

Stem cells and diabetes

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Diabetes mellitus is a metabolic syndrome characterized by increased levels of blood glucose. Type 1 diabetic patients and patients with Type 2 diabetes suffering from defective insulin secretion rely on lifelong substitution with exogenous administration of insulin. Whole pancreas and purified pancreatic islet transplantation have offered the potential for independence from insulin injections. A major obstacle, however, is the limited supply of cadaveric human islets. Success in islet transplantation-based therapies for Type 1 diabetes, coupled with a worldwide shortage of transplant-ready islets, has motivated efforts to develop renewable sources of islet-replacement tissues. Stem cells offer the greatest potential for the development of an abundant source of pancreatic islets. Insulin-producing cells for transplantation can be generated from both embryonic and adult stem cells. Before stem cell therapeutic strategies for diabetes mellitus can be transferred to clinical application in humans, stem cell biologists have to address several pressing issues related to appropriate differentiation protocols, functional aspects of insulin secretion, its regulation, cell-maturation processes and control of proliferation, along with ethical norms and safety.

Keywords: Diabetes, differentiation, insulin producing cells, stem cells, transcription factors.

DIABETES mellitus is a chronic metabolic syndrome characterized by increased levels of blood glucose, referred to as hyperglycaemia. Type 1 diabetes generally results from autoimmune destruction of pancreatic islet *b*-cells, with consequent absolute insulin deficiency and complete dependence on exogenous insulin treatment¹. Type 2 diabetes is associated with insulin resistance and pancreatic insufficiency and generally progresses to a state of insulin dependence². The current treatment of insulin does not represent a cure because insulin dosage is difficult to adjust. Exogenous insulin frequently fails to achieve optimal glucose control even when intensive regimens are used^{3,4}. In addition, intensive therapy, which uses multiple daily insulin injections or insulin pump infusion with frequent monitoring of blood glucose, often leads to an increased incidence and severity of hypoglycaemic episodes.

Pancreas/islet transplantation

Pancreas transplantation has offered the potential for a cure with total independence from insulin injections. Kelly *et al.*⁵ were the first to report the transplantation of whole pancreas on patients with Type 1 diabetes, who already had or were undergoing renal allograft in order to minimize the use of immunosuppressants as well as risks associated with surgery. Despite these favourable outcomes, pancreas transplantation can be used only on few patients because of scarcity of suitable donors and the morbidity and high costs involved. The complexity inherent in this surgical intervention and the problems associated with organ manipulation and conservation forced the search for alternative strategies, such as transplantation of purified islets injected through the portal vein. Thus, islet transplantation has generated considerable interest in recent times as a viable method of restoring endogenous insulin secretion in selected patients with Type 1 diabetes. The first trials displayed limited success owing to the use of steroid immunosuppressors such as prednisone, which is harmful for transplanted islets. However, renewed interest in this approach was stimulated in large part by the relative success of the Edmonton Protocol for islet transplantation⁶. This protocol combined steroid-free immunosuppression with at least two separate islet infusions to deliver a sufficient total islet mass to the liver to achieve insulin independence in majority of recipients. The result was a consistent and marked improvement of islet graft survival and function compared to prior islet transplant trials. Following the success of this protocol, there was an unprecedented, exponential increase in clinical islet transplant activity, with an estimated 471 patients with Type 1 diabetes treated at 43 institutions worldwide⁷. This represents a significant milestone, as more patients with Type 1 diabetes have now received islet implants in the past five years than in the entire preceding 30-year history of islet transplantation.

The main advantage of islet transplantation includes the achievement of physiologic insulin secretion in those patients who are able to achieve insulin independence. Islet transplantation is a much less invasive procedure and is considered safer than whole pancreas transplantation. Isolated islets carry a lower antigenic property than the whole organ, which may thus require reduced immunosuppression with fewer side effects. Islets can also be manipulated *in vitro* before transplantation to reduce graft rejection⁸. One of the major disadvantages is the commitment of a patient to lifelong immunosuppression that is not only

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costly, but also has potential side effects. The use of high-dose calcineurin inhibitor therapy has raised concerns because of the possibility of worsening nephropathy in long-standing diabetic patients. Moreover islet transplantation requires at least two donor organs for each recipient and therefore its broad application remains limited due to lack of sufficient cadaveric donors⁹.

Alternate sources of insulin-producing cells

Today, it is clear that the Edmonton Protocol has been a major improvement in the treatment of Type 1 diabetes, but it is necessary to find new sources of insulin-producing cells to extend transplants to the majority of potential recipients. Although cadaveric islets are currently the mainstay of endocrine cell replacement, alternative cell sources, such as stem cells hold great promise to provide a ready source of transplantable, insulin-secreting tissues that would not be limited by the supply of donor organs. Thus considerable effort is being directed towards developing alternative insulin-secreting cells for restoring endogenous insulin secretion. Realising the potential of stem cell research and its clinical relevance, the Madras Diabetes Research Foundation, Chennai jointly with the Department of Science and Technology, Government of India had convened a national level meeting in 2002 on 'cell transplantation'¹⁰. Since then a number of developments have occurred in this field, particularly in the generation of insulin producing cells (IPCs). This article reviews these developments and addresses the need for more research into stem cells, current state-of-the-art in stem-cell therapy for diabetes, ethical issues and future prospects.

Development of cell-based therapies for diabetes

In developing a potential therapy for patients with diabetes, researchers hope to develop a system that meets several criteria. For diabetes therapy, it is not clear whether it will be desirable to produce only islet **b**-cells that manufacture insulin or whether other types of pancreatic islet cells are also necessary. Studies by Soria *et al.*¹¹ indicate that isolated **b**-cells, those cultured in the absence of the other types of islet cells, are less responsive to changes in glucose concentration than intact islet clusters made up of all islet cell types. Islet cell clusters typically respond to higher than normal concentration of glucose by releasing insulin in two phases: a quick release of high concentrations of insulin and a slower release of lower concentrations of insulin. Isolated **b**-cells, as well as islet clusters with lower than normal amount of non **b**-cells, do not release insulin in this biphasic manner, with no fine-tuning for intermediate concentrations of glucose in the blood. Therefore, it is important to develop a system in which stem or precursor cell types can be cultured to produce all the cells of the islet cluster in order to generate a population

of cells that will be able to coordinate the release of appropriate amount of insulin to physiologically relevant concentrations of glucose in the blood. The main goal is not only induction of insulin biosynthesis, but also its correct processing, storage and regulated secretion in response to physiologic signals, without which such cell-therapy approaches would not be significantly advantageous over insulin administration. Along with other sources, stem cells appear as potential source of **b**-cells for research/therapy in diabetes mellitus. Table 1 summarizes the various sources of **b**-cells for cell therapy in diabetes.

Stem cells

Stem cells are defined as cells that have clonegenic and self-renewing capabilities and differentiate into multiple cell lineages. Embryonic stem cells (ESCs) are derived from mammalian embryos in the blastocyst stage (Figure 1) and have the ability to generate any differentiated cell in the body. Adult stem cells are part of tissue-specific cells of the postnatal organism into which they are committed to differentiate. Adult pancreatic stem cells are located in intra islet, nestin-positive cells, duct cells and oval cells which differentiate into pancreatic **b**-cells¹². Another advantage is that they behave as an autologous model whereby a patient's own cells can be used, thereby preventing an immune rejection¹³. The benefits of the ESCs include the possibility of propagating an unlimited number of cells that possess the ability to become fully functioning endocrine tissue. Human embryonic stem cells grow as homogeneous and undifferentiated colonies when they are propagated on a feeder layer of mouse embryonic fibroblasts (MEFs). They have a normal karyotype and express telomerase and embryonic cell-surface markers. Removal from the MEF feeder layer is associated with differentiation¹⁴.

Generation of IPCs from embryonic stem cells

ESCs proliferate indefinitely in defined culture conditions and have the potential to differentiate into any of the more than 200 cellular types present in the human body. These two properties make ESCs perfect candidates as cell and tissue regenerators for various pathologies¹⁵. In contrast to mesodermally derived cardiomyocytes and ectodermally derived neural cells, endoderm-derived cells, including **b**-cells do not differentiate spontaneously from *in vitro* culture of stem cells. Consequently, the embryonic stem cells require supplementary and specialized transcription factors to be directed into a controlled pathway of endocrine development. Several approaches have been evaluated to establish endocrine lineage.

Insulin-positive cells isolated from mouse¹⁶, monkey¹⁷ and human embryonic stem cells¹⁸ using various protocols have been reported. Assady *et al.*¹⁹ reported the existence

Table 1. Potential sources of **b**-cells for cell therapy in diabetes mellitus

Xenogenic islet cells
Human islet cells
Islet cells from cadaver donors
<i>Ex vivo</i> expanded human islets
Engineered insulin-producing cells
Rodent transformed b -cell lines
Human transformed b -cell lines
Neuroendocrine transformed cells
Somatic cells
With some b -cell properties (liver cells, intestinal cells)
Unrelated to b -cells (fibroblasts, muscle cells)
Stem cells
Embryonic stem cells
Adult stem cells
Pancreas
Pancreatic duct
Foetal tissues
Non-pancreatic stem cells (hepatocytes, enterocytes, bone marrow, cord blood stem cells, etc.)

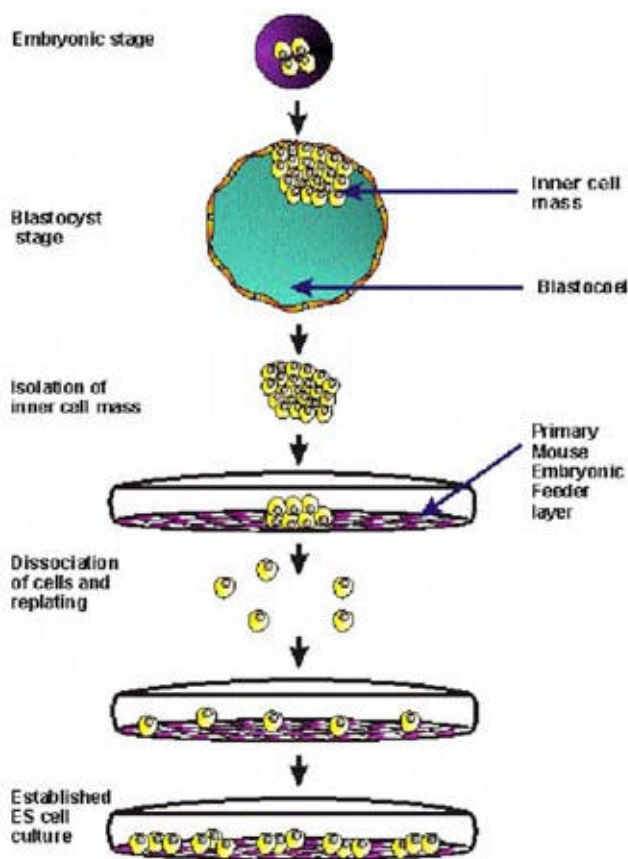


Figure 1. Steps leading to derivation of embryonic stem cells from inner cell mass of blastocysts.

of spontaneous differentiation of human ESCs into IPCs via embryoid body (EB) formation. Soria *et al.*²⁰ used a cell-trapping system based on the expression of the neomycin gene in mouse ESCs, which is driven by the human in-

sulin promoter to differentiate into insulin secreting cells. However, this procedure resulted in highly proliferating cells, and thereby potentially malignant, rather than mature, post-mitotic cells. However, this experiment showed the potential of ESCs to differentiate along the pancreatic endocrine path. This technique might be developed to provide a source of **b**-cells using more elaborate selection schemes that are compatible with normal **b**-cell development. The approach used by Lumelsky *et al.*²¹ involved the selection, expansion and differentiation of nestin-expressing cells from ESC cultures, a strategy previously used to generate neurons. Nestin is an intermediate filament protein originally expressed in the developing central nervous system and is found to be upregulated in various tissues²². Nestin-positive cells had islet-like structure, but showed more neuronal characteristics with small percentage of insulin-positive cells. However, these cells did not express Pdx-1, the homeodomain transcription factor, which not only helps in the regulation of insulin gene expression but is also required for differentiation of the mature pancreas during embryogenesis. Hnf3**b**, a critical factor in the endodermal cell-lineage development, is a transcriptional regulator of Pdx-1. Hnf6 triggers Hnf3**b** expression and Ngn3 transcription factor²³. Ahlgren *et al.*²⁴ demonstrated the expression of Isl-1, Pax-6, Nkx2.2, **b2**/NeuroD and Ngn3, which are required for the differentiation of islet cells. Ngn3 has been shown to be implicated in the development of all four endocrine cell types and it has been suggested that Ngn3-positive cells are the ideal islet cell precursors²⁵. Ngn3 induces the expression of **b2**/NeuroD, a transcription factor implicated in the insulin gene expression and in islet cell differentiation²⁶. Pharmacological manipulation by glucagon-like peptide-1 (GLP-1) was recently shown to induce differentiation of nestin-positive progenitor embryonic stem cells into insulin-producing cells, by a process of upregulation of PDX-1 expression²⁷.

Blyszczuk *et al.*²⁸ showed that the activation of Pax-4 expression in ESCs and selective differentiation via nestin positive progenitor cells increased the amount of insulin in ESCs. Over-expression of Pax-4 resulted in an increase in the differentiation status of the cells. Pax-4 expression has been shown to be one of the central elements in differentiating endocrine cells, including **b**-cells²⁹. Pax-4 activity appears essential for appropriate initiation of **b**-cell differentiation, as the loss of Pax-4 prevents the expression of Pdx-1, Hb9 and insulin in **b**-cell precursors. This role of Pax-4 is complemented with its genetic interaction with another homeobox gene, Nkx2.2.

Sipione *et al.*³⁰ have confirmed through their study that the insulin immunoreactivity detected in ES-derived cells was adsorbed from the differentiating media mostly by apoptotic cells. Insulin-positive cells were characterized and found to have a neuronal phenotype and not the **b**-cells. This clearly shows that nestin is not a marker of pancreatic endocrine cell precursors during pancreas development in mice, rats and humans. Rajagopal *et al.*³¹ provided evidence that insulin staining detected in differentiating cells could be an artifact due to insulin uptake from the insulin-containing differentiating media.

Hori *et al.*³² modified Lumelsky's protocol using specific growth inhibitors to reduce the neuronal lineage of the ESCs cells. Treatment of mouse embryonic stem cells with inhibitors of phosphoinositide-3-kinase, an essential intracellular signalling regulator, produced cells that resembled pancreatic **b**-cells. The cells secreted high amount of insulin and displayed glucose-dependent insulin release *in vitro*. Transplantation of these cells into a streptozotocin-induced diabetic mouse resulted in increased insulin release, reduced weight loss and most importantly, maintained glucose homeostasis.

Having examined the effects of several growth factors on *in vitro* differentiation of human ESCs, Schuldiner *et al.*³³ showed that hepatocyte growth factor and nerve growth factor are the only ones that induce endodermal differentiation. Because pancreatic tissue arises from the endoderm, treatment with these two growth factors could initiate the first step towards differentiation into **b**-cells. However, these cells produce low amounts of insulin compared to **b**-cells, and their potential use in transplantation has met with ethical objections as well as concerns regarding the risk of teratomas.

Another important factor may be the ability of the grafted cells to 'integrate' with the host tissue³⁴. An alternative to the *in vitro* differentiation of islets is the direct transplantation of undifferentiated ESCs into functionally compromised pancreas, where stimulus from the surrounding tissue could guide the ESCs to differentiate into the endocrine function. This type of work has been tried in Parkinson rat model by Bjorklund and his group³⁵. The problem with ESC transplantation is its tumourigenicity property, as seen in the animal models where teratomas are developed after ESC transplantation. The outcome of

ESC-derived implants may depend on the number of undifferentiated ESCs present in the graft and their ability to proliferate in an uncontrolled manner. It may also depend on a fine balance between the number of transplanted cells, their ability to escape immunological rejection and the immunocompetence of the host.

Thus, generation of **b**-cells from ESCs will require a multi-step protocol (Figure 2) that begins with induction of endoderm, followed by selection of endocrine progenitors and differentiation into mature **b**-cells. This outcome could be achieved using growth factors and extracellular matrices, selection of endocrine precursors using gene traps, forced expression of transcription factors necessary for the **b**-cell lineage and *in vivo* growth to complete the differentiation process. For an ESC-based therapy to be completely safe, the strategy developed should control the biological equilibrium between proliferative capacity, cell

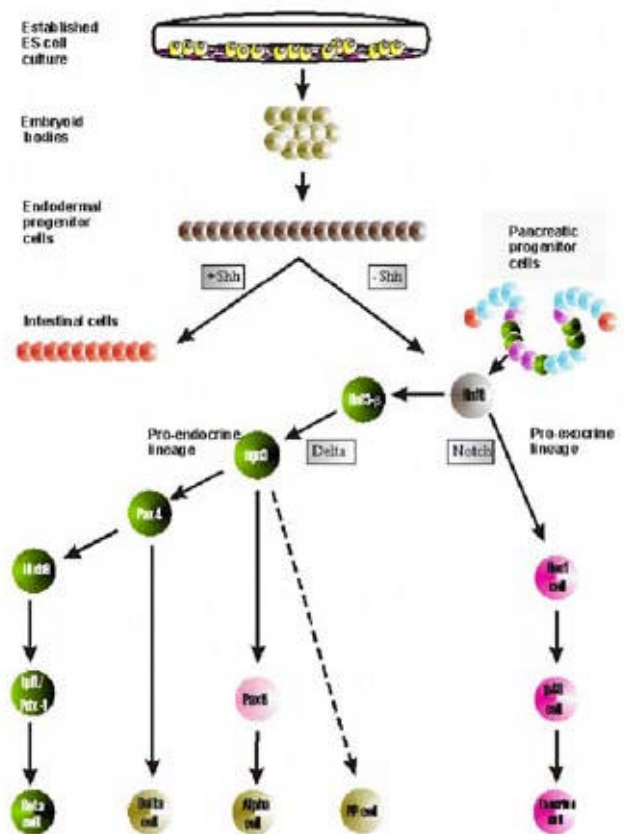


Figure 2. Schematic illustration showing how transcriptional factors regulate endocrine and exocrine differentiation. In normal pancreatic development, downregulation of Sonic hedgehog (Shh) protein is a key event in the induction of Pdx-1 expression, which in turn programs for the development of pancreatic progenitor cells. Pancreatic differentiation begins in cells expressing Hnf3b, that induces the expression of Hnf3b, which is an essential factor in endodermal cell lineage development. Neurogenin3 (Ngn3) is a 'helix-loop-helix' transcription factor that plays a central role in endocrine pancreas neogenesis from embryonic gut endoderm. Activation of signalling factors from Ngn3, such as 'delta' and 'notch' decides the differentiation of cells into endocrine and exocrine origins respectively.

death and latent teratogenesis during post-transplantation *in vivo*.

IPCs from adult stem cells

Stem cells are not only found during embryonic development, but are also present in the adult organism, where these cells are as important for maintaining of the adult organ function and tissue maintenance. Several foetal and adult tissues have been shown to contain stem cells that may have the potential to be engineered as insulin-secreting cells.

Pancreatic duct

The promising source of islet progenitor stem cells lies in the cells that line the pancreatic ducts. Some investigators believe that multipotent stem cells are intermingled with mature, differentiated duct cells, while others are of the opinion that the duct cells can undergo differentiation, or reversal to a less mature type of cell, which can then differentiate into an insulin-producing islet cell. Bonner-Weir *et al.*³⁶ reported that when ductal cells isolated from adult human pancreatic tissue were cultured, they could be induced to differentiate into clusters that contained both ductal and endocrine cells. In a month's time in culture, the cells secreted low amounts of insulin when exposed to low concentrations of glucose, and higher amounts of insulin when exposed to higher concentrations of glucose. Using immunohistochemistry and ultra structural analysis, they showed that the endocrine cells of the islet in the clusters were positive for the ductal marker cytokeratin-19, while others showed immunoreactivity for insulin and other islet hormones. Additionally, Madsen *et al.*³⁷ have shown the association of islets with pancreatic ductal epithelium during organogenesis of the pancreas.

Adult stem cells from pancreas

Another possible solution to the problem of islet supply is the use of stem cells derived from the pancreas itself. Identification and exploitation of a pancreatic stem cell or precursor cell from adult stem cells would represent a significant advancement for cell replacement therapy for Type 1 diabetes. Islet neogenesis from adult pancreatic stem cells recently received significant research attention³⁸. Many researchers focused on culturing islet **b**-cells from human adult cadavers for use in developing transplantable material. Ramiya *et al.*³⁹ reversed insulin dependence using islet generated *in vitro* from pancreatic stem cells. However, Dor *et al.*⁴⁰ have questioned the concept of adult stem cell-generated **b**-cells. They cautioned through pulse-chase experiments, that pre-existing **b**-cells were the major source of new **b**-cells during adult life

rather than pluripotent stem cells. Moreover, the efficiency of adult stem cell-derived cell types in tissue culture and their rate of differentiation into insulin-producing cells need to be improved to allow generation of significant cell numbers for transplantation.

Foetal tissue as source for stem cells

Many researchers have investigated the use of foetal tissue as a potential source of islet progenitor stem cells by grafting fresh human foetal pancreatic tissue, purified human islets and cultured islet and compared their insulin content. The insulin content was initially higher in the fresh tissue and purified islets. However with time, insulin concentration decreased in the whole tissue grafts, while it remained the same in purified islet grafts. Implantation of cultured islets resulted in an increase in insulin productivity over the course of time. These results suggested that precursor cells within the cultured islets were able to proliferate and differentiate into functioning islet tissue, but that the purified islet cells (already differentiated) could not further proliferate when grafted⁴¹.

IPCs from non-pancreatic stem cells

Both the liver and intestine share a common embryonic origin with the pancreas in the primordial gut⁴². Since the origin of liver and intestine is the same, the search for transdifferentiation of hepatocytes and intestinal cells is gaining momentum in recent years. It has been demonstrated⁴³ that certain adult cells like liver cells and enterocytes from mouse and rat respectively, do activate **b**-cell gene expression following expression of Pdx1. Yang *et al.*⁴⁴ reported the differentiation of hepatic cells into immature IPCs *in vitro* after long periods in culture. Nakajima-Nagata *et al.*⁴⁵ took this further by showing the Pdx-1 expression in insulin clusters produced from hepatocytes. Similarly, expression of neuro D, another **b**-cell transcription factor, in mouse liver cells *in vivo* resulted in reversal of hyperglycaemia. Using this concept, Zalzman *et al.*⁴⁶ reprogrammed the human liver stem/progenitor cells into IPCs, which expressed the dominant transcription factors that direct the development of endocrine pancreas. Recently this group has also succeeded in developing a stable differentiation of human fetal liver cells towards the **b**-cell phenotype by manipulating the cell culture medium with soluble factors (activin A)⁴⁷.

Another promising source of adult tissue stem cells is the bone marrow. Two recent studies have demonstrated that grafted mouse bone marrow cells can transdifferentiate into endocrine pancreas cells⁴⁸ as well as induce regeneration of endogenous islets in streptozotocin-diabetic mice⁴⁹. Banerjee *et al.*⁵⁰ demonstrated the reversal of diabetes in mice by transplanting the bone marrow stem cells. However, Lechner *et al.*⁵¹, using an animal model,

showed evidence that bone marrow stem cells do not transdifferentiate into pancreatic **b**-cells. Now investigators are interested in cord blood stem cell since it is easily accessible and also the quantity of stem cells isolated is much higher than other adult stem cells. Transplantation of these cells has been tried in rats suffering from spinal cord injury⁵². Recently, cord blood-stem cells (CB-SC) have been stimulated to differentiate into functional insulin producing cells *in vivo* and shown to have potential for elimination of hyperglycaemia after transplantation into a streptozotocin-induced diabetic mouse model⁵³. Chen *et al.*⁵⁴ isolated rat marrow mesenchymal stem cells differentiated into islet-like clusters secreting insulin and controlled blood glucose levels in diabetic rats. Recently, Hori *et al.*⁵⁵ have shown that human neural stem cells have a broad differentiation potential, and that specific *in vitro* culture conditions can divert neural stem-derived cells from neural lineages toward a fate with pancreatic-islet development and formation of clusters of glucose-responsive IPCs. Following transplantation into immunocompromised mice, these IPCs released insulin/C-peptide upon glucose challenge, remained differentiated, and did not form detectable tumours.

Cell maturation strategies

Maturation protocols represent the derivation of a final cell product for therapy from the successful transformation of precursor cells. In the context of **b**-cell replacement, the cell product has to be a post-mitotic cell expressing adequate amount of insulin and displaying regulated secretion of the hormone in response to nutrients and other factors. This requires the correct processing of insulin function of the secretory machinery and the sensing transduction pathway so that release of the hormone can be adjusted to meet extracellular demand⁵⁶. The factors that could regulate this maturation process are poorly characterized. These include characterization of growth factors and hormones and their role in the control of specific gene expression and restriction of extracellular mitogenic signals, such as lowering foetal calf serum concentration, to favour differentiation processes over proliferation. The role of specific nutrients, such as glucose, nicotinamide, antioxidants or certain amino acids, etc. needs to be studied in more detail. Some of our traditional plant-based bioactive substances may also have potential in this process. Finally, the importance of biophysical determinants in the differentiation process has to be also taken in account. Correct cell function in relation to insulin stimuli and changes in intracellular calcium levels, cAMP, and membrane potential were least studied in stem cells and need to be addressed as a part of the maturation protocol⁵⁷. Thus, cell maturation has to be focused not only on the most relevant phenotype features (i.e. insulin production and regulated secretion), but also on other impor-

tant properties, such as absence of immunogenic response, improved cell survival and precise cell function and control of cell proliferation.

The future of stem cells and diabetes

Type 1 diabetes appears to be especially difficult to cure, because the cells are destroyed when the body's own immune system attacks and destroys them. This autoimmunity must be overcome if researchers hope to use transplanted cells to replace damaged ones. Many researchers believe that at least initially, immunosuppressive therapy similar to that used in the Edmonton protocol will be beneficial. A potential advantage of stem cells, both embryonic and adult is that, in theory, they could be engineered to express the appropriate genes that would allow them to escape or reduce detection by the immune system. Others have suggested that a technology should be developed to encapsulate or embed islet cells derived from islet stem or progenitor cell in a material that would allow small molecules such as insulin to pass through freely, but would not allow interactions between the islet cells and cells of the immune system. Such encapsulated cells could secrete insulin into the blood stream, but remain inaccessible to the immune system.

The molecular mechanisms of cellular self-renewal must be understood more deeply so that we can efficiently maintain human stem cell lines in their pluripotent state. In addition, the culture methods should be improved to generate sufficient cells for clinical use. After the stem cells enter the differentiation pathway, their time clock starts ticking and they begin to lose telomerase activity and the capacity to replicate indefinitely.

While stem cells may have the potential to regenerate a variety of tissues, as indicated by a number of groundbreaking but preliminary reports, ethical issues and safety considerations seem to preclude the use of human stem cells in the clinical setting. Before any cell-based therapy to treat diabetes makes it to the clinic, many safety issues must be addressed. A major consideration is whether any precursor or stem-like cell transplantation into the body might revert to a more pluripotent state and induce the formation of tumours. These risks would seemingly be lower if fully differentiated cells are used in transplantation. Realizing the potentials of stem cell technology in modern therapeutics and biomedical research, the Indian government has taken a bold step and recommended that stem cell research and its clinical applications should be promoted in the country. It is recommended that the main source of embryonic stem cells should be from *in vitro* fertility (IVF) clinics where spare or supernumerary embryos will be available for research. However, it is cautioned that no embryo can be created for the sole purpose of obtaining stem cells. Any research on human beings, including human embryos, as subjects of medical or scienti-

fic research or experimentation done in India, shall adhere to the general principles outlined in the 'Ethical guidelines for Biomedical Research on Human Subjects' issued by the Indian Council of Medical Research (ICMR) (www.icmr.nic.in/bioethics). With regard to stem cell research in India, a separate mechanism of Review and Monitoring is proposed for Research and Therapy in the field of human stem cells, one at the National level called as National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT) and the other at the institutional level called Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). One should refer to the recent information regarding this from the 'ICMR-DBT Guidelines for Stem Cell Research and Therapy'.

Given all these challenges, there is a strong potential for developing differentiated **b**-like cells from human ESCs, although much more work is needed to realize it. However, before any kind of human islet-precursor cells can be used therapeutically, a renewable source of human stem cells must be developed. Although many progenitor cells have been identified in adult tissue, few of these cells can be cultured for multiple generations. ESCs show the greatest promise for generating cell lines that will be free of contaminants and that can self-renew. However, most researchers agree that until a therapeutically useful source of human islet cells is developed, all avenues of research should be exhaustively investigated, including both adult and embryonic sources of tissue. Thus in the field of generating new **b**-cells from stem cells, either embryonic or adult, we need to go a long way. Interestingly, each new report has been met with a mixture of excitement and skepticism. With continued efforts and rigorous assessments, hopefully the potential of generating enough new **b**-cells from stem cells will be realized in the not too distant future, thus providing a total cure of diabetes.

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