Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus

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Lechner, Andreas, and Joel F. Habener. Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus. Am J Physiol Endocrinol Metab 284: E259–E266, 2003; 10.1152/ajpendo.00393.2002.—In view of the recent success in pancreatic islet transplantation, interest in treating diabetes by the delivery of insulin-producing \( \beta \)-cells has been renewed. Because differentiated pancreatic \( \beta \)-cells cannot be expanded significantly in vitro, \( \beta \)-cell stem or progenitor cells are seen as a potential source for the preparation of transplantable insulin-producing tissue. In addition to embryonic stem (ES) cells, several potential adult islet/\( \beta \)-cell progenitors, derived from pancreas, liver, and bone marrow, are being studied. To date, none of the candidate cells has been fully characterized or is clinically applicable, but pancreatic physiology makes the existence of one or more types of adult islet stem cells very likely. It also seems possible that pluripotential stem cells, derived from the bone marrow, contribute to adult islet neogenesis. In future studies, more stringent criteria should be met to clonally define adult islet/\( \beta \)-cell progenitor cells. If this can be achieved, the utilization of these cells for the generation of insulin-producing \( \beta \)-cells in vitro seems to be feasible in the near future.

pancreatic islets; liver oval cells; embryonic stem cells; nestin; insulin; islet-like clusters; \( \beta \)-cell; transplantation

THE PREVALENCE OF THE DISABLING DISEASE diabetes mellitus is increasing at epidemic proportions throughout the populations of the world. It is estimated that 100 million individuals currently suffer from diabetes, 16 million in the United States alone. Diabetes comes about by the progressive failure of the \( \beta \)-cells of the endocrine pancreas (islets of Langerhans) to produce the hormone insulin in the amounts required to meet the body’s needs to maintain nutrient homeostasis. A lack of insulin results in elevations in blood glucose levels (hyperglycemia) and the subsequent development of premature cardiovascular disease, stroke, and kidney failure. There is no currently available permanent cure for diabetes. Blood glucose levels can be somewhat controlled by daily insulin shots or, in moderate cases of diabetes, by oral hypoglycemic (blood glucose-lowering) drugs. Diabetes is manifested in two relatively distinct forms: type 1 juvenile and type 2 adult onset. Type 1 juvenile diabetes is due to a nearly complete destruction of the \( \beta \)-cells by processes of autoimmune in which the body’s immune system mistakenly attacks and destroys the \( \beta \)-cells. The causation of type 2 adult-onset diabetes is more complex and poorly understood, but the \( \beta \)-cells fail to produce adequate amounts of insulin in the face of the accompanying resistance of peripheral tissues to the actions of insulin. All patients with type 1 diabetes require daily insulin shots to survive. Twenty to thirty percent of type 2 diabetic individuals also require exogenous insulin to control their hyperglycemia after oral hypoglycemic agents have failed.

Recently, hope for a permanent cure of diabetes has appeared, namely, the transplantation of islets isolated from donor pancreata into the livers of diabetic...
patients. The recent success of the Edmonton Protocol for pancreatic islet transplantation (45, 46) has sparked new interest in transplantation of insulin-producing cells. However, the amount of donor islet tissue is severely limited and will allow for the treatment of only a small fraction of patients with insulin-dependent diabetes, <0.5% of needy recipients. Moreover, differentiated β-cells cannot be expanded efficiently in vitro (24). Therefore, multiple approaches are now being explored to generate insulin-producing cells in vitro, either by genetic engineering of β-cells or by utilizing various potential β-cell precursor cells, stem/progenitor cells, with the ability to grow in vitro and to differentiate into β-cells. Some promising results have already been obtained with embryonic stem cells (ES cells) of both rodent and human origin (2, 38, 50). However, the potential use of ES cells for the treatment of diseases in humans is beclouded in controversy because of the ethical issues.

This review will focus on the potential of adult tissue-derived stem cells, in lieu of embryo-derived stem cells, for the treatment of diabetes. We discuss the role of adult islet stem/progenitor cells in normal physiology, highlight possible candidate cells isolated to date, and describe different approaches for stem cell-based therapy. We will also propose several criteria for the establishment of β-cell differentiation from putative stem cells, both in vitro and in vivo.

**PHYSIOLOGICAL ROLE OF ADULT ISLET PROGENITOR CELLS**

Stem or progenitor cells are defined by their capacity for self-renewal and asymmetric cell division, which leads to their differentiation and the formation of one or more mature tissues and the preservation of the stem cell population. Pluripotential cells exist in the inner mass of the blastocyst, so-called ES cells, and in the developing gonadal ridge, germ stem cells or GSCs. In some tissues, like bone marrow or intestinal epithelium, the existence of adult stem cells has been recognized for a long time and has been studied extensively (see review in Ref. 4). For other adult tissues, like brain, muscle, liver, and the endocrine pancreas, the function of adult stem cells is less well established, although substantial evidence for their existence and potential efficacy has recently been obtained.

When the embryonic development of the pancreas is studied, several sequentially expressed transcription factors are used to identify different cell populations within the forming pancreas that buds from the endodermal epithelium of the developing foregut. The homeodomain transcription factor Pdx-1 (also known as IpF-1, Idx-1, and Stf-1) is expressed early in pancreas development (embryonic day 8.5 in the mouse), and its expression is required for the pancreas to develop. The disruption of Pdx-1 expression in mice and humans results in pancreatic agenesis, a complete failure of the pancreas to develop (31, 52). The expression of neurogenin 3 (Ngn3), a basic helix-loop-helix transcription factor, at embryonic development day 9.5 defines the first identifiable endocrine cell precursors. From then on, a cascade of transcription factors leads to the formation of all endocrine lineages of the islets of Langerhans (see reviews in Refs. 11 and 54). Throughout fetal development, a massive expansion of pancreatic endocrine cells is achieved mainly by the proliferation and subsequent differentiation of progenitor cells, rather than by the division of fully differentiated endocrine cells (8, 32, 42).

The formation of new islet tissue via the differentiation of stem/progenitor cells in adult pancreata was first demonstrated in different models of pancreatic injury. This regenerative process is called neogenesis. For example, partial pancreatectomy in rodents leads to the neogenesis of new islets from cells residing within or adjacent to the epithelium of pancreatic ducts (5). In another model of injury, the treatment of rodents with the drug streptozotocin (STZ), a β-cell toxin, induces the regeneration of endocrine cells from intraslet progenitors (17, 21). Also, in the setting of a sudden increase in insulin demand, such as an experimental glucose infusion, neogenesis of endocrine cells from adult progenitor cells is a major adaptive mechanism (3). It was also shown recently that Ngn3-expressing cells persist into postnatal life and continue to contribute to islet cell development under normal physiological conditions (19). However, we suggest that the Ngn3-expressing cells are probably only transitory cells in endocrine maturation and not true stem or progenitor cells capable of self-renewal. Therefore, although the existence of adult islet progenitor cells can be inferred from these studies, the exact nature and location of these cells have yet to be definitively determined. It seems likely that distinct sets of progenitor cells and pathways of neogenesis (e.g., intraslet vs. duct to islet) exist in parallel within the pancreas. In this regard, it has been shown that the islets are polyclonal in origin (10).

A further level of complexity is added by the recent reports about the possible existence of circulating pluripotential and mobile stem cells that may take up residence within many different adult organs and tissues (4, 29). Although the uptake of circulating stem cells by the endocrine pancreas has not yet been demonstrated, the regeneration of endocrine cells from a systemic source of pluripotential stem cells seems possible.

**CANDIDATES FOR ADULT PANCREATIC STEM/PROGENITOR CELLS**

To date, no adult pancreatic stem cell has been fully characterized. However, several candidate cells have been identified, isolated, and partially characterized (Table 1). It is important to note that differentiation protocols at present allow for only small amounts of insulin production compared with pancreatic islets.

**Pancreatic Duct-Derived Stem/Progenitor Cells**

Because under certain conditions islet regeneration appears to originate from the ductal epithelium (5),
INSULIN SECRETION

Liver oval (stem) cells

Form ILCs that express insulin, glucagon, pancreatic polypeptide

Human islets

Form ILCs. Insulin secretion induced by GLP-1

Summary of studies of stem/progenitor cells and diabetes

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<tr>
<td>Ducts from NOD mice</td>
<td>In continuous expansion culture for 3 yr.</td>
<td>Lower plasma glucose levels in NOD mice (kidney capsule)</td>
<td>Ramiya et al., 2000 (44)</td>
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<tr>
<td>Human duct tissue</td>
<td>Form ducts and islet buds that express glucagon and insulin</td>
<td>Not done</td>
<td>Bonner-Weir et al., 2000 (7)</td>
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<td>Mouse ES cells</td>
<td>Select cells by insulin promoter trap</td>
<td>Normalize glycemia in STZ-induced diabetic mice</td>
<td>Soria et al., 2000 (50)</td>
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<td>Human, rat islet cells</td>
<td>Nestin positive. Form ILCs that express glucagon, insulin, Pdx-1</td>
<td>Not done</td>
<td>Zulewski et al., 2001 (64)</td>
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<td>Mouse ES cells</td>
<td>Form nestin-positive cells and ILCs that express insulin, glucagon, somatostatin</td>
<td>Successful subcutaneous engraftment. No effect on glycemia.</td>
<td>Lumelsky et al., 2001 (38)</td>
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<td>Human ES cells</td>
<td>Form insulin-producing embryoid bodies</td>
<td>Not done</td>
<td>Assady et al., 2001 (2)</td>
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<td>Liver cells (subpopulation)</td>
<td>Infection of cells with adenovirus-Pdx-1 expression vector converts them into functional β-cells</td>
<td>Restores normal glycemia in STZ diabetic mice.</td>
<td>Ferber et al., 2000 (15)</td>
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<tr>
<td>Liver oval (stem) cells</td>
<td>Form ILCs that express insulin, glucagon, pancreatic polypeptide</td>
<td>Reverse hyperglycemia in one STZ diabetic NOD-scid mouse</td>
<td>Yang et al., 2002 (60)</td>
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<tr>
<td>Human islets</td>
<td>Form ILCs. Insulin secretion induced by GLP-1</td>
<td>Not done</td>
<td>Abraham et al., 2002 (1)</td>
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NOD, nonobese diabetic; ES, embryonic stem; ILCs, insulin-producing islet-like clusters; Pdx-1, homeodomain transcription factor; STZ, streptozotocin; GLP-1, glucagon-like peptide-1.

Our laboratory has reported that cells expressing the intermediate filament protein nestin, a marker of neural stem cells, can be isolated from human and rodent islets and expanded extensively in vitro. Insulin, glucagon, and Pdx-1/Ipf-1 expression, as well as low-level insulin secretion, can be detected in cultures of nestin-positive islet-derived stem/progenitor cells (NIPs) after addition of differentiating cytokines and growth factors (1, 64). Furthermore, the expression of other neuroendocrine, hepatic, and pancreatic exocrine genes can be demonstrated by RT-PCR (64). These cells also form ILCs in vitro, a process that is markedly enhanced by the addition of the insulinotropic, neogenic hormone glucagon-like peptide-1 (GLP-1) (1).

Whereas nestin was initially believed to be a marker restricted to neural and muscular progenitor cells (63), it is now recognized that certain mesenchymal cells also express nestin under selected conditions. With regard to the pancreas, recent reports demonstrate the expression of nestin in embryonic mesenchyme (48) and adult pancreatic stellate cells, as well as in proliferating vascular endothelium (36). Data from our own laboratory confirm these observations. Thus nestin in the pancreas is not an exclusive marker for islet stem/progenitor cells, but rather, some nestin-positive cells have the potential for the differentiation into pancreatic (islet) endocrine cells, including cells that produce insulin. This finding is also supported by the observation that nestin expression occurs as an intermediate step in the differentiation of β-cells from ES cells (38). We also demonstrated recently, by flow cytometry cell sorting, that 1–3% of NIPs have a side-population phenotype similar to undifferentiated bone marrow stem cells (18, 37). This finding suggests that NIPs might contain a subpopulation of immature stem cells with a differentiating potential that extends beyond the endocrine pancreas, possibly as part of...
a population of adult multipotential/pluripotential stem cells.

**Hepatic Oval Cells**

The close anatomic association of pancreas and liver development from the primitive foregut during embryogenesis has prompted attempts to isolate pancreatic progenitor cells from adult liver. Recently, Yang et al. (60) reported the in vitro generation of ILCs from rat liver preparations enriched for hepatic oval cells. Oval cells are considered to be hepatic stem cells that can give rise to hepatocytes and bile duct cells (41). The authors induced in vivo proliferation of oval cells by a chemical injury of the liver and then enriched them to over 95% by flow cytometry cell sorting. After the in vitro expansion of the oval cells and their formation into ILCs, the expression of endocrine hormones and several other β-cell markers could be induced, including low levels of insulin secretion. In preliminary in vivo studies, the authors report the successful reversal of diabetes in one STZ-treated NOD-scid mouse (60).

**Multipotent Adult Progenitor Cells**

Recently, the laboratory of Catherine Verfaillie (Jiang et al., Ref. 29) reported the successful expansion of cells from the adult bone marrow that have the potential to differentiate into ectodermal (neuronal), mesodermal (vascular endothelium), and endodermal (liver) cell types in vitro. In vivo, these cells show similar plasticity and contribute to multiple tissues after transplantation (29). The multipotent adult progenitor cells (MAPCs) can also be isolated from brain and muscle tissue (30) and may exist in every tissue of the body. The differentiation of MAPCs into pancreatic endocrine cells has not yet been shown. If we consider, however, that MAPCs can be transdifferentiated into hepatocyte-like cells with remarkable functional properties in vitro (47), the generation of pancreatic endocrine cells from MAPCs seems to be within reach.

**STEM CELL STIMULATORS AND DIFFERENTIATORS**

Multipotential stem/progenitor cells can be induced to proliferate and to differentiate when exposed in vitro to certain growth factors or cytokines. A cell line (AR42J) derived from pancreatic ducts can be converted into insulin-producing cells by exposure to the growth factors betacellulin and activin A (39) or to the hepatic growth factor (40). Cells derived from pancreatic islets increase their rates of proliferation in the presence of basic fibroblast and epidermal growth factors (bFGF and EGF) (64). These cells can then be differentiated into ILCs by the removal of bFGF and EGF and the addition of differentiating factors such as activin A, betacellulin, hepatic growth factor (64), or the insulinotropic neogenic hormone GLP-1 (1). Nicotinamide is often used to increase the number of β-cells and to boost the production of insulin (44). GLP-1 appears to be a particularly effective agent for inducing the differentiation of both NIPs (1) and duct cells into insulin-producing cells. Notably, GLP-1 induces the expression of the homeodomain protein Pdx-1/IPF-1 in the stem/progenitor (1) and duct cells (26, 51, 59, 62), which appears to be essential for their differentiation into β-cells. GLP-1 receptors have been identified on NIPs (1). It is believed that the activation of the receptor by GLP-1 activates Pdx-1 both by its phosphorylation via MAP kinase pathways and by effecting its translocation into the nucleus (9, 13, 27, 57). In many respects, Pdx-1 is a “master regulator” of pancreas development and function (see reviews in Refs. 11, 22, and 23). Pdx-1 is required for the pancreas to develop and is a key regulator of the expression of many β-cell genes, including the insulin gene. The forced expression of Pdx-1 in a pancreatic ductal cell line renders the cells responsive to GLP-1, resulting in their differentiation into insulin-producing cells (26). Remarkably, the infection of a subpopulation of liver cells with an adenoviral vector expressing Pdx-1 converts the cells into functional β-cells and restores glycemic control in mice rendered diabetic by their treatment with STZ (15). These studies suggest that, in the milieu of a stem cell, Pdx-1 in and by itself may be capable of programming the genome of the stem cell to express the set of genes required to establish a fully functional β-cell.
from the bone marrow populate adult organs (see review in Ref. 4). Patients transplanted with gender-mismatched hematopoietic stem cells (HSCs) developed a substantial degree of chimerism (up to 7%) in the liver, skin, and gastrointestinal tract, leading to the conclusion that circulating stem cells can differentiate into mature hepatocytes and epithelial cells of the skin and gastrointestinal tract (34). The mesenchymal MAPCs in the bone marrow recently described by Jiang et al. (30) have now been found in brain and muscle. Remarkably, gene expression profiling on oligonucleotide microarrays revealed that the genes expressed by MAPCs derived from brain, muscle, and bone marrow are 99.99% identical. These findings strongly indicate that the MAPCs found in these three different organs are the same cells and are consistent with the circumstance that MAPCs are produced in the bone marrow, delivered into the circulation, and taken up by brain and muscle. This mechanism for the distribution of stem cells is further supported by several studies in rodents with specific tissue injuries in which subpopulations of HSCs were administered systemically. In rodent models of heart attack (28, 33), brain stroke (25, 61), muscular dystrophy (20), and liver injury (35, 56), the cells rapidly home in on the sites of injuries and then differentiate into the resident tissue phenotype. The extent of repopulation of some of the injured tissues was substantial. In the mouse model of brain stroke, an average 34% of the vascular endothelial cells were derived from HSCs (25). Sixty percent or more of the liver tissue of some mice with liver injury consisted of hematopoietic donor cells (56).

The property of stem cells to colonize injured tissue is also demonstrated by the recently reported study by Quaini et al. (43). Female donor hearts transplanted into male recipients rapidly became chimeric; within 4–28 days after the transplant, 15, 12, and 9% of cells in myocytes, arterioles, and capillaries, respectively, of the donor heart consisted of recipient male gender cells. Although the origin of these recipient cells was uncertain, it was suggested that they likely came from circulating stem cells of the host recipient rather than from migration of cells into the heart from the recipient tissue adjacent to the anastomoses of the donor heart (43). It is noteworthy that the effective repair of the genetically determined mouse model of liver injury, due to an inborn error of tyrosine metabolism, has also been achieved by the administration of a population of pancreas-derived cells (55). Although the pancreatic cells that resulted in a repair of the liver were not characterized, it seems rather likely that the cells are related to the duct (7, 44) and/or are islet-derived (1, 64) stem/progenitor cells. The two models proposed for the origin of adult tissue-derived stem cells are not necessarily mutually exclusive. It seems reasonable to speculate that the pluripotent stem cells in the bone marrow are derived by lifetime self-renewal of the original pluripotent cells of the blastocyst and that tissue-specific multipotent progenitor cells with restricted differentiation capacity also exist.

The physiological and cellular mechanisms responsible for the “homing” of circulating stem cells into areas of tissue injury is not understood. However, ligand-receptor interactions may be at play, as suggested by Jackson et al. (28), who conjectured that the vascular endothelial growth factor (VEGF) expressed in the injected HSCs may have directed them to the experimentally infarcted myocardium that expressed the VEGF receptor (Flk-1). In this regard, it is interesting to postulate that the expression of the GLP-1 receptor by pancreas-derived stem cells (NIPs) may promote homing in to the pancreatic islets, whose surface (mantle) is enriched in α-cells that express the proglucagon (GLP-1) gene. It seems possible to imagine that one function of the α-cells may be to “capture” circulating stem cells by virtue of their interaction with the GLP-1 hormone expressed by α-cells. If, indeed, the stem/progenitor cells in the pancreas originate from the bone marrow via the circulation, then it is possible that there may be separate distinct functions for the duct-derived compared with the islet-derived stem cells (Fig. 1). We envision that, once a pluripotential stem cell finds a niche in a particular tissue, it receives initial instructions from the environment to differentiate toward that particular tissue. Thereby, we suggest that the stem cells that are captured from the circulation by the ducts are instructed to become new islets, a concept consistent with the longstanding observations that new islets continue to bud off from the pancreatic ducts, the classic description of islet neogenesis. In contrast to the formation of new whole islets from the ducts, we conjecture that the stem cells that land in the islets are instructed to become new β-cells to replace those that undergo natural senescence. This process of β-cell neogenesis in fully formed islets may be different.
from the process of islet neogenesis that takes place in the ducts.

ENVISIONED APPROACH FOR STEM CELL-DERIVED THERAPIES FOR DIABETES

At least two distinct approaches to the use of stem cells seem feasible for the treatment of diabetes (Fig. 2). One would be to procure appropriate donor tissue (pancreas biopsy) from a diabetic “to-be” recipient of islet transplants into the liver. The stem cells could be isolated from the biopsied tissue, expanded by culture in vitro, and differentiated into ILCs, or even into fully differentiated islets, once the technology to do so is available. Such a commercial source for “growing” unlimited numbers of islets would alleviate the acute shortage of donor pancreata from which islets for transplantation are prepared.

A second approach, quite different from the preparation of islets from stem cells in vitro, is to administer multipotent stem cells systemically to patients with type 1 diabetes and to depend on their properties to home in on injured tissues, as shown in the rodent studies. The cells would be delivered into the circulation as a “cellular medicine” by short infusions or injections. This approach would require the isolation and purification of a homogenous population of stem/progenitor cells that are selected for their high propensity to want to become islet or β-cells. The coadministration during and after islet transplantation of stem cell stimulators/differentiators, such as GLP-1, would possibly enhance the efficiency of engraftment and the establishment of a permanent self-renewing population of stem/progenitor cells to provide a continued source of functional β-cells.

Aspects of the proposed stem cell therapy that are important to recognize are the recent findings of the capacity of stem cells to induce tissue tolerance, in which the host recipient recognizes transplanted foreign tissue derived from stem cells as self, and the graft is not rejected. Such induction of tissue tolerance across both allogenic and xenogenic boundaries without a requirement for immunosuppression has been demonstrated in rodents in which mixed chimerism has been established by marrow transplants (see reviews in Refs. 53 and 58) and in rat and mouse models transplanted with stem cells (14, 49). It seems possible that stem cell transplants may also be able to resist the autoimmunity of type 1 diabetes, even after they differentiate into β-cells in the recipient. Ferber et al. (16) recently reported the successful transdifferentiation of liver cells into fully functional β-cells that achieve glycemic control in the NOD mouse model of autoimmunity type 1 diabetes.

SUGGESTED CRITERIA TO DEFINE A PANCREAS-DERIVED STEM CELL

All studies of pancreatic endocrine progenitor cells published to date, including our own, fall short in fully defining their properties as stem/progenitor cells. To advance our understanding of islet biology and, more importantly, to isolate β-cell stem/progenitors that will be clinically applicable, more rigorous methods are necessary. Therefore, we would like to suggest a set of criteria that should be met in future studies. 1) The stem or progenitor cell should be clonally isolated or marked; “enrichment” of a certain cell type alone is not sufficient. 2) In vitro differentiation to a fully functional β-cell should be unequivocally established. Insulin expression per se does not make a particular cell a β-cell. The expression of many other markers of β-cells (e.g., Pdx1/IPf1, GLUT2, and glucokinase) or other endocrine islet cells should be demonstrated. 3) Ultrastructural studies should confirm the formation of mature endocrine cells by identification of characteristic insulin secretory granules. 4) The in vitro function of endocrine cells, differentiated from stem cells, should be reminiscent of the natural counterparts. For β-cells, this would imply a significant glucose-responsive insulin secretion, adequate responses to incretin hormones and secretagogues, and the expected electrophysiological properties. 5) In vivo studies in diabetic animals should demonstrate a reproducible and durable effect of the stem/progenitor-derived tissue on the attenuation of the diabetic phenotype. It should also be demonstrated that removal of the stem cell-derived graft after a certain period of time leads to reappearance of the diabetes. 6) For future clinical use, the tumorigenicity of stem/progenitor-derived tissue should be determined. Additionally, immune responses toward the transplanted cells should be examined.

CONCLUSIONS

To date, no fully defined and clinically applicable adult β-cell stem/progenitor has been isolated. Nevertheless, studies of the development and the physiology of the pancreas make the existence of pancreatic stem/
progenitor cells highly likely. Additionally, several
potential candidate cells are being studied, and although
more rigid experimental criteria have yet to be met, the
published results look highly promising. The utiliza-
tion of adult stem/progenitor cells for the generation of
insulin-producing β-cells in vitro and their use for the
treatment of diabetes, therefore, seem to be feasible in
the near future.

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