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Islet transplantation - immunological challenges and current perspectives

Immunology of islet transplantation

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Abstract

Pancreatic islet transplantation is a minimally invasive procedure aiming to reverse the effects of insulin deficiency in patients with type 1 diabetes (T1D) by transplanting pancreatic beta cells. Overall, pancreatic islet transplantation has improved to a great extent, and cellular replacement will likely become the mainstay treatment. We review pancreatic islet transplantation as a treatment for T1D and the immunological challenges faced. Published data demonstrated that the time for islet cell transfusion varied between 2 and 10 h. Approximately 54% of the patients gained insulin independence at the end of the first year, while only 20% remained insulin-free at the end of the second year. Eventually, most transplanted patients return to using some form of exogenous insulin within a few years after the transplantation, which imposed the need to improve immunological factors before transplantation. We also discuss the immunosuppressive regimens, ADLs, anti-TIM-1 antibodies, mixed chimerism-based tolerance induction, induction of antigen-specific tolerance utilizing ethylene carbodiimide (ECDI)-fixed splenocytes, pretransplant infusions of donor apoptotic cells, B cell depletion, preconditioning of isolated islets, inducing local immunotolerance, cell encapsulation and immunoisolation, using of biomaterials, immunomodulatory cells, *etc.*

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Core Tip: Type 1 diabetes (T1D) is associated with loss of beta-cell mass and insulin secretion. Regardless of its nature, autoimmune or idiopathic, the loss of own insulin

secretion is a hallmark dysfunction in T1DM; thus, therapeutic options are aimed at either replacing the missing insulin or restoring physiological insulin secretion to achieve normoglycemia and postponing micro- and macrovascular complications. Nevertheless, the need to completely replace the depleted pancreatic secretion also leads to the emergence of new therapeutic horizons, including pancreas and islet cell transplantation. However, this approach also meets several immunological challenges – cellular and antibody-mediated rejection and loss of function. To improve the outcomes, several approaches are performed: immunosuppression, ADLs, anti-TIM-1 antibodies, mixed chimerism-based tolerance induction, induction of antigen-specific tolerance utilizing ethylene carbodiimide (ECDI)-fixed splenocytes, infusion of donor apoptotic cells before transplantation, combined with anti-CD40L antibodies and rapamycin, preconditioning of isolated islets, inducing local immunotolerance, cell encapsulation and immunoisolation, using of biomaterials, immunomodulatory cells, *etc.* MSCs, as an adjunct therapy to islet transplantation, can promote long-term graft survival, possibly by reducing inflammation and enhancing immune tolerance.

INTRODUCTION

Pancreatic islet transplantation is a minimally invasive procedure aiming to reverse the effects of insulin deficiency by transplanting pancreatic beta cells [1]. Pancreatic islet transplantation can be done with autologous and allogeneic islets. While autologous islet transplantation has the advantage of being derived from the same patient, eliminating the risk of immune rejection, its widespread utilization is limited due to several drawbacks, including the need for pancreatectomy, which may have associated surgical risks, and the limited availability of functional islets from a single organ in patients with advanced disease. On the other hand, allogeneic islets are taken from different individuals of the same species, usually for treating type 1 diabetes (T1D), with followed immunological response complications [2].

Typical for T1D is the continuing pancreatic beta cell destruction, which could be autoimmune (Type 1A) or non-autoimmune (Type 1B), resulting in decreased or absent

insulin production. As a result, it increases in incidence yearly and is associated with severe hypoglycemia, ketoacidosis, and vascular complications [3]. Although exogenous insulin analogs are considered the primary treatment option for managing T1D in response to hyperglycemia, they cannot accurately resemble the timing and dosing of physiological insulin secretion. Moreover, exogenous insulin therapy is associated with an increased risk of severe side effects such as hypoglycemia, weight gain, lipodystrophy, *etc.* [4]. Therefore, there is an ongoing effort to improve the treatment options [5]. Among them, pancreatic islet transplantation is promising to become the mainstay in the treatment process [6].

As a minimally invasive procedure, islet transplantation is ideal for high-risk surgical patients burdened with cardiovascular disease [7]. It does not follow the significant complications of vascularized pancreas transplantation, and with minimal intra-operational complications, such as bleeding and portal vein thrombosis, the mortality is negligible. On the negative side, multiple donors for a single patient are needed, while the alternative whole pancreatic transplantation treatment needs 1 and rarely 2 pancreases. This makes it a rather wasteful procedure [8]. An adequate islet number must be transplanted for patients to become insulin-independent. A single transplantation is often insufficient; several sequential transplantations are needed for satisfactory glycaemic and insulin results [9]. Early attempts had been made as early as 1893. Still, the milestone that grabbed the scientific community's attention was the ground-breaking Edmonton protocol, with its non-corticosteroid immunosuppressive treatment [9] and the other studies regarding the benefits of islet transplantation on glucose metabolism improvement [10,11]. Studies have shown that 5-year insulin independence has increased manifold reaching up to 75% [12-14].

Overall, pancreatic islet transplantation has improved to a great extent, and cellular replacement will likely become the mainstay treatment. Our goal was to review pancreatic islet transplantation as a treatment for T1D and the immunological challenges faced. To prepare this narrative review, we search the main databases,

Medline, PubMed, and Scopus, in conformity with the principles of writing a narrative review [15].

ISLET TRANSPLANTATION PROCEDURE

The main procedural steps are pre-transplant assessment, pancreas procurement, islet isolation, tissue culture, transplantation, and post-transplant evaluation [8].

In pre-transplant assessment, eligible patients are chosen. Strong indications include recurrent severe hypoglycemic shocks, impaired awareness of hypoglycemia, undetectable C-peptide, age between 18-65, and a diagnosis of more than five years [16]. Additionally, previous kidney transplantation has been shown to impact the outcomes of islet transplantation positively. Studies have reported that patients who have undergone a kidney transplant before islet transplantation have higher graft survival rates, improved glycemic control, and reduced insulin requirements compared to those without a prior kidney transplant. This may be attributed to the immunosuppressive regimen used for kidney transplantation, which may enhance the success of islet transplantation by preventing the rejection of the transplanted islets [17]. Exclusion criteria include poorly controlled hypertension, heart disease, macroalbuminuria, glomerular filtration rate <80 ml/min/1.73m^2 and potential contraindications for immunosuppression. Current indications do not include the pediatric population [18]. In the transplantation of allogeneic pancreatic beta cells, ABO and human leucocyte antigen histocompatibility have to be assessed. The number of islet donors is generally limited, but new xenografts with islets from other species, typically porcine islets, and stem cell technologies could tackle this critical problem [19].

In the stage of pancreas procurement, the pancreas is removed from donors and preserved in the University of Wisconsin solution (UW) for up to 24 h. Important in this stage is the capsule to be kept intact. The pancreas is delivered to the islet isolation center when procurement is ready [20]. The islet isolation process involves the preparation of the pancreas, which is carefully cleaned of surrounding tissues and dissected to expose the islets of Langerhans. The pancreas is then cannulated and perfused with a collagenase enzyme solution for 10 minutes, which distends the

pancreas to facilitate the separation of the islets from the surrounding stroma. Next, the distended pancreas is cut and set into the Ricordi Chamber, an automated device designed to facilitate the islet isolation process. The chamber employs a series of automatic steps to separate the islets from the exocrine tissue, including filtration and density gradient centrifugation. Finally, the isolated islets are processed using a COBE 2991 cell processor, which further separates the islets from any residual exocrine tissue, and the purified islets are then cultured for transplantation [21,22].

In the hands of the proper expert, the tissue culture stage of islet isolation represents a critical step in preparing isolated islets for transplantation. This stage allows the islets to recover from the stress induced by the previous steps of the isolation procedure, during which they may have been subjected to mechanical and enzymatic stress. The tissue culture stage typically involves the placement of the purified islets into a nutrient-rich media in a controlled environment, where they are allowed to recover for several hours to several days. During this time, the islets are carefully monitored for signs of viability and function, including assessment of insulin secretion and glucose-stimulated insulin release. This stage also allows for flexibility in scheduling the subsequent transplant procedure, as the islets can be stored under optimal conditions until the transplant recipient is ready to receive them. The success of the tissue culture stage is highly dependent on the expertise of the individual performing the procedure, as optimal conditions must be maintained to ensure the viability and function of the isolated islets [23].

Before transplantation begins, the final transplantation islet site has to be decided. The liver is considered preferable for transplantation, although different places are being tested for better islet survival and function [24]. Upon islet infusion, an "instant blood-mediated inflammatory reaction" is described with platelet consumption, activation of coagulation, and the complement system [25].

The post-transplant period following islet transplantation is characterized by a prolonged period of recovery during which insulin independence may not be immediately achieved. The transplanted islets may take months to years to fully

integrate into the recipient's body [6] and establish a functional vascular supply. During this period, the transplanted islets are subject to immunological attacks from the recipient's immune system, which can compromise their function and survival. A combination of induction, maintenance, and antirejection immunosuppressive drugs are typically used to prevent rejection of the transplanted islets. However, a notable irony is that many of these immunosuppressive drugs have diabetogenic properties, which can exacerbate preexisting metabolic abnormalities in transplant recipients. As such, these drugs must be carefully balanced against the need to maintain optimal islet function and prevent rejection [26].

Recent advances in islet transplantation have focused on immunoisolation, which involves the encapsulation of transplanted islets in a protective membrane to prevent their recognition and subsequent destruction by the recipient's immune system. Encapsulation of islets for immunoisolation involves using biocompatible materials that allow for efficient nutrient and oxygen exchange while preventing immune cells from accessing the transplanted islets. Several biomaterials have been studied for this purpose, including alginate, agarose, and polyethylene glycol hydrogels [27,28]. The techniques for improving islet cell survival by encapsulation are presented in Figure 1 (A).
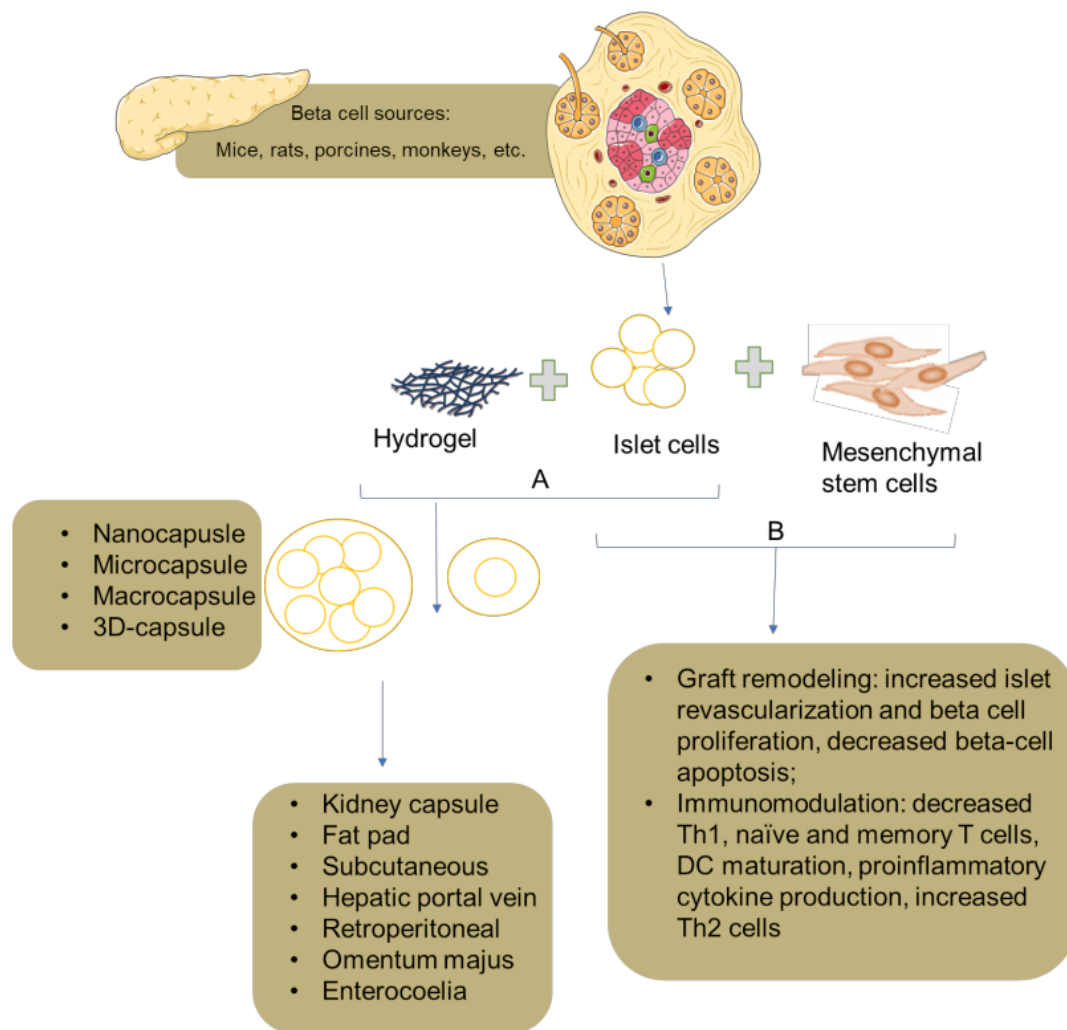
For example, alginate hydrogels are commonly used due to their biocompatibility, ease of fabrication, and ability to protect transplanted islets from the immune system. While early studies in small animal models have shown promising results, with sustained islet function and reduced immunosuppressive drug requirements, translation to larger animals and humans has been less successful, with limited long-term success and significant technical challenges in maintaining membrane integrity and permeability. Using traditional immunosuppressive regimens remains a crucial component of current islet transplantation protocols, albeit with the recognized risks of diabetogenicity and other adverse effects [11].

TYPE 1 DIABETES AND THE NEED FOR ISLET TRANSPLANTATION

Type 1 diabetes mellitus (T1DM) is a metabolic disease distinct by hyperglycemia, insulin deficiency, and a lifelong need for exogenous insulin replacement treatment [3, 29]. T1DM is an autoimmune disease that develops in genetically predisposed individuals under the influence of environmental factors, which triggers autoimmunity to pancreatic beta cells. Although it is defined as "diabetes of young age", T1DM can also affect adults [30]. In general, T1DM is divided into two subtypes, 1A and 1B [31]. While T1ADM is associated with autoantibodies against islet cells (glutamic acid decarboxylase (anti-GAD65), tyrosine phosphatases islet antigen 2 (IA-2), IA-2β insulin (IAA), or zinc transporter 8 (ZnT8)) [32], also observed in patients with T2D [33]), T1BDM, in turn, is a relatively small subtype that is not mediated by the immune system and has an unclear genesis.

T1DM is related to other autoimmune conditions such as celiac disease [34,35], Hashimoto thyroiditis, Addison's disease, pernicious anemia, etc. [36]. Moreover, patients with diabetes may have a compromised immune system, leading to a more complicated course of infections, including COVID-19 [37]. Some of the immune defects described in patients with diabetes are decreased cellular response in vitro, low complement factor 4, diminished cytokine response after stimulation, reduced chemotaxis, phagocytosis, and killing of polymorphonuclear cells and macrophages [38].

Regardless of the subtype, the loss of insulin secretion is a hallmark dysfunction in T1DM, and therapeutic options aim to replace the missing insulin or restore physiological insulin secretion to achieve normoglycemia and prevent micro- and macrovascular complications. Within the last few years, we have seen a rapid evolution in the therapy of T1DM [39]. First, tangible progress marked the discovery of insulin in 1921-22 by Banding and Macleod, saving from certain death children with diabetes. The subsequent development of new analog insulins with a better therapeutic and safety profile results in better control of hyperglycemia and a reduced risk of hypoglycemia, respectively. The introduction of insulin pumps with continuous subcutaneous insulin administration [40] and the implementation of modern technologies in diabetes control with continuous glucose monitoring systems combined with glucose prediction algorithms enabling the development of artificial pancreas delivery systems [41] marks extraordinary progress in managing T1DM.

Nevertheless, the need to completely replace the depleted pancreatic secretion also leads to the emergence of new therapeutic horizons, including pancreas and islet cell transplantation. They allow not only to achieve independence from exogenous insulin administration and the need to monitor blood sugar but also successfully to afford counterregulatory hormone secretion and pancreatic exocrine function [42].

IMMUNOLOGICAL ALTERATIONS IN TYPE 1 DIABETES

T1DM was thought to be a T cell-mediated autoimmune illness for many decades. This belief persists, but multiple recent discoveries hint at a role for beta cells beyond being a non-provoking victim of an autoimmune onslaught [38].

The interaction between genetic vulnerability and probable triggers is likely to begin at a young age, gradually leading to the loss of tolerance to self and, eventually, the development of clinical symptoms. The result is determined by genetic predisposition, decreased removal of the apoptotic cell remains, altered immune regulation, and environmental triggers (i.e., viral infections). In addition, autoreactivity may exist under

physiological settings, and illness may arise if the integrity of the complicated regulatory process is compromised [43].

The beta cells are destroyed by islet-infiltrating cells (i.e., CD8+ cytotoxic lymphocytes and macrophages), resulting in insulitis. In addition, macrophages release cytokines that are harmful to beta cells. Secondary considerations are autoantibodies, which serve as the foundation for clinical diagnosis [43].

Initially, B lymphocytes are known to play a secondary role in T1DM that even occurs in severe congenital B-lymphocyte immunodeficiency [44]. Xiu *et al* considerably delayed disease development in NOD mice by depleting B-lymphocytes using an anti-CD20 antibody. They concluded that this was not due to T effector cell reduction or T regulatory (Tregs) induction but rather to a decrease in the development of autoreactive T cells [45].

However, autoreactive T cells are part of the typical T cell repertoire. In T1D, beta cells live in an inflammatory environment and participate in their destruction. Additionally, metabolic activity is what causes beta cell malfunction and destruction. Insulitis is characterized by inflammation, associated with substantial metabolic, epigenetic, and autoantigenic alterations that expose beta cells to the immune system [46]. In line with this, immunotherapy may be insufficient to treat T1D, although beta cell therapy may help reduce beta cell immunogenicity and islet autoimmunity [47].

It was demonstrated recently that innate immunity components might play a role in T1D pathogenesis, such as pattern recognition receptors and proinflammatory cytokines [47]. Nevertheless, the accompanying inflammation of the islets leads to damaged beta cells and loss of insulin production.

Animal studies (i.e., non-obese diabetic mice) and human studies in T1D revealed defects in thymic selection, expansion of effector T cells, impaired homeostasis and FoxP3+ Tregs [48]. However, even if we accept the immune system's role in the development of T1D, science cannot assume that the disease is entirely a result of dysfunctional immunity, i.e., autoreactive T cells. Recent research focuses on the

participation of the peripheral immune system in the targeted tissue and the role of beta cells in the autoimmune process [49,50].

Indeed, when we accept this conception, it was demonstrated that T1D is usually characterized by less beta-cell mass, functional capacity and inability to control glycemia. Usually, beta cells undergo metabolic stress, inflammatory environment and other factors that increase the expression of specific adhesion molecules and other receptors, making them prone to immune attacks [46].

Islet transplantation has been considered a potential cure for type 1 diabetes by replacing the damaged beta cells. However, its effectiveness is dependent on the underlying cause of the disease. For example, if T1D results from a pancreatic dysfunction leading to the loss of beta cells, then islet transplantation may be a viable option. However, if T1D is viewed as an autoimmune disorder, the presence of autoreactive T and B cells can lead to the disease's recurrence and limit the transplantation's efficacy[47]. In such cases, alternative approaches such as immunomodulatory therapies, co-transplantation with immune cells, or encapsulation of islets can be explored to improve the success rate of islet transplantation.

RESULTS ON DIABETES CONTROL AND AVOIDING DIABETES COMPLICATIONS AFTER ISLET TRANSPLANTATION

Patients with T1D or pancreatogenic (type 3c) diabetes (also known as insulin-deficient) may benefit from islet isolation from a deceased donor followed by transplantation of allogeneic islets in the liver. This can help alleviate hypoglycemia while stabilizing glycemic lability, and maintaining glycemic control, ultimately improving quality of life and frequently eliminating the need for insulin therapy. Replacement of islet function by transplantation addresses the underlying pathophysiology of long-standing T1D with sub-total annihilation of islet alpha-cells and the associated loss of the alpha-cell response to hypoglycemia [19]. This allows for the avoidance of hypoglycemia and stabilization of glycemic lability, which would otherwise contribute to impaired awareness of hypoglycemic states. Patients with T1D uncontrolled hyperglycemia,

demonstrated by the recurring episodes of diabetes-associated ketoacidosis or quickly progressing severe complications related to the disease, might also benefit from islet transplantation [51,52].

Patients with T1D complicated by an allergy or resistance to insulin that is administered subcutaneously are a rare but essential indication for this treatment [53]. Finally, alloislets (from a viable allograft pancreatectomy) re-transplantation has been successfully executed in a patient with T1D who was initially given the pancreas transplant for hypoglycemia unawareness. Similarly, a T1D patient received simultaneous pancreas/kidney transplantation complicated by pancreas graft arterial anastomosis bleeding [54]. Notably, the degree of glycemic control achieved within the first five days after surgery determines the chances of accomplishing long-term insulin independence [55].

We analyzed the literature data published on islet transplantation focusing on the clinical outcomes [55-60]. Our results have been summarized in Table 1. The total number of included patients was 372. We established that the time for islet cell transfusion varied between 2 and 10 h. Approximately 54% of the patients gained insulin independence at the end of the first year, while only 20% remained insulin-free at the end of the second year. Most patients have received islet cells in the liver, and only 38 patients have IC harvested in the spleen. Another interesting fact we discovered was the high percentage of opioid-free patients after this intervention.

Unfortunately, the Collaborative Islet Transplant Registry reported 71% insulin independence in the first year and 24% in the third from the islet transplant centers [61]. Eventually, most transplanted patients need exogenous insulin within a few years after the transplantation [62].

Some additional factors can also improve the outcomes after islet transplantation. For example, experiments in mice and rats with Vitamin D show promising results on glycemia and TNF-α production in islet transplantation [63]. In addition, analogs of vitamin D3 are shown to prevent the autoimmune destruction of transplanted islets in

non-obese mice [64]. This is a promising direction for research on humans due to the well-known anti-inflammatory effects of vitamin D3 *in vivo* [65,66].

IMMUNOLOGICAL CHALLENGES OF ISLET TRANSPLANTATION - CELLULAR IMMUNE RESPONSE, INDUCTION OF TOLERANCE, REJECTION

At this point, the main complication after allogeneic islet transplantation is the chronic rejection conducted by activated T cells. This is also the main barrier to accomplishing long-term engraftment. One of the ways to maintain immune tolerance to the allograft is to administer immunosuppression [67].

However, this could be toxic for the islet grafts, leading to worsening long-term function of the islets, increased risk of infections, development of cardiovascular and renal diseases, de novo diabetes, neurotoxicity and malignancies [68].

The ultimate goal of islet transplantation is to achieve donor-specific immune tolerance. A recently proposed method for tolerance induction using apoptotic donor lymphocytes (ADLs) in animal models (i.e., non-human primates) [69]. ADLs employ clonal depletion, anergy, expansion of Treg cells, regulatory B cells (Bregs), *etc.* Usually, these mechanisms act together to induce and maintain tolerance. However, this approach also meets several challenges.

Initially, the immune rejection after transplantation starts with innate immune cells infiltration into the islet grafts (i.e., macrophages), followed by donor-specific lymphocyte response, consisting of T cells (CD4+ and CD8+) and B cells. In line with this, the protocol comprised of T cell depletion and anti-TNF agents may enhance short-term graft survival [67]. However, this protocol has a significant drawback – it cannot modulate antibody-mediated rejection [70,71].

Targeting Bregs (i.e., low-affinity antibodies against TIM-1, essential for B-reg development) results in considerably longer islet cell survival (about 30% of mice attained engraftment over 3 months)[72]. Surprisingly, anti-TIM-1 treatment of B cell-depleted recipients significantly increased IFN-g and prevented the typically seen rise in Th2 cytokines [72].

Furthermore, in a mouse islet transplant model, a combination of anti-CD45RB and anti-TIM-1 antibodies synergized in establishing tolerance in all recipients. Depending on the presence of IL-10-producing B cells in the recipient, the combined antibody therapy significantly increased the regulatory lymphocytes [73]. Furthermore, the study implied that B cells expressing CD19 and TIM-1 are part of tolerance development and maintenance. These results might clarify why B cell reduction decreased the effectiveness of dual antibody therapy.

Cross-reactive memory T and B cells could substantially impede immunological tolerance in animals and humans after transplantation. However, tolerance development in non-human models or humans would be more complex than in rat models, owing to cross-reactive memory immune cells. Yet, a few hopeful treatments exist, such as mixed chimerism through hematopoietic cell transplantation [74,75] or ADL exposure [76], which have led us to anticipate that immune tolerance can eventually be attained in people.

Oura *et al.* published the results of a non-human islet transplantation model where a nonmyeloablative condition regimen induced the mixed chimerism-based tolerance. The latter consisted of total body irradiation, and administration of horse anti-thymocyte globulin, monoclonal antibodies (i.e., anti-CD154, anti-CD8, *etc.*), or cyclosporine (the so-called calcineurin inhibitor-free regimen) [77]. As a result, temporary chimerism did not prompt tolerance to increase the islet graft survival. Eventually, the islet stopped functioning shortly after chimerism disappeared [77]. Oura *et al* also found that islet recipients had greater levels of inflammatory cytokines (i.e., TNFa and IL-17) in blood circulation than kidney recipients [77]. This study implies that excessive levels of inflammatory mediators following islet transplantation may impede islet graft tolerance induction. Since isolated islet grafts could induce a significant systemic inflammatory response, this should be the focus of future research to improve tolerance development and graft survival.

Induction of immune tolerance utilizing ethylene carbodiimide (ECDI)-fixed splenocytes in combination with particular antigens or peptides is a method used in

transplantation models, including islet transplantation. Kheradmand *et al* demonstrated various mechanisms (i.e., anergy, clonal depletion, employment of Tregs, *etc.*) via donor ECDI-fixed splenocytes administration. These splenocytes possess direct and indirect allospecificities that target allogeneic host responses. These mechanisms act synergistically to cause tolerance after transplantation [78]. In addition, Tregs and myeloid-derived cells that exert immunosuppression are activated and increased in number in the case of ECDI-fixed splenocytes infusion [79].

Allotransplantation in sensitized patients with pre-formed donor-specific memory lymphocytes and antibodies increases the risk of allograft rejection. Dangi *et al* showed that administration of donor apoptotic cells, anti-CD40L antibodies, and rapamycin before transplantation resulted in a considerable extension of islet graft in allosensitized patients (median survival time, 35 days) [80]. Sato *et al* confirmed that invading B lymphocytes play an essential part in the chronic rejection of the islet graft by stimulating local T cells. Therefore, ECDI-fixed splenocytes from the donor infused into sensitized recipients efficiently reduced alloreactive B cells. However, the latter could be switched by contemporary B cell invasion into the graft. As a result, in B cell-depleted patients, a method to regulate concurrent B cell invasion is required [69].

Moreover, islet grafts might be more resistant to immunological tolerance induction. Compared to kidney grafts, the considerably increased immunogenicity of islet grafts may impede tolerance induction in islet transplantation [77]. Islet grafts have relatively strong cytokine secretion activity because pancreatic islets are endocrine cells. Furthermore, cell stressors during the isolation process cause islet inflammation, increasing the immunogenicity of the islet graft before transplantation.

These conclusions imply that the stress during the separation method activates the proinflammatory gene program. Islet isolation entails many steps, including pancreatic distention, digesting with collagenase, and purification. Therefore, the islets should be injured throughout each phase by hypoxia and heated ischemia, production of activated proteolytic enzymes by acinar cells, and oxidative and mechanical stress [69].

According to estimates, around half of the transplanted islets are irreparably destroyed around the transplantation period (from hours to days). In addition, more than a quarter of islet grafts are known to be lost shortly after the portal vein infusion [81]. Therefore, the initial inflammatory response is crucial in instant transplanted islet loss due to immediate blood-mediated inflammatory reaction (IBMIR). During IBMIR, coagulation pathways are activated, proinflammatory cytokines are produced, and innate immune cells infiltrate the graft [82], all contributing to the islet's acute cell-mediated damage. Additionally, IBMIR is distinguished by coagulation and complement systems activation, fast activation and binding of platelets and leukocyte recruitment and infiltration [83].

Preconditioning isolated islets with sublethal genotoxic stress may be a potential technique for lowering islet immunogenicity and extending islet transplant life. It is reasonable to believe that preconditioning therapy for reducing graft immunogenicity will synergistically impact tolerance induction therapy, including the ADL regimen [69].

Applying the cellular treatment is a novel approach to induce local immunotolerance and avoid islet rejection. In addition, the administration of stem cell-derived beta cells during islet transplantation improves graft performance while reducing the negative consequences of systemic immunosuppression. Recent advances in T1D cell replacement treatments (i.e., non-encapsulation and local immunomodulatory techniques) are addressed in this concise review [84]. They include alteration of islet/cell, use of biomaterials that provide immunomodulation, and immunomodulatory cell co-transplantation.

Co-transplantation of pancreatic islets with MSCs is one such approach that has attracted attention. Studies have shown that using MSCs as an adjunct therapy to islet transplantation can promote long-term graft survival, possibly by reducing inflammation and enhancing immune tolerance [85]. For instance, co-transplantation of adipose tissue-derived MSCs and pancreatic islets improved glycemic control and regulation of the Th17/Treg function streptozotocin-induced diabetic mice model [86].

Encapsulation, on the other hand, is another technique that has been extensively studied for its potential to protect transplanted islets from immune rejection while allowing for efficient nutrient and oxygen exchange. In addition, Vegas *et al* demonstrated that beta cells derived from human stem cells, when implanted into mice with preserved immune competence, resulted in long-term glycemic control [87]. Thus, further investigation into these novel strategies for T1D cell replacement therapies may provide new insights and solutions to the ongoing challenges in this field.

Therefore, methods for immunoisolation or beta cell encapsulation are one approach to improving graft performance. Still, it has its own set of obstacles, which causes a loss in cell viability over time (Figure 1B). Although altering human islets in clinical applications is implausible, creating universal cells from pluripotent stem cells that can elude immune identification offers enormous promise in diabetic cell treatments. However, despite these breakthroughs, critical problems like the persistence of genomic and epigenetic modifications and cell phenotypes stability remain unanswered. Additionally, although these cells are hypoimmunogenic, their safety should be carefully maintained because cells that elude the immune system are intrinsically dangerous.

Similarly, undifferentiated stem cells can potentially develop into teratomas *in vivo* because it is well-known that both embryonic and induced pluripotent stem cells can differentiate into all three germ layers. Therefore, they can form teratomas if not fully differentiated [88]. Theoretically, the presence of a few remaining undifferentiated pluripotent stem cells can cause undesirable teratomas after transplantation. Although "suicide genes" could be incorporated into stem cells for increased safety [89], it is still uncertain how these cells would behave in people over time, necessitating additional research.

Biomaterials combined with immunomodulation give multiple instruments for locally modulating immune responses and are an intriguing way to assist cell transplantation. This technique has apparent advantages, including safety as "nonliving" materials. Furthermore, biomaterials are generally simple to mass-produce. In contrast, cell

modification or immunomodulatory cell preparation is sometimes difficult, in addition to the necessity of good manufacturing processes that must fulfill clinical requirements. Yet, given the restricted ligands and the eventual exhaustion of coated reagents, the long-term durability of biomaterials and delivery techniques remains challenging. Hence, there is a need for new approaches for the retention or restocking of the supplied reagents in the future [84].

Interestingly, immunomodulatory cells operate as "living" medicine repositories and, if engrafted, may boost functional stability by producing cytokines continuously or expressing surface markers to affect the immune system. Improvements in these immunoregulatory cells' acquisition, retention, stability, potency and localization are required to increase their effectiveness and safety. As we create T1D therapies and cures, a functioning resolution will likely need a multi-modal methodology involving several immuno-modalities and tissue engineering methods. The strategy for the 3D-engineered biomaterial tissue construct coupled with both invisible to the immune response cells and accessory cells that exert could be employed to provide long-term effective and safe cell treatments for T1D. Examining the disease's heterogeneity and customizing therapy procedures is critical to reaching the best possible outcomes [84].

Additionally, because transplanted islets are isolated from deceased donors who are not HLA-matched to recipients, the use of multiple donors and the potential need to discontinue immunosuppression in the case of a clinically failed islet-alone graft increases the risk of HLA sensitization in islet transplant recipients. Most transplant patients currently have an unexplained slow loss of islet graft function may be partly caused by allograft rejection. However, discovering anti-HLA antibodies during graft deterioration remains uncommon [90].

FUTURE PERSPECTIVES ON ISLET TRANSPLANTATION

Future pathways for improving the outcomes of islet transplantation include obtaining alternative sources of insulin-secreting cells, attempts to improve the immune protection and revascularization of the transplanted tissue, and methods for enhancing viability [91].

Islets obtained from human embryonic stem cells (hESC) are in early-phase clinical trials [92]. hESC islets should theoretically not require immunosuppression or HLA silencing, which would allow the treatment of children. However, alternative strategies, such as xenogeneic sources of islets and human-induced pluripotent stem cells [93], are also being researched.

Several therapeutical approaches to improve islet survivability are currently in the preclinical phase of research. These include cellular therapies such as mesenchymal stem cells [94], regulatory T-cells [95], as well as modulators of the liver niche with anti-inflammatory agents [96] and growth factors [97]. Mesenchymal stem cells appear promising as their anti-inflammatory and immunomodulatory properties have been used in humans for other conditions and could, in theory, enable them to reduce the immunosuppression dose [98]. In addition, improving vascularity through gene therapy [99] of the transplant has also been a sought-after strategy for future development.

Last but not least, various scaffolding methods, as well as alternative implant sites, are undergoing research to enhance the viability of the grafts. For example, dexamethasone-loaded microplate-enriched collagen-coated polydimethylsiloxane scaffolds have improved transplant outcomes and survival [100]. While the liver currently remains the localization of choice for islet transplantation, several other sites are being investigated, such as intramuscular [101], gastric submucosa [102], thymus, testes and the eyes [103].

CONCLUSION

T1DM is an immune-associated metabolic disease characterized by hyperglycemia, absolute insulin deficiency, and a lifelong need for exogenous insulin replacement treatment. The implementation of modern technologies in diabetes control with continuous glucose monitoring systems combined with glucose prediction algorithms enables the development of artificial pancreas delivery systems. Nevertheless, the need to completely replace the depleted pancreatic secretion also leads to the emergence of

new therapeutic horizons, including pancreas and islet cell transplantation. They allow not only to achieve independence from exogenous insulin administration and the need to monitor blood sugar but also successfully to afford counterregulatory hormone secretion and pancreatic exocrine function. At this point, the main complication after allogeneic islet transplantation is the chronic rejection conducted by activated T cells and autobodies-mediated rejection, the main barrier to accomplishing long-term engraftment. To improve the outcomes, several approaches are performed: immunosuppression, ADLs, anti-TIM-1 antibodies, mixed chimerism-based tolerance induction, induction of antigen-specific tolerance utilizing ethylene carbodiimide (ECDI)-fixed splenocytes, infusion of donor apoptotic cells before transplantation, therapy with anti-CD40L antibodies and rapamycin, preconditioning of isolated islets, inducing local immunotolerance, cell encapsulation and immunoisolation, using of biomaterials, immunomodulatory cells, *etc.*

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