2022

A Review of T1D: Prevalence, Current Therapies, and Cellular Therapies for the Future

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Abstract

Type 1 diabetes (T1D) is a chronic autoimmune disease that is characterized by the destruction of pancreatic β-cells and therefore, creating an insulin deficiency within the body. A deficiency of insulin within the body disrupts homeostatic glucose control leading to hyperglycemia and therefore, the need for exogenous insulin. Global incidence of T1D has been increasing for several decades and if current trajectory trends continue, incidence could double in the next year. In addition, diabetes is the seventh leading cause of death in the United States. Current therapies for the treatment of T1D include insulin injections, insulin-pump therapy, pancreatic transplant, and islet cell transplantation. However, due to these therapies not being able to replace true pancreatic function, alternative therapies are being researched, particularly cellular therapies. Stem-cell therapies, and more specifically, embryonic and human-induced pluripotent stem cell therapies are being researched and are touted as the most promising therapeutic for T1D patients in the future. However, there are several limitations with cellular therapies that need to be addressed before stem-cell therapy can be a mainstay within clinical therapeutics for T1D.

Once Sentence Summary: Reviewing the prevalence of T1D, current therapies available, and future cellular therapeutics in an effort to treat and cure T1D.

Abbreviations: TID=type 1 diabetes; glycated hemoglobin=HbA1C; ESCs=embryonic stem cells; hiPSC=human induced pluripotent stem cells; ND=non-diabetic; SC=stem cell; MHC=major histocompatibility complex

Overview

T1D is a chronic condition in which an individual produces little to no insulin. It is known to be an auto-immune disease, where the immune system attacks insulin-producing pancreatic β cells rendering them incapable of their function. As a result, hyperglycemia occurs if no exogenous source of insulin is provided (1). Historically, T1D was also known to be juvenile diabetes. This is due to the prevalence being largely among children and adolescents. However, in recent times, it has been widely accepted that age at symptomatic onset is no longer a restricting factor in the diagnosis of T1D (2). In fact, as many as 50% of cases occur in adulthood with a substantial number of adult-diagnosed cases being misclassified for type 2 diabetes rather than the correct diagnosis of T1D (3).

Although age is no longer a restricting factor in the diagnosis of T1D, it is one of the most chronic and common diseases associated with childhood. Children suspected of T1D commonly present with symptoms of polyuria, polydipsia, and weight loss; almost a third present with diabetic ketoacidosis, a life-threatening condition that must be corrected to ensure survival (4). Unlike children who have hallmark symptoms, the onset in adults is variable and may include symptoms not common to children such as: vision changes, drowsiness, or heavy breathing. In addition, the incidence of T1D is greater in boys and men whereas most auto-immune diseases disproportionately affect women.

Prevalence

Global incidence of T1D has been increasing for several decades. Incidence of T1D throughout the globe varies quite substantially. A study in 2020 extracted data from 193 articles between 1990 and 2019 to determine global incidence. Results showed that the incidence in the continental subgroups of Asia, Africa, Europe, and America was the following: 15 per 100,000,

8 per 100,000, 15 per 100,000, and 20 per 100,000 (5). Globally, T1D is most common in Finland with more than 60 cases per 100,000 people each year. Sardinia has the second highest incidence with 40 cases per 100,000 people each year (6). By comparison, T1D is rather rare in countries such as India, China, and Venezuela who exhibit around 0.1 cases per 100,000 people each year (6). Incidence trends of T1D are interesting in that they present an epidemiological conundrum due to the wide variations that occur regardless of geographical proximity. For example, the incidence in Estonia is one third of that in Finland, even though the two countries are separated by a mere 120 km (7). In the United States, the incidence is 20 per 100,000 (8). In terms of age, substantial increases have been observed in children younger than five. Not much is known about why the incidence rates are increasing; however, if they continue to increase on their current trajectory, global incidence could double over the next decade (6). Given that diabetes is the seventh leading cause of death in the United States and ninth in the world, it is important to completely understand this disease. This paper aims to discuss the diagnosis and pathophysiology, current therapies, and future cellular therapies in research that could one day be a mainstay in the management of T1D.

Diagnosis & Pathophysiology

As mentioned earlier, a substantial number of individuals are mis-diagnosed with type 2 diabetes when the correct diagnosis would be T1D or an autoimmune diabetes. As a result, the statistical incidence is often underreported. Historically, diagnosis of T1D has included the following criteria: fasting blood sugar higher than 126 mg/dL, random blood glucose of 200 mg/dL or higher with symptoms such as polyuria, polydipsia or weight loss, and/or an 2h glucose-tolerance >140 mg/dL (9). Within the last decade, the American Diabetes Association has modified their diagnosing guidelines to include HbA1C, an average value of glucose over the

last three months. An HbA1C higher than 6.4% is considered diabetic (10). Even so, the lack of standardization within diagnosing criteria between the two types of diabetes has led to improvement efforts as well as finding novel methods to accurately distinguish and diagnose cases (11).

Pathophysiology regarding T1D can be traced back to 1986 when George Eisenbarth developed and published a conceptual model for the pathogenesis of T1D. It postulates that individuals are born with various degrees of genetic predisposition to T1D. As an individual ages, the model shows loss of β cell mass and subsequently loss of function. It ends with no C-peptide being present, indicative of no insulin production in the human body (12). While this model has stood over time, it continues to be modified as it does not address the complete complexity of the pathogenesis regarding T1D.

There are a host of new factors that are considered when discussing the pathophysiology of this disease. For example, it has been proposed that environmental influences might occur from in utero to the first few years of life, affecting β cell autoimmunity. Psychological events in relation to immune system development and turnover of β cells may also be implicated in the pathogenesis (13). Immune system dysregulation, thought to be provoked by genetic susceptibility, has also been linked to the early destruction of β cells. Abnormalities within the immune system, such as a single autoantibody, does not necessarily indicate T1D (14). Rather, seroconversion to two or more autoantibodies has been implicated in early asymptomatic disease. Most individuals do not see changes for months to a decade after autoantibodies are detected as the presence of two or more autoantibodies is associated with an 84% risk of clinically diagnosed T1D (15). Symptomatic onset occurs once a critical mass of β cells is lost, resulting in hyperglycemia and the need for an exogenous source of insulin to replace the

function of the lost β cells. This is in conjunction with the loss of C-peptide, which confirms the loss of insulin production by these cells (16). Therefore, Eisenbarth's original model has been updated to include a role for disease heterogeneity, genetics, immunology, and age (Figure 1).

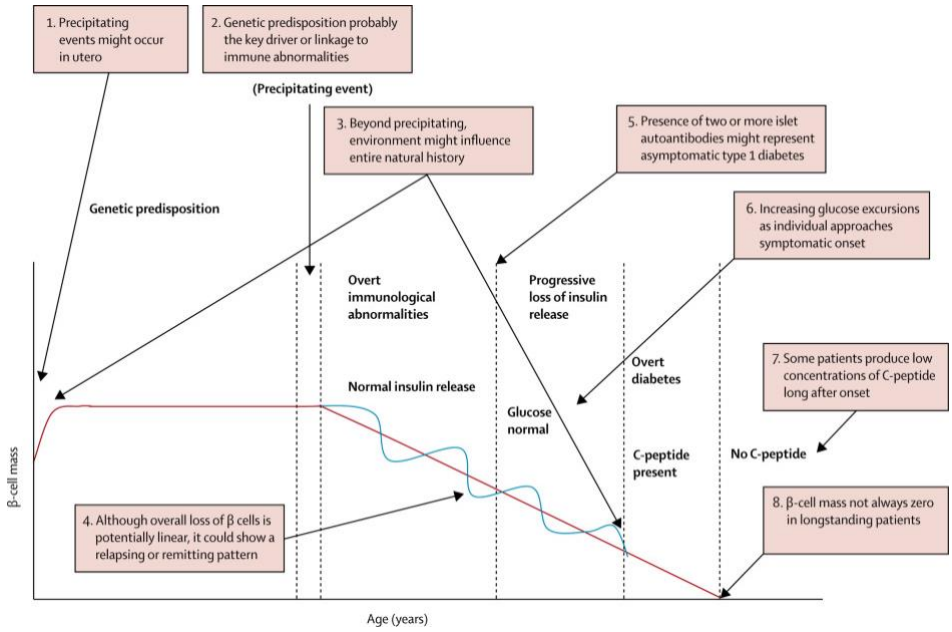

Figure 1. Visualization of Eisenbarth's original model, updated to reflect the recent advances in understanding the various factors affecting the onset and progression of T1D.

With either minimal levels or the loss of C-peptide resulting in loss of insulin production, an exogenous source of insulin must be utilized to stabilize glucose levels. The discovery of insulin in 1921 was perhaps the most influential therapeutic event in the management of T1D. However, simply having exogenous insulin does not necessarily provide the metabolic regulation that is associated with normal pancreatic function. Metabolic regulation is necessary to avoid complications that are associated with diabetes such as: neuropathy, nephropathy, retinopathy, cardiovascular disease, and hypoglycemia (17).

Current Therapies for T1D

There are several methods of insulin therapy to control glucose levels in T1D. Perhaps the most basic method is multiple daily insulin injections. This method involves two separate types of insulin, a rapid-acting version and a long-acting version (this is call basal-bolus regimen). The rapid-acting insulin is administered before a meal based on carbohydrate ratios that are determined on the number of carbohydrates consumed and with the correction of the prior blood glucose level of the individual. Long-acting insulin, also known as basal insulin, is used to control glucose outside of mealtimes, mainly in the morning or at night, and is taken once a day (18).

Over the past decade, the use of insulin pumps to continuously infuse insulin has increased substantially. For context, there were less than 7,000 users in 1990, 100,000 users in 2000, and there are over 350,000 users today (19). Insulin pump therapy continuously delivers rapid-acting insulin through a small cannula which is inserted into subcutaneous tissue and secured to the skin with adhesive tape. The infusion set is connected to the pump through small tubing through which the insulin is delivered. When an individual is about to eat or has eaten, they enter the number of carbohydrates and the blood glucose prior to the meal. This allows for the pump to determine the amount of insulin needed based on a preset ratio. When a bolus is not being delivered, insulin pumps deliver small amounts of the rapid-acting insulin in a basal fashion. In addition, if an individual has high glucose, they are able to correct it with a bolus. They are also able to alter the basal rate with a temporary basal feature which allows for more or less basal insulin based on the body's situational needs, such as illness or exercising. It is clear that the general consensus is that insulin pump therapy allows for more flexibility with eating patterns as well as maintaining a target HbA1C (18, 19).

Recent studies conducted in a randomized controlled trial have found that adults with T1D reported a lower HbA1C with pump therapy than injection therapy resulting in a larger number of patients reaching their individualized HbA1C levels which are based on factors such as age and comorbidities (20). In addition, a meta-analysis has also provided evidence that pump therapy lowers HbA1C more than daily injection therapies (21).

So far, insulin pump therapy has been discussed in an open-loop mechanism. Open-loop delivery refers to the patient administering insulin to themselves at different times of the day based on their needs. Because the patient is administering, there are still variable levels of glycemic control as a non-compliant patient may not be benefitting from the advantages pump therapy has to offer. Recently, insulin-pumps have begun to operate with sensors in a form known as sensor-augmented insulin pump therapy (22). Closed-loop systems require minimal patient interaction as a continuous glucose monitor monitors glucose in real-time and provides feedback to the insulin pump to deliver or suspend insulin delivery based on the body's needs. Early studies have reported favorable results with improvement seen in overnight control of glucose and a reduced risk of hypoglycemia due to preventative action by the system (23, 24). However, once again, the system is only as effective as the patient wants it to be as failure to bolus after meal times renders the system in a “catch-up” mode where it is not as effective in maintaining target glycemic levels.

In addition to injection and pump therapy, pancreatic transplant can be performed. Currently, this is the only therapy that can potentially reverse and cure T1D and is regarded as the gold standard. Pancreatic transplants are most common in brittle diabetics or those who experience recurrent unawareness hypoglycemia and/or hyperglycemia. If successful, it can restore normal glycemic levels and can halt, reverse, or prevent the development or progression

of diabetic complications that can result from poor glycemic control over an individual's lifetime (25). In order for a pancreas transplant to occur, a viable pancreas must be obtained from a deceased donor. Once obtained, it is surgically inserted on the right side of the abdomen and connected to a variety of blood vessels. The native pancreas remains in place (26).

While pancreatic transplants are the gold standard, there are severe limitations associated with this type of therapy. First, pancreatic transplants on their own only account for 10% of transplants (27). Over 70% of pancreatic transplants occur simultaneously with kidney transplants. These occur in patients who have had T1D for an extended period of time and have developed advanced chronic kidney disease due to diabetic nephropathy. In addition, pancreases are not readily available due to a limited number of donors, a growing waitlist, and strict guidelines regarding viability. Another limitation is that transplant recipients must be immuno-suppressed which increases susceptibility to pathogens. And, there is always a chance of organ rejection, rendering the new pancreas non-functional. Therefore, pancreatic transplants are not a therapy readily available or accessible (26, 27).

Islet cell transplantation is another form of transplant therapy that is less invasive. It was discovered in 1972 and by the 1980s, autologous islet cell transplantation performed for chronic pancreatitis showed long-term effectiveness in maintaining normal glycemic levels. Since then, studies have shown significant advances in the isolation, survival, and immune-system tolerance of transplanted islet cells. Development of the Edmonton Protocol, a steroid-free immunosuppressive regimen, has allowed for further understanding of the role of islet cell transplantation. For a successful procedure, islets must be derived from the pancreas of a deceased donor, purified, and transplanted into the recipient (28). A report by the Collaborative Islet Transplant Registry in 2014 showed short-term and long-term improved outcomes in

subjects enrolled in a study. Insulin independence was achieved in up to 80% of patients with near normalization of glucose levels. However, loss of islet function was observed in the majority of patients who initially achieved insulin independence, therefore presenting a limitation of this therapy (29).

In addition to loss of function, there are other challenges facing clinical implementation of islet cell transplantation as a therapy for T1D. First, it is difficult to procure enough islets for transplantation. To do so, more than one pancreas is often required to isolate enough islets so that insulin independence can be achieved. Isolation techniques are also of the utmost importance as improper isolation and purification of the islets can render them non-functional. Finally, the portal vein is the only location where islets can be transplanted currently. As a result, they ultimately lodge in the liver. Due to the cross-exposure between islets and portal blood, an instant blood-mediated inflammatory reaction occurs and causes the destruction of a significant number of transplanted islets, resulting in decreased insulin independence (30). Due to these reasons, islet cell transplantation is one of the lesser-used therapies in the management of this disease.

Therapies for the Future

As a result of the current limitations presented by all therapies, novel therapies and strategies are being developed. Currently, stem cell therapy is at the forefront of T1D research due to the potential limitless supply as well as discontinuation of immunosuppressants used to control rejection in transplant recipients. There appear to be multiple sources of stem cells capable of differentiating into insulin-releasing β-cells, including the pancreas, spleen, bone marrow, liver, embryonic stem cells, and human induced pluripotent stem cells. Within the last

decade, undifferentiated cells from these sources have been researched to further understand their capabilities of differentiation in hopes of regenerating functional β-cells (30).

Recently, ESCs and hiPSCs have been at the forefront of investigations in the field of T1D. Embryonic stem cells are pluripotent cells that are isolated and derived from the inner cell mass of a blastocyte, also known as the early mammalian embryo which is implanted in the uterus during development. In contrast, hiPSCs are isolated and derived from adult somatic cells that are subsequently reprogrammed to an embryonic-like state using Yamanaka factors. Both ESCs and hiPSCs are currently considered at the forefront of T1D research due to their infinite proliferative capacity and their ability to differentiate into a variety of adult cell types *in vitro* and potentially, *in vivo*.

Originally, generation of insulin producing mature β cells from ESCs and hiPSCs was based on imitation of the embryonic pancreas development *in vivo.* Embryonic pancreatic development is reliant on a host of sequential stages that are manipulated with the addition of diverse cytokines and signaling modulators at each stage to regulate the activation or inhibition of signaling pathways that contribute to the generation of mature β cells. Stages of embryonic pancreatic development include the development of the definitive endoderm, primitive gut tube, posterior foregut, pancreatic endoderm, pancreatic endocrine precursor, immature β cells, and β cells (31). A schematic of this original concept can be seen in Figure 2.

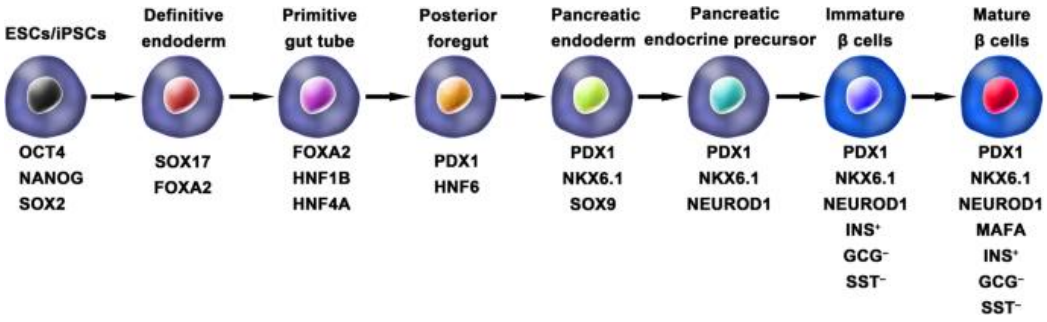

Figure 2. A schematic representation of the original differentiation protocol for the generation of insulin-producing mature β-cells from ESCs and hiPSCs by mimicking embryonic development of the pancreas.

Since the original concept, several differentiation protocols have been developed for both ESCs and hiPSCs with varying levels of success. D'Amour et al. was the first group to develop a stepwise protocol that converted ESCs to endocrine cells capable of synthesizing pancreatic hormones including insulin, glucagon, somatostatin, ghrelin, and pancreatic polypeptide. While the protocol was successful in synthesizing, it was not effective regarding quantity. At the final stage of the protocol, the average percentage of insulin-producing cells from the differentiated cells was 7.3%. In addition, these cells were not able to respond to a high-glucose stimulus, thus proving ineffective (31).

Previous studies had demonstrated that fetal human pancreatic tissues were able to develop functionally after transplantation. Therefore, Kroon et al. further expanded on D'Amour et al. to determine whether immature β cells differentiated from ESCs would be able to mature into insulin-producing mature β cells in an *in vivo* environment. Using a differentiation protocol, they generated immature β cells and transplanted them into immunodeficient mice, the standard model organism for T1D research. Results indicated that the transplanted cells successfully became mature β cells and were able to respond to glucose challenges as well as maintain glucose homeostasis for up to three months (32).

Similar to ESCs, generation of insulin-producing mature β cells from hiPSCs were also studied for their effectiveness. Tateishi et al. was the first group to demonstrate the production of islet-like clusters *in vitro* from skin fibroblast-derived hiPSCs using a differentiation protocol that mimicked *in vivo* pancreatic development. However, when testing the differentiated islet-like clusters, results demonstrated only 0.3 ng/μg of C-peptide secreted. Despite the low amounts of C-peptide expression, Tateishi et al. provided evidence of insulin-secreting cells from skin fibroblasts hiPSCs, hence raising the possibility that hiPSCs from patients could provide an avenue of treatment for diabetes in the future with more refinement and investigations (33).

Thus far, the aforementioned studies have confirmed the potential use of ESCs and hiPSCs as a therapy for T1D through their ability to differentiate into insulin-producing mature β cells. However, the various differentiation protocols have led to various levels of hormonal secretion, gene expression, and cell efficiency. Therefore, further research is warranted.

In 2014, a study published by Rezania et al. was considered a breakthrough as it illustrated a more detailed differentiation protocol and generated mature and functional insulin-producing β cells that were comparable to human β cells. There were seven sequential stages in this protocol, including definitive endoderm, primitive gut tube, posterior foregut, pancreatic endoderm, pancreatic endocrine precursors, immature β cells, and mature β cells. Analysis showed that the obtained cells displayed key markers indicative of mature β cells including INS, PDX1/NKX6.1, and MAFA. When transplanted into mice, these cells showed functional similarity to human islets as they were able to reverse hyperglycemia by secreting C-peptide and insulin. However, after further single-cell imaging and dynamic glucose stimulation, it was determined that the stage seven cells, also known as the mature β cells, were not equivalent to mature human β cells due to their differences in response to dynamic high glucose stimulation

(34). Despite these differences, the ability of these cells to respond to glucose challenges via insulin secretion at a rate of four times faster than pancreatic progenitors or cadaveric islets continued to display the ability of pluripotent stem cells as a future cellular therapy for T1D (34).

In the studies mentioned so far, the ESCs and hiPSCs generated from skin fibroblasts have been from non-diabetic patients and undergone reprogramming and differentiation via a protocol to generate insulin-producing β cells. As discussed, many of these cells are able to display some level of C-peptide expression and insulin secretion. However, their utility is limited due to a variety of reasons such as: lack of function *in vitro* and *in vivo,* mis-expression of β-cell genes, lack of correct granular structure, and in general, not resembling bona-fide β-cells. Therefore, within the field of ESCs and hiPSCs, patient-derived hiPSCs have generated great interest of recent. Patient-derived hiPSCs, particularly from T1D patients, are unique in that they can overcome traditional obstacles such as immune mismatch and rejection given their autologous nature and potentially have more defining features of β-cells.

Maehr et al. was the first to successfully generate hiPSCs from skin-fibroblasts of T1D patients. These patient-derived hiPSCs were described to resemble ESCs in the global gene expression profile and thus able to differentiate into insulin-producing β-like cells. As observed with other studies, these β-like cells were glucose responsive but once again, not comparable to human β cells. However, this study was a breakthrough as it paved the path for generating T1D patient-derived hiPSC β cells and presented an autologous method of stem cell transplantation (35).

Due to the aforementioned benefits regarding T1D patient-derived hiPSCs and their ability to differentiate into β cells, Millman et al. used this relatively novel technology to generate T1D SC- β cells and ND SC- β-cells and assess both *in vitro* and *in vivo*. To generate

both T1D and ND SC- β-cells, hiPSCs were derived using the skin fibroblasts of T1D and ND patients and underwent a differentiation protocol to produce SC β-cells (Fig. 3a,3b,3c). It was known that both the T1D and ND hiPSCs were capable of differentiating and thus, were able to co-express C-peptide/NKX6.1 and C-peptide/PDX1+ (Fig. 3d). In addition, some cells were found to express α-cell hormone glucagon (Fig. 3d). Quantification using flow cytometry found that, on average, 24±2% and 27±2% of cells co-expressed C-peptide+/NKX6-1+ for T1D and ND cells (Fig 3e) (36). Further analysis using electron microscopy confirmed that both the T1D and ND SC- β-cells contained both developing and mature insulin granules which were comparable to human β-cell granules.

Due to their roles within this field, it is important to characterize PDX1+ and NKX6.1. PDX1+ is the pancreatic and duodenal homeobox 1 transcription factor while NKX6.1 is the NK6 homeobox transcription factor-related locus 1. Both PDX1+ and NKX6.1 are considered to be hallmark regulatory factors responsible for differentiation of the definitive endoderm into pancreatic progenitors. It has been well-documented that high co-expression of PDX1+ and NKX6.1 in pancreatic progenitors is essential for the generation of mature and functional β-cells and therefore, insulin secretion.

In addition to quantifying C-peptide expression and examining physical structure, Millman et al. tested the generated cells *in vitro* with a glucose-stimulated insulin secretion assay for functionality purposes. It was found that both the T1D and ND SC- β-cells responded to glucose challenges by secreting 2.0±0.4 and 1.9±0.3 μIU of human insulin per 10^3 cells in response to 20 mM of glucose stimulation (Fig. 3f) (36). In addition, it was found that the T1D and ND SC- β-cells, on average, responded to 88% and 78% of the glucose challenges presented with insulin content being similar between the two cell types (36). With patient-derived hiPSCs

being touted as the future, Millman et al. conducted a proof-of-concept experiment where both T1D and ND SC- β-cells were treated with three anti-diabetic compounds that affect insulin secretion, namely, tolbutamide, liraglutide, and LY2608204. It was determined that treatment with each of the compounds increased insulin secretion by a factor of 2.0 on average in response to low and high glucose stimulation (Fig. 3g) (36).

In addition to testing the cells *in vitro*, Millman et al. assessed their function *in vivo* in an effort to evaluate their potential use in cellular therapy for diabetes. T1D and ND SC- β-cells were transplanted into non-diabetic immunocompromised mice (Fig. 4a). At two weeks, serum human insulin levels were measured before and thirty minutes after a glucose injection (Fig. 4b). Interestingly, insulin was detected, and the cells were glucose responsive in most, but not all, mice with 81% and 77% secreting more human insulin after glucose injection for T1D and ND SC- β-cells, respectively. The ratio of insulin secretion after glucose injection averaged 1.4 for the T1D SC- β-cells and 1.5 for the ND SC β-cells, indicating no major differences between the two cell types (36). In addition, immunostaining of the transplanted cells revealed C-peptide expression with some glucagon expression as well (Fig 4c) (36).

In order to assess their function over a period of time, the effects of the transplanted cells were measured over a period of several months with the cells continuing to respond to glucose injections via insulin secretion and displaying C-peptide expression (Fig. 4d, 4e). In addition, at this time interval, Millman et al. evaluated the ability of T1D and ND SC- β-cells to maintain normal glucose levels in the blood. A subset of mice were treated with alloxan which subsequently killed mouse β-cells but retained the transplanted human SC- β-cells. Results indicated that both the transplanted T1D and ND SC- β-cells maintained a glucose average of

less than 200 (Fig 4f), continued to secrete human insulin in response to glucose (Fig 4g), and cleared glucose after a glucose injection in a rapid and effective manner (Fig 4h, 4i).

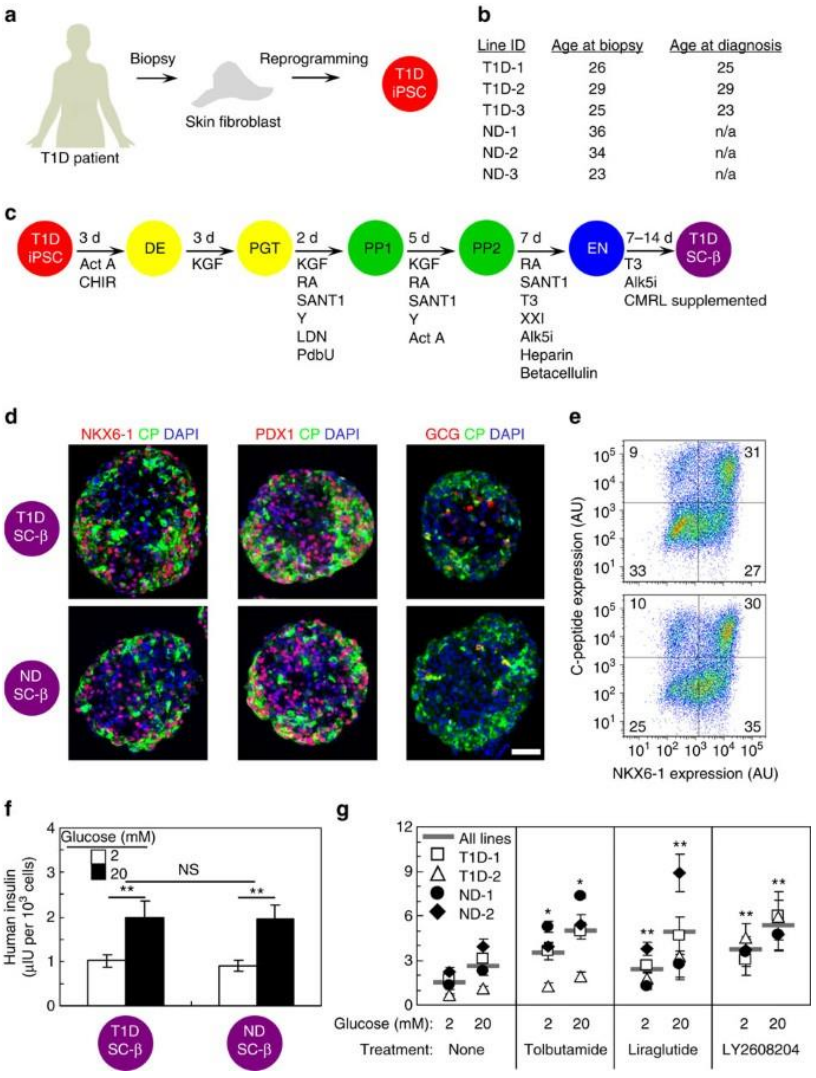

Figure 3. T1D SC- β-cells express β-cell factors and secrete insulin in response to glucose stimulation and anti-diabetic treatments *in vitro*. (a) Illustration displaying derivation of hiPSC from T1D patients. (b) Table showing cell lines used, age at the time of biopsy, and age at diagnosis, if diagnosed. (c) Illustration displaying the differentiation protocol used to generate SC β-cells. (d) Immunostaining of T1D and ND SC β-cells showing NKX6.1, PDX1, and GCG expression. (e) Quantification of C-peptide expression using flow cytometry. (f) Amount of human insulin detected in serum from both T1D and ND SC β-cells. (g) Amount of human insulin detected after treatment from three anti-diabetic drugs (36).

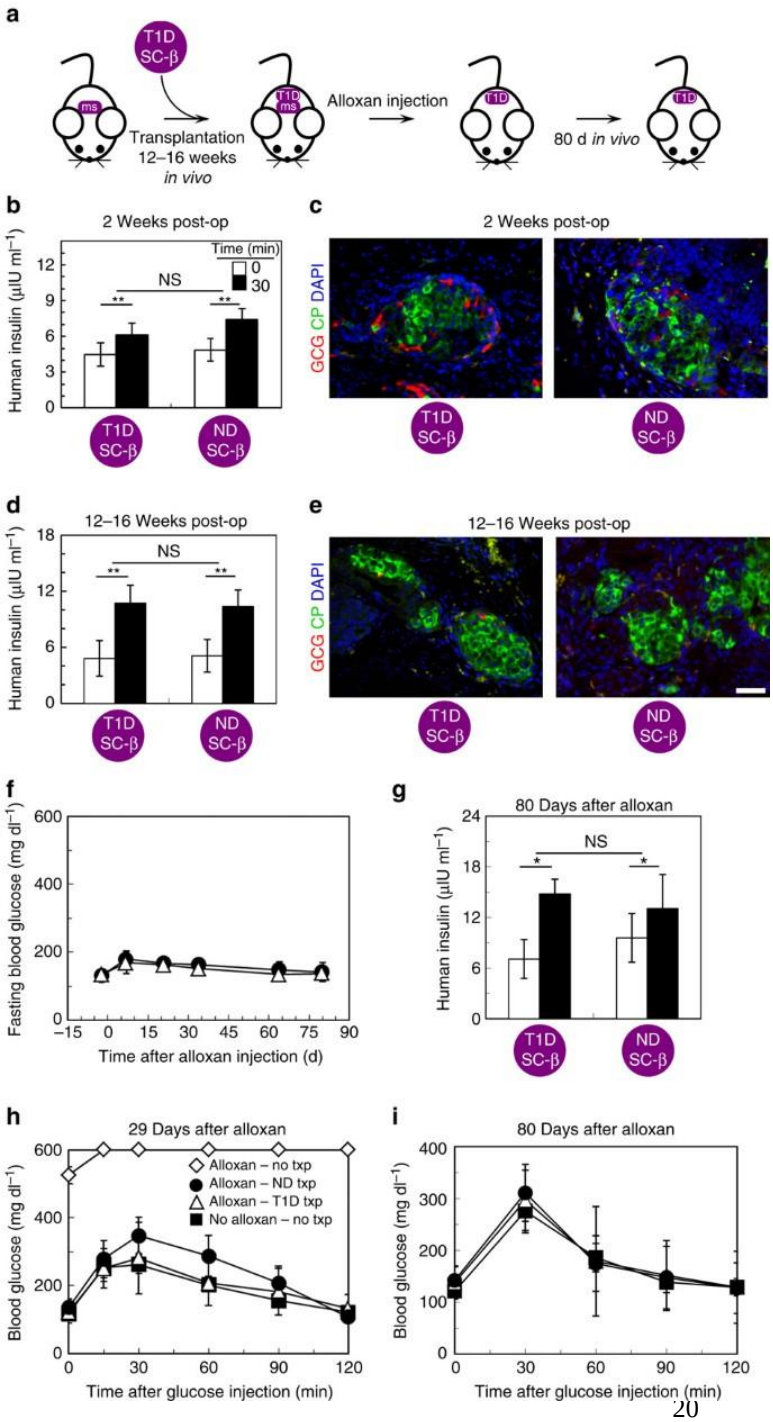

Figure 4. Analysis of T1D and ND SC β-cells after transplantation *in vivo*. (a) Illustration of *in vivo* transplantation. (b) Quantification of human insulin detected in the serum two weeks after transplantation. (c) Immunostaining of the transplanted cells showing C-peptide and some glucagon expression at a two-week time interval. (d) Quantification of human insulin detected in the serum after a period of several months post-transplantation. (e) Immunostaining of the transplanted cells showing C-peptide expression after several months of transplantation. (f)

Measurement of fasting blood glucose after alloxan injection. (g) Quantification of human insulin detected before and thirty minutes after glucose injection in mice treated with alloxan. (h) Measurement of blood glucose levels after glucose injection twenty-nine days after alloxan treatment. (i) Measurement of blood glucose levels after glucose injection eighty days after alloxan treatment (36).

Overall, Millman at al. demonstrated the ability of functional SC- β-cells to be generated from patient-derived hiPSCs. T1D SC- β-cells were shown to be functional *in vitro* and *in vivo*, responding to glucose challenges via insulin secretion as well as maintaining euglycemia. It was determined that there were no major differences between the T1D and ND SC - β-cells generated from hiPSCs and both were very similar to adult, human - β-cells. The similarity of these cells to the original, functional phenotype indicates great promise as a treatment option for patients with T1D.

Limitations

It is important to note the limitations of the studies presented with ESCs and hiPSCs. Because diabetes generally develops over a period of time, as indicated by the Eisenbarth Model (Fig. 1), the effects of ageing need to be tested. It is important to analyze the effectiveness of these cells over time longer periods of time and what defects or differences might occur. In addition, studies have shown that the TAP1 gene, a peptide transporter associated with the major histocompatibility complex (MHC) has been implicated in the development and diagnosis of T1D (37). Therefore, investigating the interaction of the generated T1D SC- β-cells with the immune system would be very informative and help prevent potential setbacks. Another aspect to account for is the diversities that exist among patients globally with T1D. Finally, while various differentiation protocols exist with varying levels of diverse cytokines, signaling modulators, and transcription factors, further research is warranted to determine the perfect combination in an effort to generate efficient and lasting insulin-producing mature β-cells.

Conclusion & Future Work

With the global prevalence of T1D, pathogenesis of the disease, limitations of the current therapies available, and the promise of future cellular therapies, it is clear that research will continue to advance the potential therapeutic options available for patients. As focused on heavily, stem cell-based therapy offers a promising therapeutic option for patients with T1D. Within the purview of stem-cell based therapy, patient derived ESCs and hiPSCs offer the most promising therapeutic as it allows for patients to be their own donor and therefore, eliminates the obstacle of immune mismatch or rejection. In addition, it offers a potentially unlimited number of cells, overcoming an obstacle seen with other stem-cell types as well as cadaveric islets. Major advances, particularly by Millman et al., have provided insight and improved the chance of re-establishing glucose-responsive insulin secretion in patients with T1D. However, as mentioned, there are several limitations to the studies presented and obstacles to overcome before these cellular therapeutics can be a mainstay in the clinical treatment of T1D. Further work in the field of stem-cell based therapy should focus on generating more mature, functional β-cells that are structurally similar to adult human β-cells. In addition, differentiation protocols should continue to be researched to improve all aspects of efficiency of insulin producing cells generated from ESCs and hiPSCs. It is also important to develop larger and more specific cell types that account for the large number of diversity within T1D patients. Finally, a protocol should be developed to generate sufficient amounts of the desired cell types, especially for the purposes of clinical transplantation. If the field of T1D research is able to overcome the presented obstacles and obtain the information needed, the application of cellular therapies could represent the greatest advancement in treating and, potentially curing T1D.

Acknowledgments:

I would like to thank my advisor, Dr. Greg Heiberger, for organizational help and discussions and Dr. Ricardo Correa for providing feedback being an expert in the field of Endocrinology.

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