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Solving the Puzzle of Immune Tolerance for β-Cell Replacement Therapy for Type 1 Diabetes

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Type 1 diabetes mellitus results from autoimmune destruction of pancreatic β cells. Insulin treatment is often inadequate in preventing devastating complications. Replacing β cells using stem cell-derived islets while fostering immune tolerance, exemplified in Yoshihara et al., holds the promise of a curative therapy for this disease.

In most patients with long-standing type 1 diabetes mellitus (T1D), β cells are irreversibly destroyed and β cell replacement therapies, such as whole pancreas or pancreatic islet transplantation, are currently the only options to restore β cell function and normal glucose. However, these approaches are limited by the shortage of transplantable organs, the high immunogenicity of allogeneic tissue, overcoming pre-existent autoimmunity to the islets, and, as a consequence, the need for life-long immunosuppression to prevent immune rejection. Thus, advancement of β cell replacement therapies will require a renewable source of β cells and effective strategies to reverse autoimmunity and to induce alloimmune tolerance to enable stable function of the β cells without immunosuppression. To accomplish this, an effective strategy will require enlisting the major mechanisms of immune tolerance of deletion, inhibition, and regulation (Figure 1).

In the last 12 years, there have been remarkable advances in the generation of β cells from human stem cells (Velazco-Cruz et al., 2020). In 2008, Kroon et al. (2008) of Novocell (now Viacyte) first demonstrated that pancreatic progenitor cells could be generated from human embryonic stem cells using *in vitro*-directed differentiation. Although these cells resembled fetal pancreatic endocrine cells and were not responsive to glucose stimulation, they matured into functional glucose-responsive insulin-secreting cells several months after transplant into immunodeficient mice (Velazco-Cruz et al., 2020). Since then, several research groups have refined the process to generate more mature β cell clusters *in vitro*. Recently, Yoshihara et al. (2020) reported that the addition of WNT4 during the last stage of *in vitro* differentiation improved maturation and function of stem cell-derived β cells. However, β cells generated with this process have reduced expression of key mature β cell markers and the culture contains a large number of non-endocrine cells. Others have developed processes to produce more mature and enriched β cells using cell sorting or by manipulating the cytoskeleton during endocrine differentiation (Velazco-Cruz et al., 2020; Hogrebe et al., 2020). These recent advances have brought us ever closer to producing fully functional β cells from stem cells.

The progress in creating stem cell-derived β cells increases the urgency to develop an effective strategy to prevent tissue rejection. The conventional approach has been the use of combination immunosuppressive therapies to block autoreactivity and the alloreactivity. Since islets do not need to form physical contact with other cells to function, one way to avoid immunosuppression is to shield these organoids in immune-protective capsules. Although it is possible to insulate islets from the immune system, the capsules often limit efficient vascularization of the grafts and β cells die in the capsules from ischemia. Thus, to date, islet encapsulation procedures have not been successful in humans.

Advances in genome engineering in recent years present new opportunities to generate immune-privileged tissue for transplantation. The principal driver of rejection of transplanted foreign tissue from another human (i.e., allografts) is T cell recognition of the highly polymorphic human leukocyte antigens (HLA). Frequencies of T cells capable to recognizing allogeneic HLA are estimated to be orders of magnitude higher than those for self-antigens in autoimmune patients. Thus, transplanted allogeneic tissue is swiftly rejected by the immune system in the absence of immunosuppression. Abolishing HLA expression in stem cells before differentiating them into β cells can hide the cells from this major form of rejection. However, total elimination of HLA may allow tumor outgrowth on the one hand or tissue rejection by NK cells which are normally inhibited by HLA. Moreover, autoantigens, along with minor alloantigens, can be shed and cross-presented by the recipients' own antigen-presenting cells, leading to indirect immune activation and β cell destruction. Thus, even if the direct HLA-specific allogeneic response can be contained, blocking recurrent autoreactivity may be a much more challenging problem to address as these activated memory cells are more difficult to shut down. Combination treatments may be required to overcome autoimmune memory and complement this strategy. As discussed below,

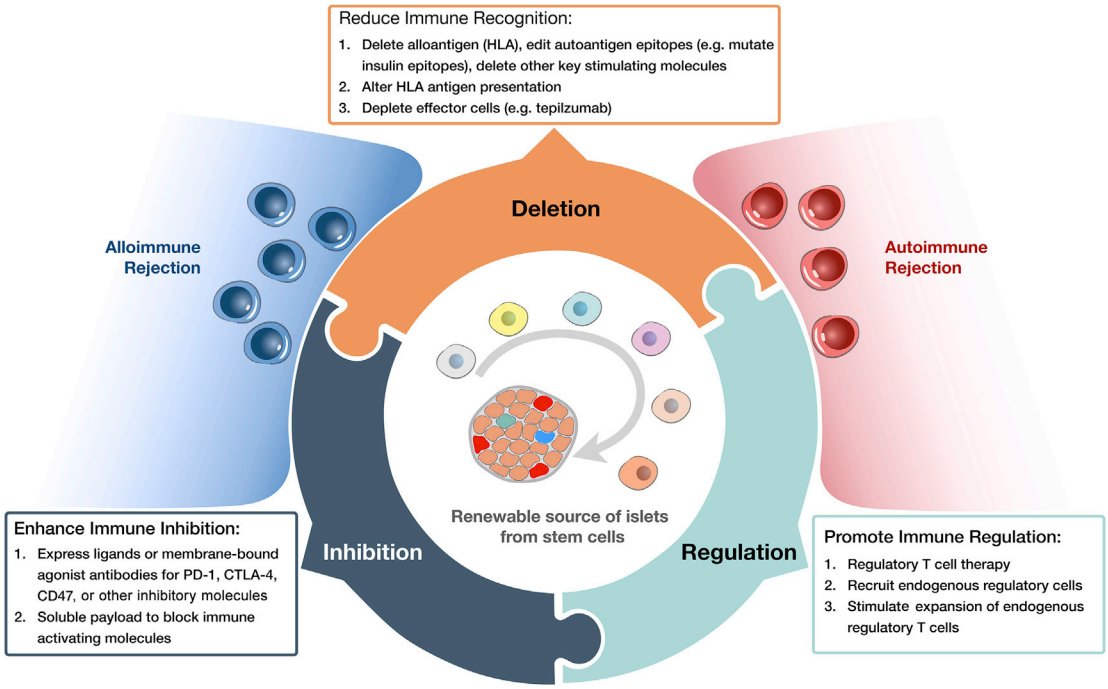

Figure 1. Strategy for Immunosuppression-free β Cell Replacement Therapy for T1D
A renewable source of β cells engineered to reduce their immunogenicity supported by therapies that enlist immune tolerogenic mechanisms of deletion, inhibition, and regulation may block alloimmune and autoimmune rejections to allow β cells to function without maintenance immunosuppression.

immune-depleting drugs such as teplizumab can eliminate autoreactive cells and complement other gene manipulations in the stem cell-derived β cells.

An attractive strategy to control the residual immune response is to engage the cell-intrinsic inhibitory pathways that control immune activation. These pathways include the checkpoint pathways, such as PD-1-PD-L1/L2, CTLA-4-CD80/CD86, and CD47, that have been targeted to break tolerance in cancer patients (Casey et al., 2016). One approach has been the development of a new class of drugs against inhibitory molecules expressed on effector T cells. These agonists engage immune checkpoint molecules on T cells, including CTLA-4 and PD-1, to deliver "off" signals to T cells. The importance of these inhibitory pathways in preventing autoimmune attack of islets in humans is underscored by the clinical observation of eruptive onset of T1D in some cancer patients treated with PD-1/PD-L1 blockers (Quandt et al., 2020). Another approach to engage immune inhibition has been the direct expression of PD-1 ligand 1 (PD-L1), CD47, or membrane-bound anti-CTLA-4 binding domains on the stem cell-derived islets to fend off attacks by T cells, NK cells, and myeloid cells. In fact, overexpression of PD-L1 on islet cells slows T1D progression and promotes islet transplant tolerance in mouse models. In this regard, Yoshihara et al. (2020) showed that PD-L1 overexpression or treatment of human stem cell-derived β cells with interferon γ led to the survival of human islet organoids in immune-competent mice and in immunodeficient mice reconstituted with human immune cells. However, rejection mediated by diabetogenic autoimmune cells was not addressed in this study. Therefore, immune engineering of stem cells can be a very effective tool in reducing immunogenicity of β cells but likely insufficient to completely evade immune rejection.

Re-education of the immune system to be tolerant to self and transplanted tissue has been a long sought-after goal. Resetting the immune system through allogeneic bone marrow transplantation has been shown to be feasible in humans and can restore tolerance without the need for lasting immunosuppression (Mahr et al., 2017) and in mouse studies of T1D. However, the extensive, and often harsh, recipient conditioning, risks of graft-versus-host disease, and the need for donor hematopoietic stem cells are significant barriers for adopting this strategy to induce tolerance to islet transplantation in T1D. Subtler approaches, such as short-term anti-CD3 (teplizumab) treatment, has been shown to delay T1D in high-risk humans and is effective in preventing islet allograft rejection in mice (Herold et al., 2019; You et al., 2012). The therapy works by deleting and inactivating recently activated autoimmune and alloimmune effector cells, while preserving regulatory T cells (Tregs) that are essential for maintenance of tolerance. Similarly, infusion of purified, expanded Tregs, alone or in combination with effector cell depleting agents, can halt immune attacks in various autoimmune and transplant animal models and are being actively developed in early-phase clinical trials (Esensten et al., 2018).

Taken together, in order to cure T1D using immunosuppression-free β cell replacement therapy, a renewable source of functional β cell and a strategy to prevent rejection by autoimmune and alloimmune cells is essential. An effective strategy to evade a destructive immune response will likely need to engage the

major mechanisms of immune tolerance of deletion, inhibition, and regulation (Figure 1). The roadmap to success will undoubtedly depend in part on the manipulation of the stem cell-derived β cells themselves to facilitate engraftment and tolerance induction. Reducing HLA expression can be an effective alternative to massively depleting or inactivating immune cells likely needed to block alloimmune rejection. The autoreactive cells and cells reactive to residual minor alloantigens can then be controlled by inhibition through engaging immune checkpoint molecules and by promoting immune regulation to ensure long-term tolerance and β cell function.

DECLARATION OF INTERESTS

J.A.B. is the A.W. and Mary Margaret Clausen Distinguished Professor in Metabolism and Endocrinology and a member of the Scientific Advisory Boards of Arcus Biosciences, Celsius Therapeutics, and VIR Biotechnology. J.A.B. is a member of the Board of Directors of Rheos Medicines and Provention Bio, a company actively involved in the development of Teplizumab and other T1D-directed therapies. J.A.B. is a co-founder, President, and CEO and Q.T. is a co-founder and advisor of Sonoma Biotherapeutics, a company involved in developing novel Treg-based cell therapies for the treatment of autoimmune diseases. J.A.B. and Q.T. hold stock and are compensated by Sonoma Biotherapeutics. J.A.B. holds stock and is compensated by Provention Bio, Arcus Biosciences, Celsius Therapeutics, Rheos Medicines, and VIR Biotechnology. Q.T. is an advisor of Encellin, eGenesis, and Qihan Bio.

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