REVIEW ARTICLE

Status of stem cell therapy in type-1 diabetics

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For years, researchers have painstakingly studied type-1 diabetes, a complicated disease caused by the destruction of insulin-producing islet cells of the pancreas. Despite significant attempts to understand the contributing disease mechanisms for diabetes, there is still no solution for a cure. Investigators are continuously working on strategies for pancreatic transplantation and islet cell replacement. Adult stem cells that appear to be precursors to islet cells and embryonic stem cells that produce insulin seem to have caught the attention of researchers worldwide. However, the universal consensus is that until a therapeutically useful source of human islet cells is developed, a persistent search for a potential surrogate replacement needs to be pursued. The other challenge that needs to be overcome is autoimmunity. This is perhaps why type-1 diabetes still continues to exact its toll on humankind.

KEY WORDS: Glutamic acid decarboxylase, Stem-cell Transplant, Type 1 Diabetes,

DOI: 10.4103/0973-3930.66502

Introduction

Banting and Best received, in the shortest time in history, the Nobel Prize in 1923 for their discovery of insulin in 1921–22. Nearing a complete century since the discovery of insulin, it is right time to expect a change in the management strategies in type 1 diabetes. Type-1 diabetes is an autoimmune disease that results in the destruction of insulin-producing β-cells of the pancreas.[1] Type-1 diabetes is not exclusively a childhood problem; the adult incidence of type-1 is noteworthy.

Despite considerable efforts, finding a curative treatment continues to remain a daunting expedition. To begin with, a thorough understanding of the fundamental complexities and a realistic approach coupled with unbiased introspection of failures is the need of the hour.

Although clinical research involving genetics, research in animal models, human trials are underway aiming at a revolutionary breakthrough, human body is much more complicated and follows totally different rules of the game. Medical science can never be certain of the future unless there are answers to why, what and how?

This review is a sincere attempt to extrapolate and analyze recent approaches with regard to immune intervention, β-cell regeneration and replacement, in an attempt to find a cure for type-1 diabetes.

Continued need for an alternative

Pancreatic islet transplantation has demonstrated that long-term insulin independence may be achieved in patients suffering from type-1 diabetes mellitus (T1DM). However, because of limited availability of islet tissue, new sources of insulin-producing cells that are responsive to glucose are required.[2] Chronic shortage of donor organ, lifelong immunosuppressive therapy and failure to achieve sustained insulin independence (limited life span of islet cells) are the other issues. The need for an essentially limitless supply of a substitute for primary human islets of Langerhans has led to a research on the suitability of stem or progenitor cells to generate insulin-producing cells for use in replacement therapies for diabetes.

Biology of stem cells

A variety of tissues harbor progenitor or stem cells and if it were possible to isolate and expand these cells *in vitro* and then differentiate them to adopt a β-cell phenotype, they would be a potential source of substitute tissue for transplantation.

Stem cells are totipotent in the early stages after

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Manuscript received: 01.03.2010; Revision accepted: 27.04.2010

fertilization as they can form any type of cell, including cells necessary for an embryo such as placenta cells.[3] Several days after conception, the stem cells become pluripotent. Such cells are versatile enough to form any kind of cell found in adults. However, they cannot become cells necessary for an embryo to develop into a human. Pluripotent cells become multipotent or adult stem cells. These cells can only develop into certain specific cell type within a tissue, organ or system.

Researchers believe that multipotent cells may remain undivided until they are required to create necessary new cells, thereby demonstrating a quality called plasticity. Comprehension of fundamental aspects of stem cell biology and behavior remains crucial to define the rationale for their therapeutic application.

Stem cell therapy – Progress and promise

The use of stem cells to treat T1DM has been proposed for many years. One way is to downregulate the immune system subsequently preserving β cells and another way is to offer personalized therapy with differentiation of stem cells into functional insulin secreting β cells.

Although seemingly idealistic, many aspects of these scenarios are already possible.

Etiological perspective – targeting autoimmunity

Scientific understanding of the cause of T1DM began with the discovery of inflammatory insulitis by von Meyenburg.[4] Subsequently, Gepts and others recognized that insulitis, an inflammatory lymphocyte infiltrate, was specifically associated with the islets of children with diabetes.[4]

The work of Doniach, Bottazzo, and Drexhage then revealed that people with T1DM circulate antibodies, often present long before the disease onset, which target constituents of the β cell and even insulin itself.[4]

Since the identification of the autoimmune etiology of T1DM in the late 1970s, immunosuppressive agents began to be used. In 1981, Eliot and colleagues treated newly diagnosed children with prednisone with the aim of stopping pancreatic β-cell destruction by the autoimmune process.[5] The chronic toxicity of immunosuppression and the loss of the metabolic benefits after the withdrawal of these agents limited their use. Since 2000, reports on acute immunomodulating therapies that are theoretically aimed at providing longer immunologic effect have been published. Such studies have shown several degrees of β-cell mass preservation; however, none resulted in insulin independence.

Cell therapy for T1DM was conducted using autologous umbilical cord blood cells at the University of Florida. Such cells are able to secrete cytokines that promote a decrease in the population of cytotoxic T lymphocytes and increase the population of regulatory T cells but are unable to regenerate. The study conducted included a group of 21 patients (average age of 5 years) with a history of diabetes (average of 9 months), paired with a control group of patients receiving usual insulin therapy. No significant differences in the C-peptide levels and in the insulin doses were observed after a follow-up of 1 year.[6]

Recently, Food and Drug Administration (FDA) has approved the use of rituximab, an anti-CD20 monoclonal antibody. A randomized, double-blind study evaluating selective depletion of B lymphocytes with a four-dose course of rituximab resulted in partially preserved β-cell function over a period of 1 year in patients with T1DM.[7] The finding that B lymphocytes contribute to the pathogenesis of T1DM may open a new pathway for exploration of novel treatments.

Success story – Role of autologous hematopoietic stem cell transplantation

By the end of 2003, cell therapy for T1DM started to be administered to humans, and the world's first study was performed by a research team headed by Júlio C Voltarelli at the Divisions of Immunology and Endocrinology of the Hospital das Clínicas, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil.[8] The basic inclusion criteria for this study were: age between 12 and 35 years and a diagnosis of T1DM less than 6 weeks prior to inclusion, confirmed by positive serum levels of anti- GAD (Glutamic Acid Decarboxylase). antibodies.

For autologous hematopoietic stem cell transplantation (AHST), the patient's hematopoietic stem cells are mobilized from bone marrow to the blood with the use of low dose cyclophosphamide and granulocyte colony-stimulating factor. Then, the harvested peripheral blood hematopoietic Stem C ell are reinjected intravenously after conditioning with high dose chemotherapy [cyclophosphamide (200 mg/kg)] and rabbit antithymocyte globulin (4.5 mg/kg). These reinfused cells do not have immunologic memory to regenerate a new immune system that will not attack

pancreatic β cells. This is a lymphoablative scheme, as most of the patient's lymphocyte clones, both autoreactive and non-autoreactive, are destroyed, and immunologic system is recovered.

Up to December 2008, 20 out of the 23 patients included remained insulin free for some period. Of them, 12 have been continuously insulin-free since treatment, 8 became transiently insulin-free and 3 maintained daily insulin doses. In the continuously insulin-free group, many stopped daily insulin injections soon after stem cell infusion [mean period free from insulin is 31 months (14–52 months)]. There was a significant reduction in hemoglobin A1c (HbA1c) compared with pretreatment values, with all measurements below 7% during follow-up.

In the transiently insulin-free group, there was an increase in C-peptide levels from 0.6 nmol/L pretreatment to 1.7 nmol/L, 4 years after treatment ($P < 0.05$) which is considered most prolonged period of C-peptide increase (up to 4 years) in interventional trials aiming at β-cell preservation.

Only three patients did not experience any period free from insulin. One presented with diabetic ketoacidosis at diagnosis and received glucocorticoids to prevent rabbit anti-thymocyte globulin reactions, one developed diabetic ketoacidosis before enrollment and one had inadvertently received steroids (300 mg hydrocortisone) along with stem cell infusion. In spite of the progressive increase in daily insulin doses (>0.8 IU/kg/day) none achieved HbA1c levels less than 7%.

Assumptions tested – Affirmations derived

Firstly, transplant is a misnomer as in actual sense it involves 'immunologic resetting' involving stem cell rescue.

Further, it is important to emphasize that hematopoietic stem cells are unable to differentiate into β cells and although no β cells are regenerated, those cells not yet destroyed are preserved, that is to say, neither there is transdifferentiation of hematopoietic stem cell into β cell nor does directed differentiation into β cell take place.

Secondly, it is also imperative to note that only patients with considerable β-cell reserve would benefit from strategies of immunomodulation, that is, by blocking the immunologic aggression of T cells against β cells and allowing an endogenous regeneration of β cells. Therefore, only newly diagnosed patients, that is, patients who still have residual β-cell mass to be preserved, need to be considered for ASHT.

Following the procedure, the secretion of endogenous insulin is maintained or increased, long-term increase in C-peptide levels is noted, metabolic control is improved with an obvious reduced risk of chronic complications without suspension of insulin therapy. Finally, with a cure unachieved, patients should continue to maintain routine monitoring of glycemia and carbohydrate counting. Treatment-related complications range from transplant-related morbidity and mortality, autoimmune disease, secondary leukemias and short-lived remission. Compliance with these guidelines is a tough challenge. Disappointingly true though at this juncture is that a cure continues to remain elusive.

However, in August 2008, in Chicago, an international multicentric randomized research protocol project was started to test the effect of 'immunologic resetting' more widely. This study is being evaluated by the US FDA and by American research ethics organizations.[9]

Bridging the crucial step – Seeking a functional surrogate

Implantation of surrogate β cells, or 'cell-replacement therapy,' encompasses all methods that involve the creation or expansion of insulin-producing cells *in vitro* followed by their implantation in the patient. Stem cell therapy here implies the replacement of diseased or lost cells from progeny of pluripotent or multipotent cells. Both embryonic stem cells and adult stem cells have been used to generate surrogate β cells. Perhaps the most important issue is the choice of the appropriate starting material.[10]

Building the ideal surrogate β cell – Challenges ahead

For use in cell replacement therapy, stem cells must be reproducibly made to:

- proliferate extensively and generate sufficient quantities of tissue;
- differentiate into the desired cell type(s);
- survive in the recipient after transplant;
- integrate into the surrounding tissue after transplant;
- function appropriately for the duration of the recipient's life and
- avoid harming the recipient in any way.

Approximately 2–4 × 10^9 cells will have to be derived from a proliferative precursor population that can be expanded *in vitro* before differentiation into mature phenotype. The replacement cells must have the ability to synthesize,

store and release insulin in response to changes in ambient glycemia.[11] The proliferative capacity of these cells must be tightly controlled to avoid the development of hyperinsulinemic hypoglycemia as the β cells expand *in vivo*. Finally, to overcome transplant rejection, they should possess similar functional phenotype but remain developmentally and immunologically distinct. Complex architecture poses a challenge as adult pancreatic islets have cells preferentially located in the islet core and other types of cells in the periphery. Single β cells are less responsive than electrically coupled cells; therefore, a functional aggregate of β cells is essential.

The β cell is a remarkably sophisticated and highly differentiated cell and should be faithfully mimicked by surrogate cells. If the cell cannot accomplish this, we will be left with faint hope. As fluctuations in secretory demand are likely to vary both on a day-to-day basis and in the long term, the hurdles presented to the engineered β cell and to the scientists are enormous.

Experimenting with adult stem cells

Bone marrow stem cells have been studied most extensively because a variety of cell surface and genetic markers have helped delineate various stages of their differentiation during hematopoiesis.

But there are several drawbacks that make adult stem cells less attractive than embryonic stem cells as a source. The cells are difficult to isolate from adult tissues, are few in number, and it is difficult to keep them proliferating in culture. To date, it appears that cultured adult stem cells give rise to only a limited number of cell types. Finally, they are adult cells and have been exposed to a lifetime of environmental toxins and have also accumulated a lifetime of genetic mutations.[12] Despite these apparent drawbacks, research on adult stem cells should be pursued vigorously because these problems may be overcome with new techniques and insights.

Exaggerated hype – Embryonic stem cells' experience

In 1998, capitalizing on nearly 20 years of experience with mouse embryonic stem cells, scientists at the University of Wisconsin isolated stem cells from the inner cell mass of human blastocysts and grew them in tissue culture for prolonged periods of time.[13] This study led to an explosion of research on human embryonic stem cells (hESCs). Many approaches were used to obtain insulin-producing cells from embryonic stem cells: 1) cell trapping with antibiotic resistance driven by the insulin promoter, or more recently the Nkx6.1 promoter, to select cells at an early stage; 2) expression of key transcription factors such as Pax 4 and pancreatic duodenal homeobox factor-1 (PDX-1); and 3) selection by manipulating the culture conditions. Controversy regarding success of these methods remained unresolved.

Researchers from the University of Calgary found that the insulin-producing cells derived from ESCs are not the 'β cells' needed to reverse diabetes. While the cells produced some insulin, they did not do so in response to changes in glucose levels; when placed in mice they did not reverse diabetes but formed (tumors) teratomas.[14]

Hopes ran high when researchers claimed to have produced cellular structures similar to pancreatic islets from ESCs in 2001, and when injected into diabetic mice, the insulin-producing cells undergo rapid vascularization and maintain a clustered, islet-like organization.[15] Then, in 2003, researchers showed that the first team probably had not managed to create insulin-producing cells at all; rather, the cells had only released the absorbed pre-existing insulin from their culture medium.[16]

Several study results have suggested that the environment needed to provide the appropriate stimulatory or inhibitory extracellular factors for inducing differentiation and maturation of embryonic stem cells into insulin-producing cells is difficult to create *in vitro*.

Because of the difficulty in getting ESCs to differentiate into desired tissues, the risk of tumor formation, the genetic instability in culture, and other ethical problems, they cannot be expected to provide treatments for juvenile diabetes.In his speech on August 9, 2001, President Bush recognizing the value of research on hESCs announced that 62 hESC lines were available in labs around the world, and all subsequent federally supported research would be confined to these existing lines.[17]

A relentless pursuit

In 2006, a Chinese group from the University of Naijing started a protocol in which stem cells were infused, 50% into the peripheral vein and 50% straight into the pancreas through arterial catheterization. Of the five patients initially treated less than 3 months after diagnosis, four became insulin-independent (two only transiently) and one had a 50% decrease in insulin dose.[9]

A group of researchers from Argentina, China and the United States performed cell therapy using stem cells from the patient's own bone marrow (including a conglomerate of mesenchymal stem cells and hematopoietic stem cells), obtained in a bone marrow biopsy. The authors did not publish complete data at all.[18]

Approaches with new cell lines – Still in infancy

In January 2006, a team of South Korean researchers, headed by Prof. Kang Kyung-sun of Seoul National University, have successfully grown pancreatic β cells from umbilical cord blood stem cells of newborn babies. This achievement was published with the title "Cord Blood Stem Cell Breakthroughs: Cure for Diabetes?" by The Biochemical and Biophysical Research Communications, the US-based weekly, that documents breakthrough papers in biotechnology.

In September 2008, scientists from the University of North Carolina at Chapel Hill School of Medicine announced their success in transforming cells from human skin into cells that produce insulin.[19] The skin cells were first transformed into stem cells and then had been differentiated into insulin-secreting cells.

However, the research papers fail to detail the new cells' glucose responsiveness and the amount of insulin they are capable of producing.

In 2008, a group of researchers at the Medical School of Ribeirão Preto, University of São Paulo, Brazil, started pioneering studies in humans with T1DM using mesenchymal stem cells. So far, two patients have been included in this protocol and following proper follow-up, the results will be published.[9]

Conclusions

Stem cell therapy has provided new hope for a cure of T1DM. Recent advancements point to the great promise of research in this area, and hopefully, with further analysis and research, an unlimited source of β cells for islet transplantation will be found, moving a step closer to a cure. President Obama has optimistically expressed his support for this potential biomedical breakthrough by lifting the ban on federal funding for embryonic stem cell research on cell lines created after August 9, 2001. Last but not the least, this effort should go forward because we simply will not know the answers unless we do the research.

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Source of Support: Nil, **Conflict of Interest:** None declared