Pancreatic Stem Cell Transplants In The Treatment of Diabetes Mellitus Type 1

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Type 1 diabetes Mellitus (T1DM) is a chronic autoimmune disease in which a destruction of the β-cell of the pancreas results in the loss of endogenous insulin production. Already one of the most prevalent and chronic childhood diseases, T1DM in children and young adults is projected to continue to increase over the next several decades. Although advances in technology have facilitated glucose monitoring and insulin delivery, no curative therapy is currently available for the young patients. Moreover, patients continue to experience long-term complications such as blindness and kidney failure, even with the most intensive insulin regimens. Consequently, medical costs of this disease are substantial, considering that 1.6 million patients with T1DM were identified in 2015 and considering the estimated lifetime medical cost and cost due to lost income average respectively $133.7 and $289 billion. There are diseases, similar to T1DM, in which patient cells or tissues, once destroyed, can only be adequately replaced only by tissue or organ transplants. This is where stem cells may be able to regenerate tissue and even cure diseases for which there is no adequate therapy. Can pancreatic stem cell transplant be used as a cure for T1DM, an autoimmune disease, and how does it stand against other cell transplants and other treatment modalities in the US?

Replacement of the damaged β-cells has shown considerable potential in the treatment of T1DM, but lack of adequate donors may also represent a barrier. The literature suggests that embryonic and adult stem cells can offer promising alternatives in long-term treatment of diabetes. However, any successful strategy should address the need for β-cell replacement and the control of the autoimmune response to cells in need of insulin.

T1DM predominantly originates when insulin-producing beta (β)-cells in the pancreas are destroyed due to an autoimmune response initiated by the auto-reactive T-helper 1 (Th1)

cells. Interestingly, the antigens responsible for this immune response is “glutamic acid decarboxylase”, normally present in the β-cells; however, their destructive capabilities are restrained by the regulatory mechanisms of the body. When a person has certain genetic factors (e.g. the absence of protective histocompatibility locus antigen) or other environmental factors, they may be predisposed to injure the β-cells. Furthermore, the odds of presenting with T1DM may diminish with treatment with immune-suppressive regimens that specifically target T-cells. As a result, Th1 cells dominate the protective regulatory T-cells. In turn, Th1 cells secrete cytokines (interleukin 2) and interferon gamma, which initiate inflammation of the islet cells resulting in T1DM.

Current T1DM treatment options include thus far:

1. ***Pancreas–kidney transplantation***

Throughout the years, this attractive alternative to treating certain diabetic patients (uremic) has been discussed. In this sequential transplant, most commonly a living donor’s kidney followed by a cadaver pancreas is transplanted. Transplantation improves quality of life by not only removing the need for dialysis and insulin therapy, but also by reducing the complications of T1DM.

2. ***Human islet cells transplantation***

Islet cells transplantation has its origin over a century ago. The concept arose in 1894, when minced sheep’s pancreatic xenografts and other extracts in glycerin were used in an attempt for oral and subcutaneous therapy. Even though this attempt failed, islet allografts in humans commenced in 1980. The potential of islet transplantation in humans with T1DM was recognized when in 2000 Shapiro et al. reported a complete success in their 7-patient study, and later

presented a study from multiple islet transplantation centers throughout the world.

Their primary endpoint was defined as insulin independence with adequate glycemic control one year after the final transplantation. It has become evident that in order to achieve successful islet tissues transplantation, it was essential to have adequate amounts of islet cells. Unfavorable outcomes and complications from islet cells transplantation can arise, including the inability to achieve access to the site, portal venous hypertension, allo-rejection, ongoing immune damage, intra-abdominal-bleeding, portal venous thrombosis, and

abdominal pain. Unfortunately, insulin independence is usually not sustainable and therefore further studies are needed to improve the quality and productivity of transplanted islet cells.

3. *Xenografts/Xenotransplantation*

In Xenotransplantation, similarities between some animal species are used to treat various diseases. In the case of T1DM, islet cells of species such as pig and bovine are used to generate insulin production in human pancreatic β-cells. The most studied animal so far is the pig, due to its pancreatic β-cells 'potential to accept genetic modification so that immune-rejection is curbed. In order to reduce or eliminate immune-rejection, pigs 'islet cells have been encapsulated via bio-acceptable material. However, due to this encapsulation, problems such as inability to produce insulin, and most importantly, fears of the porcine endogenous retro virus (PERV) activating itself in other animals, raised questions and fears that led to a halt of this process. In addition, it was also noticed that a risk of PERV infection still exists in human cells owing to the short-term contact of primary porcine liver cell supernatants with primary human cells during the transplantation. Different suggestions have been made to avoid the rejection of this graft, and the most important was when transgenic pigs were developed that lacked the alpha-1,3-galactosyl transferase enzyme.

Another approach was the use of an immune-suppressant that specifically targets T-cells. In one study of 140 pigs (64 juvenile and 76 adult), xenotransplantation results showed an increased amount of the mean collagen in juveniles compared to adults. However, no significant correlation was found between the collagen content between the juvenile and adult pigs and the percent of islet encapsulation. Further studies are needed to explore this avenue to minimize immune-rejections and the risk of PERV.

4. *Stem cells*

In 2001 it was found that diabetes might be the first disease for which stem cell therapy is an option, when insulin-producing embryonic stem cells of R1-mice were transplanted into streptozotocin-induced diabetic animals and normal blood glucose levels were achieved. Minor complications in R1-mice aside, this event once again renewed the quest to find more practical and functional means to create human islets to treat diabetes.

4.1 Embryonic stem cells

Embryonic stem cells (ESCs) have the potential of replicating into countless numbers of any types of cells in the body. This includes all three germ layers cells both in vivo and in vitro. These cells are pathogen-free and can be engineered for different purposes as long as the right environment and precursors are provided for their growth. Essentially research on ESCs began in the late 19th and early 20th centuries, and primarily concentrated on avian and mammalian embryos. In 1995, isolation of the first human ESCs (hESCs) was achieved by Thomson et al. Rhesus monkeys ’ESCs were developed and used to prepare the medium for the culture, which allows the growth of hESCs. This historical breakthrough was given a further boost when, in 1998, Shamblott et al. were able to grow the first pluripotent hESCs that possessed many characteristics of the current hESCs. In addition, in the early stages of human development, the blastocyst contains the inner cell mass from which hESCs are derived.

4.2 Adult stem cells

Adult stem cells compared to ESCs have a lower capability of proliferation, differentiation, and regeneration of tissues. β-Cell neogenesis in adults has been reported in animal models of experimentally induced pancreatic damage, suggesting the presence of adult stem/progenitor cells. Adult stem cells do not have the ethical difficulties that are associated with hESCs.

4.3 Umbilical cord blood cells

Umbilical cord blood contains stem cells and is readily available in large amounts and therefore is a probable source for the generation of insulin-producing cells. Graft rejection is seen as a low risk. Human cord blood cells can be induced to express endocrine markers such as Isl-1, Pdx-1, Pax4, and Ngn-3. Transplantation of human cord blood cells to diabetic mice lowers blood glucose levels. Another study showed that stem cells isolated from human cord blood, which express stage-specific embryonic antigen-4 and the stem cell marker octamer-4, differentiate into insulin- and C peptide-positive cells.

4.4 Liver and intestinal cells

The intestinal and liver epitheliums are derived from gut endoderm growth like the pancreas; therefore, the production of islets from both intestinal cells and liver has been tried as a potential source of insulin-producing cells. Hepatic oval stem cells can be differentiated into

insulin-producing islet-like cells in vitro in the presence of high glucose. These cells express pancreatic β-cell markers such as Pdx-1, Pax4, Pax6, Nkx2.1, and Nkx6.1, and reverse diabetes when transplanted into diabetic mice . Another study demonstrated that fetal human liver progenitor cells differentiate into insulin-producing cells when engineered to express Pdx-1 and transplantation of these cells reverses hyperglycemia in diabetic mice . Similarly, differentiation of adult hepatic progenitor cells by overexpression of Pdx-1 results in insulin secretion in response to glucose. Based on these results, it is proposed that the controlled differentiation of liver or intestinal cells into insulin-producing cells is possible, and this avenue may also provide an alternative source of β-cells.

4.5 Pancreatic stem cells

Many studies show that adult pancreatic ducts are the main site of β-cell progenitors. The islets cells turn over slowly and new, small islets are continuously generated by differentiation of ductal progenitor cells. Islet cells are seen close to ducts in T1DM and partially pancreatectomized rodents. It was found that islet-like aggregates are generated from mouse pancreatic ducts and ductal tissue-enriched human pancreatic islets, and these aggregates release insulin after glucose stimulation and express islet proteins. It was observed that multi-potent precursor cells clonally identified from adult pancreatic islets and ductal cell populations can differentiate into cells with β-cell function. It is because of ectopic expression of Ngn-3, a critical factor for the development of the endocrine pancreas in humans, that pancreatic ductal cells result can change into insulin-expressing cells. In addition, treatment of human islets containing both ductal and acinar cells with a combination of epidermal growth factor and gastrin induces neogenesis of islet β-cells from the ducts and increases the functional β-cell mass. Based on these results, it is possible that these cells will become a source for new islets.

4.6 Human induced pluripotent stem cells and differentiation into insulin-producing cells

This new option has been considered as a promising alternative since first reported in 2007. The advantage of human induced pluripotent stem (iPS) cells is based on their independence from human oocytes, which always presents ethical issues owing to the destruction of blastocytes to establish hESC lines. Interestingly, iPS cells can be derived from somatic cells, which presents the possibility of generating patient-specific pluripotent cells. It might be

possible to generate patient-specific iPS cells from an individual's somatic cells and then differentiate them into functional pancreatic islet cells. This process would avoid problems with immunogenicity. Several studies have reported the differentiation of insulin-producing cells from iPS cells. Tateishi et al. reported the generation of hESC-like iPS cells from human skin cells by retroviral expression of the transcription factors OCT4, SOX2, c-MYC, and KLF4. It has also been demonstrated that human iPS cells can be differentiated into insulin-producing cells. With a chemically defined induction system it has been shown that iPS cells express key markers similar to hESCs generated by a stepwise differentiation process. Finally, differentiated human iPS cells co-expressed PDX1 and C-peptide, suggesting that these cells possess the characteristics of mature pancreatic β-cells. iPS technology has shown the potential of human iPS cell-derived production of insulin-producing cells, and opens new avenues for future diabetes cell therapy. However, before induced islet cells can be successfully used for diabetes cell therapy, further research is required, especially to examine the effects of iPS cells-derived islet cell transplantation into human pancreas.

4.7 Transplantation of adipose tissues

It is well known that visceral adipose tissue can induce harmful metabolic effects. Both subcutaneous white and brown adipose tissues have the potential to improve glucose homeostasis and increase energy consumption. The ability of adipose-derived stem cells within adipose tissue to differentiate into multiple lineages might be of value for the repair or replacement of various detriment cell types. Adipose tissue transplantation has been primarily used as a tool to study physiology and for human reconstructive surgery. Today, transplantation of adipose tissue is considered a possible tool to promote the beneficial metabolic effects of both subcutaneous white and brown adipose tissue as well as of adipose-derived stem cells. Vanikar et al. reported the first successful treatment of T1DM with co-transplantation of insulin-secreting adipose tissue derived mesenchymal stem cells and hematopoietic stem cell transplantation. This prospective, open-labeled clinical trial of co-transplantation in 11 diabetic human subjects showed promise in treating T1DM but further studies are required.

Development of β-cells

The pancreas is a dual organ that is home for its exocrine and endocrine subsets, with exocrine being 80% of its composition and comprised of ductal and acinar cells . The endocrine portion consists of hormone-producing cells β, γ, α, ε, and F; they synthesize and secrete insulin, somatostatin, glucagon, ghrelin, and pancreatic peptide, respectively (1,3,7). The pancreas is developed from definitive endoderm that contains common progenitor cells, which are considered to be cells that give rise to acinar, ductal, and islet cells . Furthermore, the most important transcription factors that lead to the formation of the pancreas are Pdx1 (pancreas duodenal homeobox-1) and Ptfla (pancreas specific transcription factor). Pdx1 is the homeodomain-containing transcription factor that develops in the 5th week of human embryonic development, and in the 8.5th week, development of the mouse embryo in early pancreatic progenitor cells. To determine the validity of Pdx1 being one of the most important transcription factors in the development of pancreas, experiments have resulted in pancreatic agenesis. Thus, it is known that Pdx1 is not only an efficient marker of early pancreatic progenitor cells, but also is necessary for the differentiation of pancreatic cells that end up being insulin-producing β-cells.

First more specific endocrine factor Pax4 was discovered in nestin-positive islet derived progenitor cells, and second, 3-dimensional culture conditions were developed that led to normoglycemia in diabetic mice. It is accepted that hESCs provide a major alternative in creating islet cells that are able to produce insulin and treat T1DM, and possibly T2DM. Ethical issues related to hESCs aside, the following limitations have to be overcome before stem cell therapy could become a reality. Other human pancreatic beta cell lines show some potential for utilization in functional studies. The year 2011 marked the emergence of two new beta cell lines, 1.1B4 and EndoC-βH1.

In humans, to date, the only clinically acceptable treatment for type I diabetes, other than insulin replacement, remains islet transplantation under the cover of pharmacologic immunosuppression. A very recent safety trial has begun using an anti-CD3 antibody, but the results require confirmation and further safety analysis. In 2001 it was found that diabetes might be the first disease for which stem cell therapy is an option, when insulin-producing embryonic stem cells of R1-mice were transplanted into streptozotocin-induced diabetic animals and normal blood glucose levels were achieved. Minor complications in R1-mice

aside, this event once again renewed the quest to find more practical and functional means to create human islets to treat diabetes. Ethical issues and politics aside, the use of stem cell therapy has shown great promises for the treatment and cure of T1DM and possibly Type II diabetes.

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References:

1. Agardh, E., Lundstig, A., Perfilyev, A., Volkov, P., Freiburghaus, T., Lindholm, E., . . . Ling, C. (2015). Genome-wide analysis of DNA methylation in subjects with type 1 diabetes identifies epigenetic modifications associated with proliferative diabetic retinopathy. BMC Medicine, 13, 182-182. doi:10.1186/s12916-015-0421-5
2. Alnek, K., Kisand, K., Heilman, K., Peet, A., Varik, K., & Uibo, R. (2015). Increased blood levels of growth factors, proinflammatory cytokines, and Th17 cytokines in patients with newly diagnosed type 1 diabetes. Plos One, 10(12), e0142976-e0142976. doi:10.1371/journal.pone.0142976
3. Shapiro, A. M James. "Islet Transplantation in Type 1 Diabetes: Ongoing Challenges, Refined Procedures, and Long-term Outcome." The Review Of Diabetic Studies: RDS 9.4 (2012): 385-406.
4. Newby, Brittney N., Naohiro Terada, and Clayton E. Mathews. "In Search of a Surrogate: Engineering Human Beta Cell Lines for Therapy." Trends In Endocrinology And Metabolism: TEM 25.8 (2014): 378-80.
5. Montanucci, Pia, Alessia Alunno, Giuseppe Basta, Onelia Bistoni, Teresa Pescara, Sara Caterbi, Ilaria Pennoni, Vittorio Bini, Roberto Gerli, and Riccardo Calafiore. "Restoration of T Cell Substes of Patients with Type 1 Diabetes Mellitus by Microencapsulated Human Umbilical Cord Wharton Jelly-derived Mesenchymal Stem Cells: An in Vitro Study." Clinical Immunology (Orlando, Fla.) 163 (2016): 34-41.

6. Stanekzai, Jamil, Esma R. Isenovic, and Shaker A. Mousa. "Treatment Options for Diabetes: Potential Role of Stem Cells." Diabetes Research And Clinical Practice 98.3 (2012): 361-68.
7. Pereyra-Bonnet, Federico, María L. Gimeno, Nelson R. Argumedo, Marcelo Ielpi, Johana A. Cardozo, Carla A. Giménez, Sung-Ho Hyon, Marta Balzaretti, Mónica Loresi, Patricia Fainstein-Day, León E. Litwak, and Pablo F. Argibay. "Skin Fibroblasts from Patients with Type 1 Diabetes (T1D) Can Be Chemically Transdifferentiated into Insulin-expressing Clusters: A Transgene-free Approach." Plos One 9.6 (2014): E100369.
8. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145–1147.
9. Street, Cale N., Simonetta Sipione, Lisa Helms, Tanya Binette, Ray V. Rajotte, R. Chris Bleackley, and Gregory S. Korbutt. "Stem Cell-based Approaches to Solving the Problem of Tissue Supply for Islet Transplantation in Type 1 Diabetes." The International Journal Of Biochemistry & Cell Biology 36.4 (2004): 667-83.
10. Tateishi K., He J., Taranova O., Liang G., D'Alessio A. C., Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. The Journal of Biological Chemistry. 2008;283(46):31601–31607. doi: 10.1074/jbc.m806597200
11. Vanikar AV, Dave SD, Thakkar UG, Trivedi HL. Cotransplantation of adipose tissue-derived insulin-secreting mesenchymal stem cells and hematopoietic stem cells: a novel therapy for insulin-dependent diabetes mellitus. Stem Cells Int. 2010 Dec 20;2010:582382. doi: 10.4061/2010/582382. PMID: 21197448; PMCID: PMC3010655.