogawa et al. (75) administered exendin-4 together with ALS to diabetic mice, achieving complete remission in 88% of the mice within 75 d. This was accompanied by improvements in islet histology, insulin content, and normalization of glucose tolerance. With ALS alone the remission was only 40%. These data suggest that if autoimmunity can be curtailed, regeneration can occur. Recently, combined gastrin and EGF treatment demonstrated islet regeneration and restoration of normoglycemia in C57Bl6/J mice treated with alloxan (76). In addition, betacellulin (a member of the EGF family) and activin A (a member of the TGF-β family) promoted regeneration of β-cells, increased β-cell mass, insulin content, induced β-cell neogenesis, and improved glucose metabolism in neonatal rats treated with STZ (103).

Potential human studies

Herold et al. (104) conducted a pilot trial in humans with new-onset type 1 diabetes treated with anti-CD3 antibody to preserve C-peptide production. Encouraging results have been noted up to 18 months following therapy, leading to its consideration for use with exendin-4 in an attempt to induce regeneration. Anti-thymocyte globulin (ATG) also holds promise. Seven adult patients with type 1 diabetes of long-standing duration (>25 yr) were given cultured human islet cells intraportally. Only three of seven graft recipients remained C-peptide-positive for more than 1 yr, two of them becoming insulin-independent. Those patients received ATG therapy at the time of transplantation (105), thus, providing evidence for a long-term immunomodulatory effect. Although there are considerable data on the use of ATG in adults, there are very little data on its use in children. Because most of the patients with type 1 diabetes are children, its safety and efficacy in this population is critical. A retrospective study used thymoglobulin (Imtix, Lyon, France) in pediatric cardiac transplant patients over a 13-yr period. In these pediatric heart-transplant patients, the antibody reagent was effective in reducing rejection rates and patient survival and was found to have a good safety profile (106). This study, albeit limited, is encouraging. Clearly, more studies are needed in animals in conjunction with regenerating supplements such as

exendin-4 prior to any consideration of clinical trials in the United States. Further, it is also crucial to investigate more products/genes for their potential to promote *in vivo* regeneration to strengthen our arsenal of regenerating reagents for clinical use. However, regeneration therapy in humans at this time remains an exciting concept with a lot of information yet to be garnered.

Conclusion

Cell replacement therapies are potentially promising approaches to treating several diseases, including diabetes, Parkinson's, Alzheimer's, and other autoimmunity-related diseases among others. Besides stem cell-derived islets for reversing type 1 diabetes, other approaches such as *in vivo* trans-differentiation (not discussed in this review) and regeneration are becoming more attractive. In patients with poor residual regenerative capacity, stem cell-based or allogeneic islet replacement therapy may be a suitable option, providing there are great strides made in both the understanding of the differentiation process and reversal of both alloimmunity and autoimmunity. We predict that regeneration approaches will soon be used in clinical trials, concurrent with the acquisition of a large amount of data in animals. It is hoped that the ongoing clinical trials in patients with type 2 diabetes treated with exenatide and LAF237 will lay the foundation for regenerative clinical trials in patients with type 1 diabetes.

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