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Cellular Therapies for Type I Diabetes

David D. Lee^{*,@}, Eric Grossman^{*}, and Anita S. Chong^{*}

Werner A. Scherbaum, M.D., Ph.D. [Editor-in-Chief]

Director and Chair Department of Endocrinology, Diabetes and Rheumatology University Hospital
Düsseldorf Moorenstrasse 5 40225 Düsseldorf, Germany Phone: [+49] 211-81 18 502 Fax: [+49]
211-81 18 772 E-mail: hmr@thieme.de

^{*}Section of Transplantation, Department of Surgery, The University of Chicago, IL 60660

[@]Department of General Surgery, Rush University Medical Center, IL 60612

Abstract

Type 1 diabetes mellitus (T1DM) is a disease that results from the selective autoimmune destruction of insulin-producing β -cells. This disease process lends itself to cellular therapy because of the single cell nature of insulin production. Murine models have provided opportunities for the study of cellular therapies for the treatment of diabetes, including the investigation of islet transplantation, and also the possibility of stem cell therapies and islet regeneration. Studies in islet transplantation have included both allo- and xeno-transplantation and have allowed for the study of new approaches for the reversal of autoimmunity and achieving immune tolerance. Stem cells from hematopoietic sources such as bone marrow and fetal cord blood, as well as from the pancreas, intestine, liver and spleen promises either new sources of islets or may function as stimulators of islet regeneration. This review will summarize the various cellular interventions investigated as potential treatments of T1DM.

Introduction

Type 1 diabetes mellitus (T1DM) is characterized by the selective autoimmune destruction of insulin-producing β -cells. There is a substantial genetic component in the etiology of T1DM, and nearly 40% of familial cases of T1DM can be traced to the major histocompatibility complex on chromosome 6p21.3 [1]. Although auto-reactive CD4 T lymphocytes are instrumental in the autoimmunity of T1DM [2], anti-cytoplasmic islet cell antibodies and anti-insulin antibodies have both been implicated in the pathogenesis as well as detection of T1DM [3]. The treatment of this disease process demands the maintenance of glycemic control as well as the avoidance of life-threatening hypoglycemia [4]. When poorly controlled, T1DM can result in a variety of vascular afflictions such as cardiovascular disease, peripheral vascular disease, cerebrovascular disease, as well as kidney and other end-organ damage [5]. Hypoglycemic unawareness, a potentially devastating complication of T1DM, can lead to life-threatening comas and seizures. However, strict glycemic control can significantly reduce the incidence of diabetic complications [6,7].

Because of the challenges and limitations of exogenous insulin therapy, curative solutions have been sought, including pancreas and islet transplantation. However, both are complicated by life-long immunosuppression, organ shortages and in the case of islet transplantation,

Correspondence to: Anita S. Chong.

Requests for reprints should be addressed to: achong@surgery.bsd.uchicago.edu, University of Chicago, 5841 South Maryland Ave, SBRI J547 [MC 5026] , Chicago, IL 60637.

difficulties in islet cell preparation [8]. The disease manifestations of T1DM are due to a lack of insulin production from β -cells; and because of the single cell nature of this disease process, cellular therapy has been proposed. Exploration of various stem cell sources as well as the possibility of stimulating islet regeneration has led to a number of potential curative solutions.

The contribution of animal models has been critical in the advancement of cellular therapies for T1DM. Two mouse models have been repeatedly utilized in the investigation of T1DM. The non-obese diabetic (NOD) mouse is a model for spontaneous autoimmune-mediated diabetes, and its use has greatly advanced the understanding and pathogenesis of T1DM [9, 10]. In this model, T lymphocytes recognizing pancreatic islet antigens mediate the destruction of insulin-producing β -cells; and the reversal of diabetes requires the simultaneous control of autoimmunity as well as the restoration of islet β -cells. Streptozotocin (STZ), an agent which selectively destroys insulin-producing β -cells, has been used to create another model for β -cell-mass loss without concurrent autoimmunity [13-15]. Thus this model has been used primarily to monitor the function of transplanted β -cells or the process of regeneration. Through the use of these two models, cellular therapies that functionally restore the pancreas's insulin producing abilities have been explored in order to further advance the curative options for T1DM.

Islet transplantation

Islet transplantation was first explored in rats as early as 1972 by Paul Lacy at Washington University in St. Louis [16]. Through the use of rat models, the process of islet isolation was improved and eventually applied to mouse models shortly thereafter. With the use of STZ and syngeneic models, islet viability was confirmed. Studies in islet transplantation have now been used with syngeneic, allogeneic or xenogeneic grafts from human, rat or pig islets in both NOD and STZ induced-diabetic mice [17]. These studies have allowed for significant advances in islet isolation, survival, and immunotolerance, as well as furthering the understanding of autoimmunity in T1DM. In addition, various sites for islet transplantation have been utilized, including the portal vein [18,19], intraperitoneal, omental pouch, and kidney capsule injections [20,21]. Recent reports have revealed that islet transplantation may also play a role in facilitating islet regeneration of the endogenous pancreas [22-24], however this effect in mice is not observed when exogenous insulin is provided in the form of subcutaneous slow-release insulin tablets (unpublished data).

While the possibility of islet transplantation holds a great deal of promise [8,25]. Clinical islet transplantation performed under the Edmonton protocol, a steroid-free immunosuppressive regimen first described by Shapiro *et al.* in 2000 [26], represented a groundbreaking innovation in the field of islet transplantation. Insulin independence with tight glycemic control was achieved for up one year following islet cell transplants in all enrolled subjects. However, in a recent publication, Shapiro *et al.* reviewed the feasibility and reproducibility of the Edmonton protocol at nine international centers, and the results were less encouraging. At one-year post islet transplant, only 44% of enrolled patients achieved insulin independence with adequate glycemic control, and 28% of enrolled subjects had achieved partial graft function with clinical improvement of diabetic control. Unfortunately, loss of islet function was observed in the majority of patients who initially achieved insulin independence [27].

There are numerous challenges currently facing the clinical application of islet cell transplantation. The first is harvesting sufficient islets for transplantation. Frequently, more than one pancreas is required in order to isolate enough islet cells to achieve insulin independence in one recipient. Islet isolation techniques and limited survival of islet grafts post-transplantation are also potential limitations of islet transplantation [8]. Following islet harvest and purification, islets are currently transplanted into the portal vein, where they

ultimately lodge in the liver. The exposure of islets to portal blood triggers an instant blood-mediated inflammatory reaction (IBMIR) that causes the acute destruction of a significant portion of the transplanted islets [28,29]. In addition, in the early period of implantation, localized ischemia may result from engraftment-induced islet embolism within the liver [30]. Lastly, islet grafts have been shown to induce steatosis of surrounding hepatocytes [31], and there is evidence that intrahepatic transplanted islets display increased levels of glucagon unresponsiveness to hypoglycemia when compared to both islets transplanted intraperitoneally and to control animals [32]. In an attempt to control IBMIR and circumvent the early complications associated with intrahepatic transplantation, low-molecular weight dextran sulfate, and surfacing coating with heparin have been suggested [28], and additional sites of implantation are being explored [33,34].

Perhaps the most challenging problem facing clinical islet transplantation is the inexorable loss of islet function in the first 5 years post-transplantation leading to only 10% of recipients being insulin-free [35]. This loss of function is not completely understood but believed to be caused by a combination of insufficient transplanted β -cell mass, allograft rejection, recurrent autoimmunity, incompatibility of the islet implantation site, and immunosuppressive regimens that are toxic to the islet grafts [36,37]. Although NOD mouse studies implicate recurrent autoimmunity in preventing the long-term survival of islet and pancreas allografts [38], the magnitude of this problem is less clear in humans where pancreas allografts enjoy relatively long-term survival under current immunosuppressive regimens [39]. It is possible that islet allografts are more susceptible to recurrent immunity than pancreas allografts. Immunosuppressive regimens that are non-toxic to the islet allograft have emerged as a vital component of islet cell transplantation. Indeed the success of the Edmonton protocol is attributed, at least in part, to the immunosuppressive protocol that omitted steroids, which are known to be toxic to islet β -cells [26,40]. Nonetheless, it is possible that the current immunosuppressive strategies still have negative effects on islet graft function or are unable to completely control reactivity. Thus new strategies to reverse autoimmunity and induce long-term tolerance, including those that target co-stimulatory and adhesion molecules as well as growth factors, are being explored in mouse and non-human primate models [41-48].

Stem Cell Therapies

Introduction

Islet cell therapy is limited by the supply of islets cells available for transplantation. Stem cell therapy promises a nearly limitless and reproducible supply of transplantable and functional islets. The use of the patient's own adult stem cells would allow for the discontinuation of immunosuppression to control rejection although issues of recurrent autoimmunity would have to be resolved [49]. Islets derived from allogeneic stem cells will likely elicit alloreactive immune responses that also have to be controlled. There appear to be multiple sources of stem cells capable of differentiating into β -cells located in the pancreas or in other organs, including the liver and intestine. In addition, the spleen, bone marrow and umbilical cord blood, as well as embryonic stem cells, are potential sources for undifferentiated cells and their ability to differentiate into β -cells are being explored [50].

Pancreatic stem cells

The existence of the pancreatic stem cells in adults has recently become contentious, but there is substantial data to support its existence. During embryogenesis, stem cells within the pancreatic duct epithelium give rise to both the endocrine and acinar cells of the pancreas [51]. Adult pancreatic duct cells retain the capacity for expansion and differentiation suggesting that adult pancreatic ducts may still harbor stem cells capable of differentiating into β -cells *in vivo* [52-54]. This notion is supported by the ability of ductal tissue *in vitro* to be harvested,

expanded, and cultured to form islet-like structures capable of glucose responsive insulin secretion [54]. The identification of nestin, a neural stem-cell specific marker found in some pancreatic cells, as a marker for pancreatic stem cells has improved efforts to expand beta cells *in vitro* [55,56]. Ramiya *et al.* [52] cultured and expanded nestin-positive adult pancreatic duct cells from prediabetic NOD mice. Budding cell clusters expressing both glucagon and insulin were identified from monolayer cultures of mouse pancreatic ducts and found to demonstrate glucose responsive insulin secretion. After expanding the cell culture for over three years, the cultured islet-like cells were transplanted under the kidney capsule of diabetic NOD mice yielding resolution of hyperglycemia for 55 days [52]. Ogata *et al.* [57] also derived a similar subset of islet-like insulin secreting cells from pancreatic duct cells. Pseudoislet structures were derived from cultured neonatal rat pancreatic duct cells after incubation with betacellulin, a known promoter of pancreatic β -cell growth and differentiation, and Activin A, a peptide known to be involved in the regulation of cellular differentiation and proliferation. These pseudoislets demonstrated tolbutamide sensitivity, and glucose responsive insulin secretion. Transplantation of 50 pseudoislets into the portal vein of STZ-induced diabetic nude mice (blood glucose greater than 400 mg/dl), demonstrated improved glucose levels of approximately 200 mg/dl for two weeks [57]. Lastly, Hao *et al.* [58] isolated stem cells in the adult human pancreas from the nonendocrine fraction of cells that remained after islet isolation and through selective culturing. These cells produced only modest amounts of insulin *in vitro* however, when transplanted with fetal islet-cell clusters under the kidney capsule of immunodeficient mice, a significant transformation into endocrine cells was appreciated. The transformed endocrine cells exhibited a loss of epithelial markers, such as CK19, and an upregulated expression of the endocrine hormones, insulin, glucagon and somatostatin. The insulin positive cells also expressed markers of mature β -cells such as C-peptide, phogrin, glucokinase and synaptophysin [58]. These *in vitro* and *in vivo* studies collectively support the existence of pancreatic progenitor cells residing in the adult pancreas capable of differentiating into glucose responsive insulin secreting cells that are able to maintain normoglycemia *in vivo*.

The existence of stem cells as the major source of new islets *in vivo* was recently questioned by studies of Dor *et al.* [59]. Using an approach of lineage tracing of newly forming adult β -cells, they reported that β -cell regeneration occurred primarily from the replication of existing β -cells during the normal maturation of a mouse, and after a 70% pancreatectomy. Their data concluded that terminally differentiated β -cells retain proliferative capacity, and that these adult β -cells, and not stem or progenitor cells, are primarily responsible for β -cell regeneration and β -cell turnover *in vivo* [59]. More recently, Brennand *et al.* [60] reported that all β -cells contribute equally to growth and maintenance and that no specialized subset of β -cells capable for replication existed in the adult mouse. Collectively, these observations stand in contrast to previous notions of neogenesis of β -cells from progenitor cells, located within the pancreas or from non-pancreatic compartments, as the primary source of β -cell regeneration *in vivo*. One argument could be that the normal growth in non-diabetic mice or following 70% pancreatectomy are not sufficient stimuli to illicit stem cell regenerative abilities, and that under specific conditions of β -cell destruction, stem cells may contribute more significantly to β -cell regeneration. Regardless of their role *in vivo*, if progenitor stem cells could be expanded and differentiated into β -cells *in vitro*, they could be used therapeutically in islet cell transplantation and thus remain an important area of investigation [61].

Liver stem cells

Both the liver and the ventral pancreas arise from the same cell population within the embryonic endoderm [62]. The plasticity of liver stem cells is well known [63]. Through manipulation with transcription factors, hepatic stem cells have been shown to differentiate into insulin-producing cells [64]. Yang *et al.* [65] derived islet-like clusters capable of secreting insulin *in*

vitro in a glucose sensitive manner from rat hepatic oval cells, which are considered to be hepatic stem cells. Two hundred of these cell clusters were transplanted into STZ induced diabetic NOD-*scid* mice and restored normoglycemia for 10 days. Zalzman *et al.* [66] transformed human-telomerase-immortalized fetal hepatocytes (FH-B-hTERT) transduced with pancreatic and duodenal homeobox gene-1 (Pdx1), via lentivirus, into insulin-producing cells. Pdx-1 is a transcription factor expressed in all progenitor cells that eventually become exocrine and endocrine pancreas. No pancreas tissue develops in Pdx-1-deficient mice, however the expression of Pdx-1 in hepatocytes does not result in the formation of a functional endocrine pancreas, suggesting that the Pdx-1 transcription factor is necessary but not sufficient to induce the differentiation into pancreatic tissue [64]. Nonetheless, Zalzman *et al.* [66] reported that the transplantation of these virally transfected cells were transplanted under the kidney capsule of STZ induced diabetic NOD-*scid* mice resulted in the restoration of normoglycemia for greater than 80 days. When subjected to an intraperitoneal glucose tolerance test, these mice also showed a similar rate of glucose clearance as control mice. When the transplanted grafts were removed from these mice, blood glucose immediately rose to greater than 400 mg/dl, demonstrating that normoglycemia was a direct result of the transplanted insulin-producing cells. Collectively these studies illustrate the potential of using progenitor cells derived from the liver as donors for pancreatic β -cells.

Splenic stem cells

Faustman and colleagues first proposed the idea that the spleen may harbor stem cells capable of differentiating into β -cells in 2003. Her group investigated a complex protocol for curbing the autoimmunity in the NOD mouse model [67] involving complete Freund's adjuvant (CFA), 2×10^7 splenocytes, and either insulin or encapsulated islets. CFA, an inducer of TNF- α , was hypothesized to induce apoptosis in autoreactive T cells, thus enabling the prolongation of islet graft survival. Initially, splenocytes were postulated to lessen autoimmunity by providing a continual source of antigenic stimulation, which allowed for the stable reselection of tolerogenic memory T cells. Later work by Faustman *et al.* suggested that these splenocytes not only contribute to the reversal of autoimmunity, but may directly contribute to islet regeneration by differentiating into β -cells [68],[71]. Spontaneously diabetic female NOD mice were treated with a modified protocol, which included CFA, islet transplantation under the kidney capsule, and either irradiated or non-irradiated male splenocytes. After 120 days, the islet graft was removed and recovery of the endogenous pancreas via islet regeneration was demonstrated. By fluorescence *in situ* hybridization (FISH) splenocytes from the male donors within the newly regenerated islets were identified. In contrast, the NOD mice that received irradiated splenocytes were able to maintain normoglycemia due the transplanted islets, however there was markedly slower islet regeneration, further implicating the direct role of live splenocytes in islet regeneration.

Due to the potential clinical implications of splenic stem cells, three independent groups sought to reproduce these data, with mixed results. Suri *et al.* [69], Nishio *et al.* [70], and Chong *et al.* [23] reported that the same experimental protocols reversed autoimmune diabetes in 58%, 73%, and 68% of NOD mice, respectively. In the mice that showed recovery from the autoimmune attack, 7.5%, 15%, and 27% respectively showed evidence of endogenous islet regeneration after graft removal. However, all of these groups failed to show evidence of splenocytes directly contributing to the endogenous regeneration, raising questions regarding the spleen as a critical source of stem cells for β -cells.

To further investigate the role of the spleen, we utilized the STZ-induced diabetes model with C57B/6 mice to develop a model for islet regeneration [24]. Once diabetes was induced, a splenectomy was performed at the time of islet transplant. In a subset of mice, after splenectomy, splenocytes were given intravenously. Transgenic mice with the green

fluorescent protein reporter system under the mouse insulin promoter (MIP-GFP) were used as splenocyte donors to determine if the splenocytes from the MIP-GFP were the progenitors of the insulin-producing β -cells. Although, our data showed that the spleen played a role in facilitating β -cell regeneration, splenocytes did not play a direct role by differentiating into the β -cells. How the spleen influences islet cell regeneration is currently under investigation.

Gene Therapy of Intestinal cells

Within the gut lining are several cell subtypes capable of hormone production. K cells, a subset of enteroendocrine cells, were initially investigated for their ability to produce glucose-dependent insulintropic polypeptides, which works to potentiate insulin release postprandially [72]. Recently, Han *et al.* [73] genetically engineered a cell line [Gi-INS cells] from K cells in a mouse intestinal endocrine tumor cell line (STC-1 cells). These cells were altered to produce insulin in a glucose sensitive manner and then transplanted into STZ-induced diabetic NOD-*scid* mice. These transfected Gi-INS cells were able to reverse STZ-induced diabetes within 20 days, when transplanted under the kidney capsule. The change in blood glucose was gradual and dependent on insulin production by the transfected Gi-INS cells, as the removal of these transplanted cells by nephrectomy resulted in blood glucose returning immediately to diabetic levels. These studies raise the possibility of genetically engineering non β -cell lines into cells capable of expressing insulin in response to glucose stimulation and acting as surrogate β -cells in the treatment of diabetes.

Hematopoietic stem cells

There is much excitement regarding the potential of bone marrow derived stem cells because of their plasticity and ease of procurement. Indeed, there are numerous reports suggesting that the bone marrow harbors cells capable of differentiation into β -cells [74-77]. In 2003, Tang *et al.* derived insulin-producing cells from bone marrow of BALB/c mice and transplanted these cells under the kidney capsule of STZ-induced diabetic mice with successful correction of hyperglycemia [78]. In the same year, Ianus *et al.* transfused via the tail vein, bone marrow cells marked with enhanced green fluorescent protein (EGFP) into irradiated NOD-*scid* mice and demonstrated that these cells were capable of differentiating into insulin-producing cells with features similar to β -cells [74]. These exciting findings, however, have not been corroborated in various *in vivo* diabetes/ β -cell injury mouse models in which bone marrow transfusion alone yielded minimal to no recovery of endogenous insulin [79-84]. In one such study, Mathews *et al.* [80] reported that bone-marrow derived cells were not capable of differentiating into β -cells following STZ induced β -cell injury. Endothelial progenitor cells (EPCs), a bone marrow-derived endothelial cell, was utilized from a ubiquitously expressing EGFP transgenic mouse. Using the EGFP as a reporter, they demonstrated that bone marrow derived cells localized to the pancreatic ducts and periislet locations, however no direct contribution of EPCs among the β -cells was observed. More importantly, these mice required exogenous insulin during the entire year of the study and showed no signs of β -cell regeneration.

Promoting regeneration rather than direct differentiation of the bone-marrow cells has been proposed other investigators who showed that intravenous transplantation of bone-marrow derived stem cells can modestly improve the hyperglycemia caused by STZ induced β -cell injury. Hess *et al.* [82] noted a reduction of blood glucose within 15 days of GFP-positive bone marrow infusion. Although insulin-positive bone marrow-derived cells were present within the pancreas after recovery, these cells were absent during the onset of hyperglycemic reduction, did not express Pdx-1, and were unlikely to be responsible for the reduction in blood glucose. In a similar model, Hasegawa *et al.* [83] did not demonstrate GFP-positive bone marrow cells in regenerated islets, however, islet regeneration was dependent upon bone-marrow cells capable of division and mobilization out of the bone marrow. Lee *et al.* [84]

showed that human bone marrow stem cells (hMSCs) infused into STZ-induced diabetic NOD-*scid* mice helped lower blood glucose and increase β -cell mass, however there was no significant hMSC differentiation into β -cells. Collectively these series of reports suggest that bone marrow cells can indirectly facilitate regeneration of β -cells, although the precise mechanisms by which this is achieved has not been elucidated.

In contrast to the indirect role of bone marrow cells in facilitating regeneration, Kang *et al.* [79], suggested that bone marrow cells play an important immunological role in diabetes prevention. Using the NOD mouse model, intraperitoneal islet transplantation, along with bone marrow transfusion was performed in order to prevent and reverse autoimmune diabetes. In contrast to the controls which received the intraperitoneal islet transplants alone, NOD mice that received bone marrow cells became normoglycemic for greater than 2 months. These observations confirmed studies by Zorina *et al.* [85], who generated bone marrow chimeric mice by irradiating diabetic NOD mice and then providing T-cell depleted MHC-mismatched bone marrow cells. An islet transplant was performed under the kidney capsule and subsequently removed 17-26 weeks after transplantation revealing restoration of endogenous pancreas insulin production. Bone marrow transfusion was concluded to have cured the mice of their autoimmune diabetes and allowed for the endogenous β -cell mass to recover. Steptoe *et al.* [86] similarly demonstrated that autoimmunity could be cured by performing a syngeneic bone-marrow transplant of engineered hematopoietic stem cells. Transgenic hematopoietic stem cells that express proinsulin on antigen presenting cells were transfused into irradiated prediabetic NOD mice, which subsequently prevented autoimmune diabetes.

In summary, the published reports point to three potential roles of bone marrow cells in the treatment of diabetes, namely as a source of stem cells for β -cells, as a facilitator of β -cell regeneration and as an immune modulator. Further experimentation is required to determine whether these activities can be replicated and harnessed to cure T1DM in humans.

Umbilical cord blood-derived stem cells

Human umbilical cord blood is a readily available source of stem cells with the potential for differentiation into a wide range of cell fates [87,88]. Similar to bone marrow derived stem cells, the utility of these cord stem cells in diabetic mice is still not completely understood. Markers for pancreatic progenitors can be identified on human umbilical cord blood cells [89]. *In vitro* cultures of human umbilical cord blood has also yielded islet-like structures capable of insulin and C-peptide production [90], however the *in vivo* behavior of these cells remains unknown. Ende *et al.* [91,92] reported in two separate studies that infusions of human umbilical cord blood improved hyperglycemia and diabetic nephropathy in obesity induced diabetic mice. In addition, NOD mice were also protected from developing insulinitis and diabetes. Yoshida *et al.* [93] demonstrated that human cord blood-derived cells could be transfused into 2 day old NOD-*scid* mice and that they eventually differentiated into islet cells. Using FISH and reverse transcription-polymerase chain reaction, human derived cells within the adult pancreas of the NOD-*scid* mice was confirmed. These studies suggest great promise for the utility of human umbilical cord blood stem cells, however, the mechanism for their effect requires further elucidation.

Embryonic stem cells

Another promising source of new β -cells is embryonic stem (ES) cells [94-96]. Its potential use is currently prevented by the difficulty in generating functional insulin-secreting cells from these totipotent stem cells. In 2000, Soria *et al.* [97] derived insulin-secreting cells from mouse ES cells capable of reversing hyperglycemia in STZ-induced diabetic mice. A cell trapping method driven by antibiotic resistance to enrich for insulin-secreting cells was utilized, and 1×10^6 of these cells were injected into the spleens of diabetic mice. Restoration of

normoglycemia was noted within 1 week following infusion and was maintained in 60% of the diabetic mice for greater than 16 weeks. Lumelsky *et al.* [98] generated islet-like clusters from mouse ES cells through a five-step process of enriching culture conditions. These clusters were glucose sensitive and behaved very similarly to β -cells *in vitro*. When transplanted subcutaneously in the shoulder of STZ-induced diabetic mice, these cells experienced vascularization and maintained their islet-like clustering. Although the amount of insulin produced by these clusters was not sufficient to correct hyperglycemia, the implanted cells did impart a survival and health benefit to its recipient. Schroeder *et al.* [99] generated C-peptide/insulin-positive islet-like clusters that release insulin upon glucose stimulation through a three step culture process; these clusters were also able to induce the normalization of glycemia in STZ-diabetic mice.

Despite reports that ES cells can differentiate into cells capable of insulin expression, there is considerable controversy as to whether the data are sufficiently robust to infer successful β -cell differentiation. Notably, it has been demonstrated that many of the insulin-positive cells in ES cell cultures may be the product of insulin uptake from the culture medium, and not from endogenous synthesis [101,102]. In addition, Milne *et al.* [100] reported that insulin production by cells differentiated from ES were fated to become the extra-embryonic endoderm, and were not from cells that had correctly differentiated into endodermal-derived β -cells. These data suggest that the means for generating β -cells from ES cells is still early in development. Indeed, many groups are continuing to optimize the *in vitro* conditions that induce the differentiation of ES cells toward a more islet-like structures that are capable of releasing insulin in a glucose- and secretagogue-sensitive manner. It is important to note that, even with success in differentiating ES cells in β -cells, an important caveat of ES transplantation is the oncogenic potential of the undifferentiated ES cells, and risks of malignant transformation must be carefully considered prior to its clinical use [103]. Finally the issue of the immunogenicity of islets differentiated from allogeneic ES cells remains unresolved [104,105].

Conclusions

Cellular therapies in diabetes hold considerable promise. The field of islet transplantation has evolved rapidly since its birth over thirty years ago, and culminating in its successful use to treat T1DM clinically. Many controversies and challenges remain, especially the means to generate unlimited supplies of β -cells, the ability to regenerate β -cells, the existence of islet β -cell stem cell precursors in adults, achieving long-term efficacy of islet transplantation, and the ability to generate islet β -cells from ES cells. Successes in the generation of β -cells from stem cells, improvements in islet transplantation and the ability to reverse autoimmunity and prevent immune reactivity directed against the transplanted islets are likely to shape the future of cell-based diabetic therapy.

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