The Role of Stem Cell Therapy in Treating Type 1 Diabetes and Scientific Advances in Evading an Immune Response

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Abstract

Considering the increasing prevalence and healthcare costs associated with diabetes mellitus (DM), the disease has become an essential subject of continuing research. In particular, type 1 diabetes (T1D) has garnered great interest since current treatments are limited to following a strict diet and insulin regimen or involve approaches that are inaccessible or inappropriate for use by the public. While studies have shown promising effects of stem cell therapy in treating diabetes-induced nephropathy/retinopathy, further research is underway to find a cure for the disease itself. Human embryonic stem cells hold promise for treating T1D because they can effectively differentiate into endocrine and pancreatic cells without encapsulation. However, the likelihood of rejection has shifted focus onto novel techniques that could allow stem cell-derived β-cells to circumvent the immune system. Targeting Human Leukocyte Antigen (HLA) molecules using gene editing techniques such as CRISPR/Cas9 system would allow for an increased graft tolerance. This promising system, combined with encapsulating stem cells to physically separate them from the immune system, could support long-term cell survival and thus increase the likelihood of finding a cure for T1D.

Introduction

Approximately 415 million people around the world are affected by diabetes mellitus (DM) and this number is expected to grow to 642 million by 2040 (1). The worldwide expenditure from diabetes is estimated to be 802 billion U.S. dollars in 2040, with 423 billion dollars attributable to type 1 diabetes in particular (1, 2). Diabetes affects nearly every body system and often leads to microvascular complications such as nephropathy, retinopathy, and neuropathy, and thereby reduces life expectancy by an average of 13 years (3-7). DM is identified as a metabolic disease that results in hyperglycemia, characterized into two etiologies: type 1 and type 2.1 Type 2 diabetes (T2D) is associated with insulin resistance and an inability of the pancreatic beta (β) cells to meet insulin demand, perpetuated by environmental factors such as obesity, smoking, and lack of physical activity.8 Approximately ten percent of the population currently affected by DM presents with type 1 diabetes mellitus (T1D) which is characterized by the autoimmune destruction of insulin-secreting beta-cells, which requires exogenous insulin to avoid deleterious glycemic fluctuations (9).

Effectively controlling elevated glucose levels with exogenous insulin decreases the risk of secondary complications, but comes with increased risk of

hypoglycemia, stemming in part due to inconvenience and poor patient acceptability of multiple insulin injections each day (10). As a result, there is a distinct need for effective therapies beyond exogenous insulin that more closely mimic the native physiological response to hyperglycemia (6). Novel therapies, such as pancreatic transplantation, are limited by donor availability (11, 12). Human pluripotent stem cells (hPSCs) has driven intense research, as their pluripotency allows them to differentiate into insulin-producing β-cells (11). Therefore, a patient can use their own regenerative stem cells to differentiate into large numbers of insulin-producing β-cells reactive to glucose levels, bypassing the problem of donor scarcity (11, 12). Novel therapies, such as pancreatic transplantation, are limited by donor availability and the need for immunosuppression (8). As a result, techniques like genetic engineering and islet cell encapsulation for transplantation are being explored to evade the immune system response that often results in graft rejection and therapy failure (12).

This paper reviews advancements in the treatment of T1D, exploring current transplantation strategies and stem cell-derived therapies. We discuss the immune response associated with diabetes and stem cell therapies, current methods limiting the need for immunosuppression, and newer techniques like genetic modifications with CRISPR/Cas9 and encapsulation delivery methods, highlighting their potential benefits.

Current Therapeutic Strategies for Treating T1D

As healthcare costs and human costs related to diabetes mellitus continue to rise, it is essential to consider options to treat and cure the disease (6). T1D results from the autoimmune destruction of insulin-producing β-cells (9). Symptoms include polyuria and polydipsia, confirmed with tests revealing high levels of blood glucose, C-peptide deficiency, increased amount of hemoglobin glycosylation (HbA1c), and the production of autoantibody markers (13-15). The current treatment regimen for T1D combines intensive diet treatments (such as limiting sugar intake) with the need for lifelong exogenous insulin administration, either via multiple daily doses or using insulin pumps (16). A recent FDA-approved intervention involves an infusion pump providing precise doses of insulin and glucagon, responding to glycemic fluctuations; however, the infusion pump was found to increase the risk of severe hypoglycemic responses (6).

Pancreatic and islet cell transplantation have emerged as alternatives to exogenous insulin administration. Pancreatic transplantation is generally only considered when a patient has severe complications of diabetes mellitus with frequent and severe hypoglycemia and poor quality of life refractory to insulin (17). Risks include thrombosis, bleeding, graft pancreatitis, graft failure, pancreatic-enteric fistula, intra-abdominal abscess, and graft rejection, managed with immunosuppressants (17). Stronger immunosuppressive agents may allow functional graft survival for several years, with patients achieving metabolic control with little to no insulin administration (8). However, pancreatic transplantation is not a primary treatment option for children and adolescents, as they rarely suffer the severe sequelae of type 1 diabetes that would qualify them for transplantation (18). Furthermore, donor availability is on the decline as well.19 Transplantation requires young, non-diabetic, non-obese donors; with the increasing rates of diabetes and obesity in the USA, the availability of deceased donors suitable for pancreatic transplantation has undoubtedly been affected (19). Even if donor availability is not an issue, transplantation and immunosuppression face minor complications such as mouth ulcers, diarrhea, and acne, as well as

longer-term risks like malignancy and infection (20). The Edmonton Protocol, which infuses isolated pancreatic islets into the portal vein of adults with T1D, is now clinically implemented worldwide. Coupled with consistent immunosuppression and induction, the Edmonton Protocol has significantly improved insulin independence over the last ten years (8). While most patients in the first clinical trial achieved over one year of insulin independence, only about 10% maintained insulin independence after five years (8). All other participants achieved insulin independence for an average of 15 months, with the primary cause of failure being antibody development (8). To avoid islet cells trapping in portal capillary sinusoids, research continues in other administration sites, including muscle, the renal subcapsular space, pancreas, omentums, eyes, and testes (6). Overall, current treatments and their complications have shifted focus to other avenues, such as stem cell and cellular replacement therapies to treat T1D.

Stem Cells in Treating Diabetes

While long-term stem-cell based solutions have shown promising results in treating primary T1D through transplanting islet cells reactive to blood glucose levels, these results stem from clinical trials that are years away from being incorporated in clinical practice (21). Therefore, short-term solutions that alleviate diabetes-induced microvascular complications through stem cell-derived therapies remain the focus of this review. Bone marrow-derived mesenchymal stem cells (BM-MSCs) for autologous cell transplant have shown therapeutic value in treating diabetic nephropathy through their vascular repair abilities, which offsets the pathogenesis of diabetic sequelae (7, 22). In one preclinical trial, T1D-induced mice regained renal function after one dose (25 million per kilogram of body weight) of systemically administered mesenchymal stem cells (MSCs), ultimately regenerating β-cells and avoiding renal damage (5).

Autologous BM-MSCs have shown to be effective and safe in treating diabetic retinopathy, particularly during the nonproliferative stage (7). MSCs have also delivered promising results in treating diabetic neuropathy through regulation of spinal neuroinflammatory cascades and reversing associated sensorial dysfunction in diabetic mice (4).

As more studies look at stem cells as a possible treatment for diabetes-induced microvascular complications, several optimistic studies focus on a long-term treatment for the disease itself. Currently, islet and pancreatic cell transplants risk rejection and tumor formation (12). Also, donor availability is limited (11, 12). In theory, MSCs derived from adipose tissue (hAD-MSCs), bone marrow (BM-MSCs), or from the umbilical cord (UC-MSCs), which all have similar morphology, phenotypic expression, self-renewal capabilities, and multi-lineage potential, as well as induced pluripotent stem cells (iPSCs), would concurrently address rejection risk and donor availability (6, 23, 24).

Human embryonic stem cells (hESCs) and iPSCs have been differentiated into functional islets through expression of specific pancreatic transcription factors such as PDX-1, MAFA, Neurod1, and NGN3 (Figure 1) (25, 26). Despite the theoretical risk reduction in autologous transplantation, iPSCs may still be rejected due to neoantigens or other epigenetic factors (27). Ensuring that stem cell-derived β-cells are identical to those endogenous to the pancreas remains a challenge.

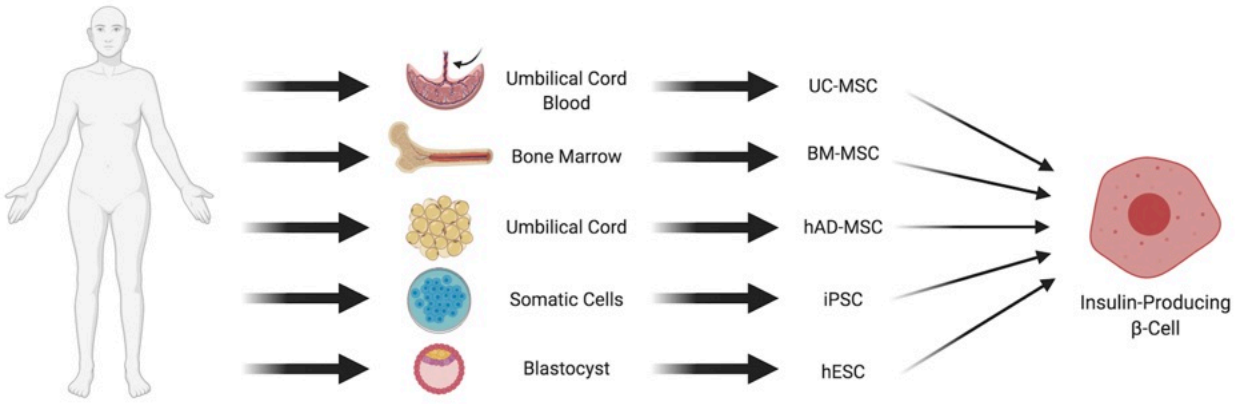

Figure 1: Sources of Stem Cell-Derived β-cells. Mesenchymal stem cells (MSCs) can come from a variety of sources in the human body. Adipocytes and bone marrow MSCs are primarily used to suppress the immune response against β-cells and improve diabetic retinopathy, respectively. Induced pluripotent stem cells (iPSCs) can come from any somatic cell in the body, while human embryonic stem cells (hESCs) are derived from the blastocyst. Both iPSCs and hESCs are differentiated through the expression of pancreatic transcription factors - PDX-1, MAFA, Neurod1, and NGN3 (25, 26). Image created with BioRender.com.

hAD-MSCs appear to be more promising in terms of mitigating rejection caused by neoantigens without entirely suppressing the immune system. hAD-MSCs elicit an upregulation in regulatory T-cells (Tregs) and TGF-β1, which diminishes the autoimmune response in T1D, and suppresses $CD4$ TH1 (T helper) cells that are responsible for destroying pancreatic islets in T1D (28). However, hAD-MSCs have not shown long-lasting effects in preclinical trials; after nine weeks, blood glucose levels steadily rose in mice, reaching concentrations above 300 mg/dL (28). Furthermore, while hAD-MSCs are considered immune-privileged, it is imperative to consider that recognition and eventual rejection of these cells by the immune system cannot be disregarded in the long term (28). Until they are effective in not only avoiding an immune response altogether, but also remaining functional overtime, we must rely on other methods of treatment, such as genome editing via CRISPR/Cas9 and encapsulation. Encapsulation of stem-cell derived β-cells is a promising approach in protecting these cells from the immune system.

The Immune Response to Stem Cell-Derived β-Cells

Transplanted stem cell-derived β-cells are attacked by the innate and adaptive immune systems through autoimmune and alloimmune mechanisms (12). The pathogenesis of type 1 diabetes involves an intricate interplay between the immune system and the β-cells of the pancreas (12). Autoreactive T cells recognize self-determining molecules Major Histocompatibility Complex (MHC) or Human Leukocyte Antigen (HLA) on the surface of cells. Studies on the NOD mouse, which is highly prone to developing T1D, reflect the essential role of MHC class II allele I-Ag7 in the development of

autoimmune diabetes (29). In humans, T1D heritability was found to be linked to two HLA class II haplotypes, HLA DR3 (DRB1*0301-DQA1*0501-DQ*B10201) and HLA DR4-DQ8 (DRB1*0401-DQA1*0301-DQB1*0301) (30). A study on identical twins and HLA-identical siblings showed that the individual with T1D rejected islet transplants from their non-diabetic sibling, showing that the disease process is due to an autoimmune process (31). This process is characterized by autoantibody production and an infiltration of lymphocytes in pancreatic tissue (29). Once bound to these molecules, autoreactive T-cells can either directly destroy β-cells or indirectly through an innate response, driven by natural killer (NK) cells and macrophages, leading to β-cell destruction (12). Evidence shows that MHC class I expression on the surface of β-cells plays a role in initiating the process of β-cell autoimmune destruction by activating CD8 T-cells (32). Antigens presented by HLA class II molecules on the surface of antigen-presenting cells (APCs) are recognized by CD_4 T cells, which are then activated to produce chemokines and cytokines, leading to inflammation (27). The damage to β-cells due to inflammation does not immediately alter glucose levels. A compensatory process of hormone secretion occurs in order to maintain glucose homeostasis until there is a significant decrease in β-cells, at which point T1D manifests (33). Diabetes-associated MHC class I and II alleles also play a role in allowing self-reactive lymphocytes to avoid the negative selection process in the thymus. Autoreactive CD_4 T cells detect specific MHC molecules and destroy the β-cells by a similar autoimmune process (34).

Alloimmunity occurs when the immune system encounters a cell that presents non-self HLA molecules at its surface, such as is the case with transplanted allogeneic stem cells. Similarly to autoimmunity, alloimmunity is largely mediated by CD_4 and CD8 T cells (12). To bypass the alloimmune response, the HLA markers of the donor must match the markers found on the recipient. However, HLA genes have one of the most polymorphic loci in the human genome, thus rendering the mechanisms of susceptibility difficult to clearly elucidate.30 Further illustrating this sensitivity, it has been noted that in order to avoid donor-derived cells from being targeted by the host's T cells, the transplanted cells should not strictly express any mismatched HLA since T-cell receptor αβ (TCRαβ), which is required to bind to MHC class I for the initiation of islet allograft destruction, is extremely sensitive to HLA complexes on the target cell (11, 35).

Novel Methods to Circumvent a Stem Cell-Induced Immune Response

To date, chronic immunosuppression remains necessary in circumventing graft failure. Despite its essential role in protecting the graft from an immune attack, immunosuppression does not seem to be strictly beneficial (11). It significantly increases the risk of infection with pathogens and is strongly correlated with diabetogenicity and β-cell dysfunction (11). Recent studies uncover the benefits of targeting antigen presentation and cytotoxic T lymphocyte activation to subvert chronic immunosuppression in patients with T1D who undergo stem cell therapy. hESCs expressing low levels of HLA were protected from the effects of an autoimmune response.36 However, this same study showed that increased IFNIγ and hESCs differentiation to β-cells both upregulated HLA expression, which was directly correlated with a higher vulnerability to an autoimmune attack.36 Genome editing techniques, such as CRISPR/Cas9, have emerged as promising therapeutic strategies in the treatment of T1D through a selective targeting of HLA genes (37). Overall, zinc finger endonucleases, CRISPR/Cas9, and encapsulation have all shown potential in improving immune tolerance of transplanted stem cells (6, 35, 37). A 2012 study shows that zinc finger endonucleases can

eliminate HLA-A gene expression in T-cells, allowing them to evade destruction by other healthy cytotoxic T cells (35). Low or no levels of HLA-A, however, were not sufficient to completely avoid an immune response. In fact, allogeneic cells that present no HLA molecules are prone to attacks by natural killer (NK) cells according to the "missing self" theory (35, 38, 39). This NK recognition was avoided in the 2012 study by enforcing the expression of non-classical HLA molecules in the transplanted cells such as HLA-A2 , providing HLA compatibility and rendering them unrecognizable by the immune system (35,39). The results from this initial experiment on T cells were used in hESC transplantation, which is usually complicated by HLA matching between the donor and the recipient, and even to autologous iPSCs, which may also induce an immune response as mentioned earlier (27, 35).

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein 9 (CRISPR/Cas9) system is a simple and efficient method for targeting virtually any locus in the genome by directing the Cas9 endonuclease using short-guide RNAs to the specific gene locus (11). This technique allows the disassembly of MHC molecules in β-cell-derived stem cells with the same goal of circumventing the autoimmune and alloimmune responses (12). One preclinical trial showed that human iPSCs become minimally immunogenic after their MHC class I and class II molecules are inactivated by knocking out the accessory chain beta-2-microglobulin (B2M) and by targeting its transcriptional master regulator using CRISPR/Cas9.38,40 Another recent study used CRISPR/Cas9 to eliminate both human MHC class I genes HLA-A and HLA-B from iPSCs. The remaining HLA-C was sufficient to avoid detection by T-cells and destruction by NK cells while maintaining the ability to present antigens (41). In the future, we believe CRISPR could be used endogenously to increase the expression of pancreatic transcription factors (TFs) such as PDX1, MAFA, Neurod1 and Neurog3 (42). These necessary factors are found in endocrine progenitor cells and control cell differentiation into islet cells (42). Using CRISPR/Cas9 in a mouse model, one preclinical trial identified RNLS as a gene that makes β-cells resistant to autoimmune killing (43). Therefore, combining immunomodulating gene therapy with CRISPR/Cas9 could significantly improve stem cell therapy in patients with T1D by improving immune tolerance of stem-cell derived β-cells without subjecting patients to immunosuppressive therapies.

While gene editing techniques are extensively studied, encapsulation, a novel method for delivery of stem cells, is a newer therapeutic approach for T1D. The main goal of encapsulation is to eliminate the need for chronic immunosuppression in patients undergoing islet transplantation, by using a physical barrier to protect β-cells.6The early results of Phase I/II clinical trial indicate promising results (6). In these studies, an encapsulation device surrounds hESCs and, after implantation in subcutaneous space, selectively allows for diffusion of nutrients while preventing direct cell-cell contact with immune cells (6). The most recent encapsulation device allows for external vascularization, which improves oxygenation of the implanted cells but increases access of transplanted β-cells to immune cells (6). Therefore, continued research is necessary to fine-tune the balance between allowing for oxygenation of transplanted cells while minimizing autoimmune response, with the ultimate goal in mind of eliminating an autoimmune response entirely.

Conclusion

In short, the pursuit of stem cells as an alternative therapy for treating T1D arises in part from the relative dearth of available donors for organ and

islet cell transplants, but also from the complications associated with current therapies. Consistent exogenous insulin administration via an artificial pancreas seems to greatly increase the risk of a hypoglycemic response. Moreover, islet cell transplantation has its own issues since transplants have a risk of rejection as well as a limited shelf life of the transplanted islet cells. With over 200 million people anticipated to be newly diagnosed with diabetes mellitus over the next twenty years, developing new treatments is of the utmost concern.

The flaws associated with current treatments may potentially be solved with the use of stem cells. MSCs allow for an individual's own body to become the solution to their disease. Bone-marrow derived MSCs have been shown to improve diabetic nephropathy and retinopathy. Adipose-derived MSCs have been shown to minimize an autoimmune response and to mitigate β-cell destruction through multiple mechanisms, however, they are ineffective in the long run. hESCs and iPSCs have also been transformed into insulin-producing β-cells. Graft failure due to elimination by the immune system is a major hurdle to successful stem cell therapy in treating diabetes and diabetes-induced complications. Currently, the only treatment to avoid autoimmune and alloimmune attacks on stem cells is the use of immunosuppression in engrafted patients. While effective in reducing the immune response, immunosuppression is associated with a significant increase in infection risk and is strongly correlated with β-cell dysfunction. Two recent scientific advances – genetic engineering and cell encapsulation – have shown promise in decreasing chances of graft failure by evading the immune system. Inducing tolerance with genetic engineering and protecting cellular cargo with encapsulation can potentially circumvent the immune system in patients with diabetes treated with stem cells. Genetic editing using novel CRISPR/Cas9 technology would allow an individual to maintain the integrity of their immune system while ensuring the protection of stem cell-derivedβ-cells. This could be achieved by specifically targeting HLA class I and class II genes, considering that low levels of MHC molecules on the surface of cells minimize the chance of an immune response. Encapsulating stem cell-derived β-cells in hypoimmunogenic capsules also seems to be a promising therapeutic strategy for type 1 diabetes by creating a barrier between the therapeutic cargo and the host immune system. Further research must be done to verify the safety of these techniques in a clinical setting, but we remain optimistic that science is leading us in the right direction to ultimately find a cure for type 1 diabetes.

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