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Review

Stem Cell Educator therapy in type 1 diabetes: From the bench to clinical trials

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ABSTRACT

Type 1 diabetes (T1D) is an autoimmune disease that causes a deficit of pancreatic islet β cells. Millions of individuals worldwide have T1D, and its incidence increases annually. Recent clinical trials have highlighted the limits of conventional immunotherapy in T1D and underscore the need for novel treatments that not only overcome multiple immune dysfunctions, but also help restore islet β-cell function. To address these two key issues, we have developed a unique and novel procedure designated the Stem Cell Educator therapy, based on the

Abbreviations: AA, alopecia areata; AIRE, autoimmune regulator; APC, antigen-presenting cells; Arg, arginine; BCR, B cell receptor; B1R, kinin B1 receptor; B2R, kinin B2 receptor; BTLA, B and T lymphocyte attenuator; CB-SC, cord blood-derived multipotent stem cells; COVID-19, coronavirus disease 2019; CPM, carboxypeptidase M; CRH, corticotropin-releasing hormone; CRHR, CRH receptor; CXCR4, C-X-C motif chemokine receptor 4; DC, dendritic cells; EPC, endothelial progenitor cells; f-Mϕ, fibroblast-like macrophages; Gal-9, Galectin-9; GMP, good manufacturing practice; GVHD, graft-versus-host disease; HbA1C, glycated hemoglobin A1C; HOMA-IR, homeostasis model assessment (HOMA) of insulin resistance; HSC, hematopoietic stem cell; iPS, induced pluripotent stem cells; Ig M, immunoglobulin M; IND, investigational new drug; iNOS, inducible nitric oxide synthase; MHC, major histocompatibility complex; Mo/Mϕs, monocytes/macrophages; MSC, mesenchymal stem cells; NK, natural killer cells; NKT, natural killer T cells; NO, nitric oxide; NOD, nonobese diabetic; PD-L1, programmed death ligand 1; PD-1, programmed death 1; RBC, red blood cell; SCE, stem cell educator; SDF-1, stromal cell-derived factor-1; SSEA-3, stage-specific embryonic antigen; TGF β1, transforming growth factor β1; T1D, type 1 diabetes; T2D, type 2 diabetes; T_{CM}, central memory T cells; T_{EM}, effector memory T cells; T_{RM}, resident memory T cells; TNFα, tumor necrosis factor α; Tregs, regulatory T cells..

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Autoimmune regulator
Immune cells
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Mitochondria
Extracellular vesicle

immune education by cord-blood-derived multipotent stem cells (CB-SC). Over the last 10 years, this technology has been evaluated through international multi-center clinical studies, which have demonstrated its clinical safety and efficacy in T1D and other autoimmune diseases. Mechanistic studies revealed that Educator therapy could fundamentally correct the autoimmunity and induce immune tolerance through multiple molecular and cellular mechanisms such as the expression of a master transcription factor autoimmune regulator (AIRE) in CB-SC for T-cell modulation, an expression of Galectin-9 on CB-SC to suppress activated B cells, and secretion of CB-SC-derived exosomes to polarize human blood monocytes/macrophages into type 2 macrophages. Educator therapy is the leading immunotherapy to date to safely and efficiently correct autoimmunity and restore β cell function in T1D patients.

1. Introduction

Human islet β cells are the group of specialized cells in pancreatic islets that can maintain the stability of blood sugar levels and metabolic homeostasis through releasing the hormone insulin and coupling with other groups of islet cells including glucagon-producing α cells, pancreatic polypeptide-producing PP cells, somatostatin-producing δ cells, and ghrelin-producing ε cells [1–3]. In type 1 diabetes (T1D), islet β cells are predominantly damaged where autoimmune cells directly target them, leading to the absolute shortage of islet β cells [4]. However, this conventional immune concept has been recently challenged by increasing clinical evidence of the complexity of T1D autoimmunity. Such evidence includes the remarkably low incidence of insulitis, the islet autoantibody-positive population without T1D, and the observation that different age groups of T1D patients showing different profiles of immune cells and clinical progression [5–9]. T1D-related immune dysfunction has been traced to multiple immune cells, including T cells, B cells, regulatory T cells (Tregs), monocytes/macrophages (Mo/Mϕs), dendritic cells (DC), natural killer (NK) cells, and natural killer T (NKT) cells [10]. Furthermore, increasing evidence reveals that there is damage or loss of pancreatic islet nervous system function in newly-diagnosed T1D patients [11] and in autoimmune-caused diabetic animal models [12–14]. Therefore, T1D etiology appears to be multifactorial, including genetic, epigenetic, physical, social, and environmental factors [4,15]. The detailed mechanisms underlying T1D pathogenesis needs to be further explored in order to find a cure for T1D.

Ideally, therapeutic approaches for treating or curing T1D should address many or all of the underlying causes of the disease. Due to the multifactorial nature of T1D-related autoimmune responses and the global challenges of immune regulation in T1D patients, comprehensive immune modulations are needed to fundamentally halt the autoimmunity at both the local pancreatic islet and systemic body levels. Over the last 15 years, we have developed Stem Cell Educator™ therapy for its immediate clinical translational potential to successfully counteract and reverse the autoimmunity of T1D [16,17] and other immune dysfunction-associated diseases such as alopecia areata [18] and type 2 diabetes(T2D) [19,20]. This comprehensive review will focus on the current progresses of Stem Cell Educator therapy for the clinical treatment of T1D and molecular mechanistic studies with a special focus on its immune modulations on the different types of immune cells and therapeutic potential to overcome the shortage of islet β cells.

2. Introduction of Stem Cell Educator™ Therapy

2.1. Characterization of cord blood-derived multipotent stem cells (CB-SC)

Human cord blood has been utilized as an alternative source for hematopoietic stem cell (HSC) transplantation since the first clinical transplant in 1988 [21,22]. There are different kinds of stem cells in human cord blood such as $CD34^+$ HSC, mesenchymal stem cells (MSC), endothelial progenitor cells (EPC), and monocyte-derived stem cells [23,24]. We identified multipotent CB-SC in human cord blood [25], with features that distinguished CB-SC from monocyte/macrophage-derived stem cells [23], MSC [24], and HSC (Table 1). They are uniquely capable of adhering to non-tissue culture treated petri dishes and they express embryonic cell markers, OCT-3/4, SOX2, stage-specific embryonic antigen (SSEA)-3, and SSEA-4, and leukocyte common antigen CD45, but lack blood cell lineage markers [25]. CB-SC express very low levels of major histocompatibility complex (MHC) antigens and can give rise to multiple cell lineages in response to physiological growth factors and inducers [25]. The detailed characterization of CB-SC has been described in previous reviews [4,24].

2.2. The closed-loop and open-loop system of Stem Cell Educator™ Therapy

CB-SC not only display the nature of stem cells, but also manifest the properties of immune modulators, which have been demonstrated by preclinical and animal studies [4,26–28]. Stem Cell Educator therapy (Educator therapy) has been utilized with a closed-loop system (Fig. 1A) and open-loop system (Fig. 1B). With this patented technology, a patient's blood is circulated through a blood cell separator, wherein the patient's immune cells (mononuclear cells) are co-cultured with adherent CB-SC in vitro, after which CB-SC-"educated" immune cells (designated Gleukocell™) are returned to the patient's circulation through infusion. This one time dialysis-like, ex-vivo treatment "resets" a patient's immune system using CB-SC. For the closed-loop system, it can be utilized at the patient's bedside, thereby minimizing the transportation of patient's blood products and protecting the medical staff from the transmittable diseases. The advantage of the open-loop system of Educator therapy is the increased incubation time of CB-SC with patient's immune cells from 8 to 17 h. The final products in both Educator therapies are the "educated" autologous immune cells which are then returned to the patient's circulation, without transplant of CB-SC. These "educated" immune cells can Educate other immune cells after infusion, thereby reverse the root cause(s) of the autoimmune disease and resulting in the long-lasting clinical efficacy of Educator therapy

Table 1
Phenotypic comparisons of CB-SC with other types of stem cells.

	CB-SC	HSC	MSC	f-Mϕ*
Morphology	Oval, big size	Round, small size	Spindle	Spindle
Attaching surface	Hydrophobic	Hydrophilic	Hydrophilic	Hydrophilic
Cell detachment	Tightly, resistant to EDTA/trypsin	Sensitive to EDTA/trypsin	Sensitive to EDTA/trypsin	Partially sensitive to EDTA/trypsin
HSC marker CD34	Negative	Positive	Negative	Negative
Leukocyte common antigen CD45	Positive	Positive	Negative	Positive
Thy-1 antigen CD90	Negative	Negative	Positive	Negative
Endoglin CD105	Negative	Negative	Positive	N/A
Immunogenicity	Very low	High	High	High

Note: *f-Mϕ, fibroblast-like macrophages [23].

[16,18,29].

2.3. Brief summary of clinical safety and efficacy of Stem Cell Educator Therapy from international multicenter clinical trials

To date, both the closed-loop and open-loop systems of Educator therapy have been approved by the US FDA for phase 2 clinical trials to treat T1D (IND No. 019247), alopecia areata (AA) (IND No. 019246) and the viral infection-caused hyperinflammation in severe COVID-19 patients (IND No. 019679). The safety of Educator therapy has also been demonstrated by the international multicenter clinical trials in T1D [16,17], type 2 diabetes (T2D) [19] and AA [18] in the United States, China and Spain. The procedures were well acceptable in all patients aged from 3 to 70 years old, without any significant adverse events and safety concerns during the treatment. Educator Therapy modifies rather than destroys immune cells responsible for autoimmunity, without increasing the chances of infection and tumor formation as demonstrated by the long-term (4 years) follow-up study of Educator therapy [29]. Educator therapy has been utilized over the last 10 years to treat subjects of different ages (from 3 to 70 years old) among different races (Chinese, Caucasian, Spanish, and Indian), and multiple diseases (diabetes, alopecia areata, lupus, Sjogren Syndrome, Hashimoto thyroiditis, and psoriasis). Specifically, Educator therapy offers comprehensive immune modulation at both the local (pancreatic islets) and systemic body levels [16,17]. Our multi-center clinical studies in the United States, China, and Spain have demonstrated the clinical safety and efficacy of Educator therapy for the treatment of T1D in patients aged from 3 years old to adults with long term disease [16,17]. Our 4-year follow-up studies [29] revealed that a single Educator therapy provides lasting reversal of autoimmunity, leading to regeneration of islet β cells and improvement of metabolic control in subjects with long-standing T1D. Several T1D patients had their islet β-cell function restored and stopped taking insulin after Educator therapy [29].

Mounting evidence highlight the multiple immune dysfunctions contributing to the insulin resistance of type 2 diabetes (T2D) [30–34], suggesting that immune modulation may be an effective tool in clinical management of T2D [20,35]. The failures of using conventional approaches to controlling inflammation and immune dysfunction of T1D should enlighten the development and testing of approaches in T2D. To determine the clinical efficacy of Educator therapy in T2D, Zhao and colleagues found that about 70% of T2D patients achieved the improved metabolic control, with marked reduction of median glycated hemoglobin (HbA1 C) values from 8.61% ± 1.12 at baseline to 7.9% ± 1.22 at 4 weeks post-treatment ($P = 0.026$), and to 7.25% ± 0.58 at 12 weeks post-treatment with Educator therapy ($P = 2.62E-06$) in long-standing T2D subjects [19]. Homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) revealed that insulin sensitivity was improved, as well as significantly reduced inflammation markers after receiving Educator therapy. Notably, the islet β-cell function in long-standing severe T2D subjects (15–24 years) was markedly recovered, as established by the restoration of C-peptide levels at both fasting and post glucose challenging [19]. This clinical efficacy could be retained over four years after receiving one treatment with Educator therapy [29]. Therefore, Educator therapy holds great promise for improving diabetic treatment and finding a cure for diabetes.

Alopecia areata (AA) is one of the most common autoimmune diseases affecting more than 6.8 million people in the United States. The quality of life in AA patients has been significantly affected by the disappointing outcomes, side effects, and relapses with current conventional therapies, including topical and systematic applications of immunosuppressive regimens (such as corticosteroids and cyclosporine) or immune modulators (e.g., dithranol and diphenylcyclopropenone (DPCP)). To date, curative therapy for AA does not exist. There are no FDA-approved treatments for AA. Recently, Janus kinase (JAK) inhibitor tofacitinib was an effective treatment for severe AA [36,37]. However, for all those patients who do respond, relapses are common after discontinuation of treatment, due to the existing of autoimmune memory T cells [38,39]. Therefore, AA is a serious condition with physical, emotional and social impacts, having an unmet need [40]. Notably, Educator therapy has demonstrated potential for correcting additional autoimmune diseases such as AA [18]. A phase 1/2 study ($N = 9$) demonstrated the safety, feasibility, and hair regrowth in AA patients

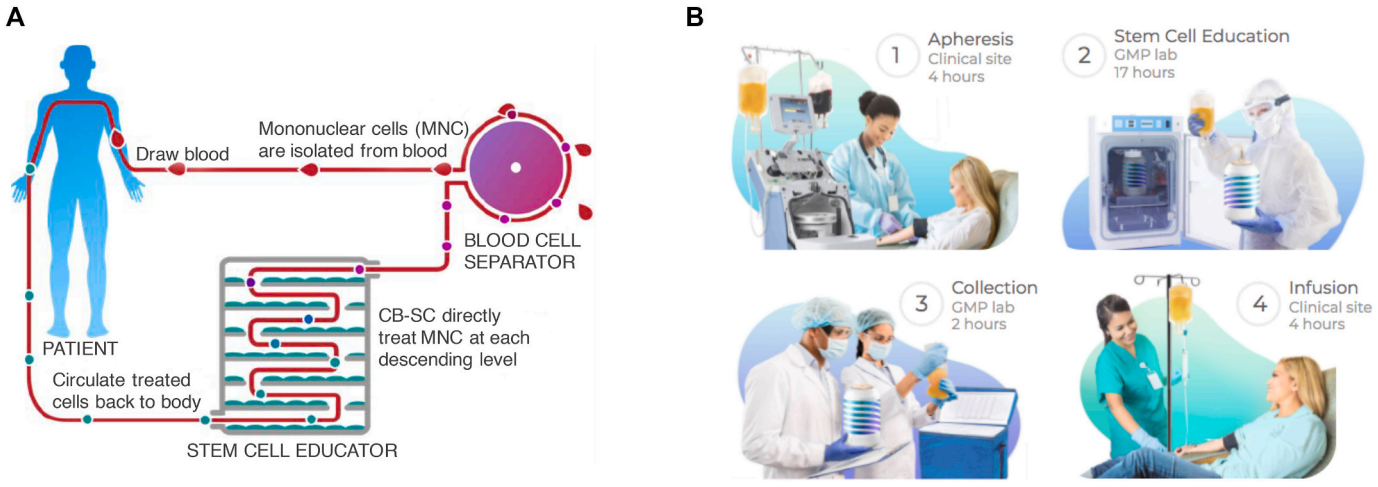

Fig. 1. Fig. 1. Clinical protocols of Stem Cell Educator therapy. A. Scheme of the Educator therapy in a close-loop system. CB-SC are initially prepared from human cord blood and cultured inside the Educator device (FDA-registered, class I medical device) for 2–3 weeks at a GMP facility and shipped to the clinical site for bedside treatment. Briefly, a patient's blood is passed through a Blood Cell Separator (apheresis) that isolates the lymphocytes and monocytes (mononuclear cells) from peripheral blood, these collected immune cells are transferred into the Educator device where they encounter CB-SC (green layer) as they slowly transit through the chambers from the top to bottom layers; after that, only the "CB-SC-educated" autologous cells (designated Gleukocell™) are returned back to the patient via infusion at a dorsal vein in the hand (note: CB-SC remain tightly attached inside the Educator device). This is a closed-loop system and blood is continually processed for a total of about 7–10 L. The whole procedure takes about 8–9 h. B. Educator therapy with an open-loop system. The clinical procedure includes four steps: 1). A patient's white blood cells (mononuclear cells) are collected through a blood cell separator and sent to the GMP lab for treatment. 2). The mononuclear cells are incubated in the Educator device which is coated with the CB-SC that resets the defective immune cells. 3). Only the "Educated" mononuclear cells (Gleukocells) is transported back to the clinical site. The stem cells do not leave the Educator device. 4). The Educated cells (Gleukocells®) will be infused back into the patient. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

following Educator therapy [18]. The broad applicability of Educator therapy enhances its clinical potential to treat multiple autoimmune and inflammation-associated diseases.

3. Modulations of Stem Cell Educator® Therapy on different immune cell compartments

Increasing evidence substantiates that T1D-related immune dysfunctions has been tracked to multiple cell types, including T cells, regulatory T cells (Tregs), B cells, monocytes/macrophages (Mo/Mϕs), dendritic cells (DC), natural killer (NK) cells, and natural killer T (NKT) cells [10]. Due to these multiple contributing factors of T1D-associated autoimmunity and immune modulation in T1D subjects, conventional immune therapies that target only one or a few components of the immune cell compartments are likely to fail. Therefore, there is a need for approaches that can produce comprehensive immune modulations at both the local pancreatic islets and systemic body levels. Educator therapy takes this broader approach based on the immune modulation of CB-SC (Fig. 2). Readers are encouraged to refer to prior review [24].

3.1. Monocytes and macrophages

Monocytes/macrophages (Mo/Mϕ) are front line innate immune cells protecting humans against viral and bacterial infections and maintaining homeostasis, with diverse plasticity and heterogeneity [23,41,42]. Based on their functional profiles, macrophages are divided into two sub-populations: type 1 macrophages (M1, pro-inflammation) and type 2 macrophages (M2, anti-inflammation) [43]. For decades, T1D has been thought to be due to dendritic cell (DC)-initiated, T-cell-mediated autoimmune destruction of islet β cells [15,44]. However, recent characterization of non-obese diabetic (NOD) mice indicated that T-cell dysfunction in T1D is initiated by M1 F4/80$^+$CD11c$^+$ islet

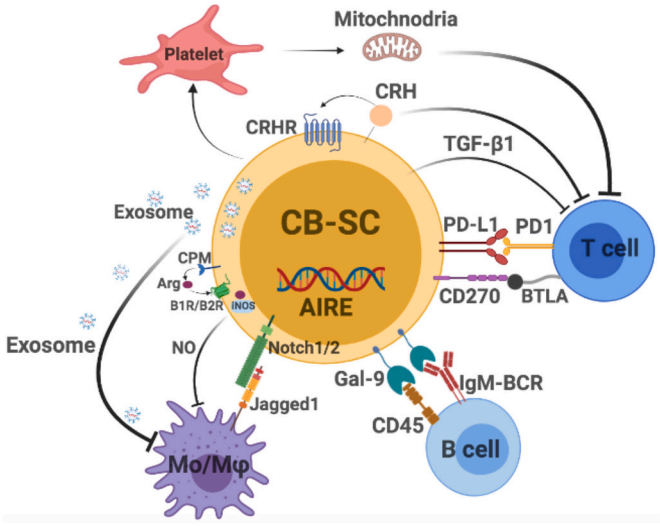

Fig. 2. Outline of the molecular mechanisms for the immune modulation by CB-SC. Mechanistic studies revealed that CB-SC could correct the autoimmunity and induce immune tolerance through multiple molecular and cellular mechanisms such as the expression of a master transcription factor autoimmune regulator (AIRE) in CB-SC for T-cell modulation, an expression of Galectin-9 on CB-SC to suppress activated B cells, and secretion of CB-SC-derived exosomes to polarize human blood monocytes/macrophages into type 2 macrophages. AIRE, autoimmune regulator; Arg:arginine; BCR, B cell receptor; B1R, kinin B1 receptor; B2R, kinin B2 receptor; BTLA, B and T lymphocyte attenuator; CPM, carboxypeptidase M; CRH, corticotropin-releasing hormone; CRHR, CRH receptor; Gal-9, galectin-9; Ig M, immunoglobulin M; iNOS, inducible nitric oxide synthase; Mo, monocytes; Mϕ, macrophage; NO, nitric oxide; PD-L1, programmed death ligand 1; PD-1, programmed death 1; TGF-β1, transforming growth factor-β1.

macrophages [45–47]. Importantly, Mϕ are antigen-presenting cells (APC) that are physically located in pancreatic islets and interacinar stroma at 98% of islet CD45$^+$ cells, with no dendritic cells (DC) [45]. A significant body of evidence has demonstrated that islet Mϕ initiate the T cell-mediated autoimmune destruction of islet β cells in T1D [46,47] and that Mo/Mϕ dysfunction contributes to the pathogenesis of diabetes and other autoimmune diseases [32,48–51] by releasing TNFα and IL-1 and recruiting autoreactive T cells [46,47]. In contrast, Xiao et al. reported that M2 macrophages promoted the proliferation of islet β cells via the upregulation of SMAD7 signaling pathway [41,52]. Therefore, these data highlight the distinct roles of M1 and M2 macrophages in the immune surveillance and maintenance of normal β-cell function. Considering all conventional and current approaches for the prevention and treatment of T1D, there are no therapies, either under investigation now or at the beginning of the pipeline, that directly focus on the modulation of pancreatic islet macrophages.

Indeed, we have shown that the percentage of monocytes expressing an M1 macrophage marker was markedly decreased in type 2 diabetic (T2D) patients four weeks after Educator therapy and that co-culture of CD14$^+$ monocytes with CB-SC significantly down-regulated numbers of inflammation-related genes, including chemokines, multiple cytokines, and matrix metallopeptidases [19]. To further explore the molecular mechanisms underlying the immune modulation of Educator therapy on monocytes/macrophages, our recent study demonstrated that CB-SC could release exosomes (designated cbExosomes) that promoted the M2 differentiation of monocytes [53,54]. Thus, CB-SC counteract this autoimmune damage by releasing cbExosomes, which promote monocyte differentiation into anti-inflammatory M2 macrophages, contributing to the control of autoimmunity in islets and improving clinical outcomes of Educator therapy in T1D.

Exosomes belong to a family of nanoparticles with diameters ranging 30–150 nm [55] and are enriched with many bioactive molecules including lipids, mRNAs, proteins and microRNAs (miRNA), which play an essential role in cell-cell communications. During the Educator therapy, patient's immune cells were treated with CB-SC for about 8 h in a closed-loop system or 17 h in an open-loop system respectively. The Educator-treated monocytes carried the CB-SC-derived exosomes back into the body, which could contribute to the M2 differentiation and the expansion of the induction of immune tolerance in pancreatic islets or other tissues, leading to the improvement of clinical outcomes in T1D patients after the treatment with Educator therapy. Additionally, it is expected that CB-SC-released exosomes may enter into blood circulation and directly target the islet M1 macrophages, leading to their differentiation into M2 macrophages. In line with this expectation, the differentiated M2 macrophages may stimulate the expansion of residual islet β cells as reported by Xiao et al. [41,52], which are consistent with the improved β-cell function in diabetic patients after receiving Educator therapy [16,19].

3.2. T cells

To date, there are more than eighty autoimmune diseases that have been characterized with either systematic or organ-specific damage. Importantly, T cell dysfunction was associated with the chronic pathogenesis of most autoimmune diseases [56–58]. Based on the phenotypic differences, human T cells have been recognized as different sub-sets by flow cytometry [59]. Here, we summarized the immune modulations of Educator therapy on different types of T cells.

3.2.1. Regulatory T cells (Tregs)

Tregs play a crucial role in maintaining immune tolerance through releasing immunosuppressive cytokines interleukin-10 (IL-10) and/or transforming growth factor-β1 (TGF-β1). Increasing evidence demonstrates that defects of Tregs, either in cell number or in function, contribute to the initiation and progression of T1D patients. Therefore, targeting Tregs for treatment of T1D is an attractive approach. Using the

autoimmune-caused NOD mouse model [27], Zhao et al. showed that treatment with the purified CB-SC-modulated $CD4^+CD62L^+$ Tregs (modulated CD4CD62L Tregs) could reverse overt diabetes and resulted in a marked reduction of insulitis, restored Th1/Th2 cytokine balance in peripheral blood, and induced apoptosis of infiltrated T cells in pancreatic islets [27]. Notably, pancreatic histological studies established the proliferation of residual islet β cells with high percentage of $Ki67^+insulin^+$ β cells in the modulated CD4CD62L Tregs-treated diabetic mice, relative to that of CB-SC-unmodulated CD4CD62L Tregs group [27]. Moreover, after double immunostaining with β-cell-marker insulin and α-cell-marker glucagon, confocal microscopy revealed that pancreatic islets in diabetic NOD mice treated with the modulated CD4CD62L Tregs exhibited a similar pattern of α- and β-cell distribution as that noted in normal islets of non-diabetic NOD mice at 7 weeks. In contrast, islet architecture was completely damaged with almost complete disappearance of β cells in islets of diabetic mice treated with unmodulated CD4CD62L Tregs [27]. Thus, treatment with modulated CD4CD62L Tregs can correct hyperglycemia of T1D mice by promoting β-cell regeneration and reconstitution of islet cell architecture. In line with these animal data, clinical data indicated that Educator therapy could upregulate the percentage of human $CD4^+CD25^+Foxp3^+$ Tregs and promote the regeneration of islet β cells in long-standing established T1D patients, as demonstrated by an increase in fasting and glucose-challenged C-peptide levels (a by-product of insulin production from islet β cells) and improved metabolic control [16]. However, there were no such improvements in control subjects who received the sham therapy with no CB-SC inside of device [16].

3.2.2. *Autoimmune memory T cells*

Overcoming autoimmune memory is crucial for eliminating autoimmunity in T1D and other autoimmune diseases. Memory T cells (central memory T cells (T_{CM}), effector memory T cells (T_{EM}), and resident memory T cells (T_{RM})) are critical components of the immune system, reacting quickly upon re-exposure to their cognate antigens to eliminate the reinfecting pathogens. However, substantial evidence demonstrates that autoimmune memory T cells also constitute the most significant barriers to curing autoimmune disease T1D [60]. Whereas several immunotherapies exist that target the general immune population (e.g. by CD2, CD3 and CD20 mAbs), these therapies cause broad cytotoxicity and usually lead to an overall decline in T and B cells, making patients more vulnerable to pathogens and raising concerns about clinical safety. Thus, new approaches are needed to eliminate inappropriate autoimmune T cell memory without ablating the entire T-cell compartment. Our studies demonstrated that Educator therapy offers such an approach.

Delgado et al. reported that Educator therapy provides lasting reversal of autoimmune memory without compromising T-cell function as a whole. Analysis of T_{EM} ($CD45RA^-CCR7^-$) cells revealed that both $CD4^+$ T_{EM} cells and $CD8^+$ T_{EM} cells were considerably decreased in Educator-treated T1D patients at 18 weeks and 26 weeks post therapy [17]. In contrast, the percentage of $CD4^+$ T_{CM} ($CD45RA^-$ $CCR7^+$) cells was markedly and consistently increased starting at 18 weeks after Educator therapy. The percentage of $CD8^+$ T_{CM} cells also increased at 18 weeks, but returned to baseline levels at further follow-ups. Notably, the percentage of naïve $CD4^+$ T ($CD45RA^+$ $CCR7^+$) cells was significantly increased at 26 weeks after Educator therapy and maintained through the final follow-up at 56 weeks post-treatment. The percentage of naïve $CD8^+$ T ($CD45RA^+$ $CCR7^+$) cells did not exhibit significant changes at any follow-ups. Furthermore, previous study demonstrated an up-regulation of C—C chemokine receptor 7 (CCR7) expression on $CD4^+$ T_{CM}, $CD8^+$ T_{CM}, $CD4^+$ T_{EM}, and $CD8^+$ T_{EM} cells after receiving Educator therapy in T1D subjects [17], which outcomes may lead to the removal of autoimmune memory T cells from the insulitic lesions through lymphatic vessels which endothelial cells express CCL19 and CCL21, the two ligands of CCR7 [61]. Additionally, the enhancement of CCR7 expression on Naïve $CD4^+$ and naïve $CD8^+$ T cells may contribute to the redistribution and polarization of T cells and result in the restoration of homeostasis in the immune system [17]. Notably, using the HLA-DR expression as a marker activated T cells, flow cytometry confirmed that both percentages of activated $CD4^+HLA\text{-}DR^+$ T and $CD8^+HLA\text{-}DR^+$ T cells were all declined at 26 weeks post Educator therapy [17]. Thus, Educator therapy delivers not only the recovery of homeostasis in pancreatic islets, but also the comprehensive immune balance for the whole body.

3.2.3. *Expression of the master transcription factor autoimmune regulator (AIRE) in CB-SC*

Maintenance of T cell-mediated immune tolerance is the critical step to prevent and treat autoimmune diseases. AIRE is a master transcription factor that is mainly expressed in the medullary thymic epithelial cells (mTEC) and has key roles in governing T-cell development and immune tolerance through negative selection [62–64]. Mutations of AIRE gene cause a rare autoimmune disease designated autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) [65], also known as autoimmune polyglandular syndrome type 1. The Mechanistic studies confirmed the expressions of AIRE gene and protein in CB-SC [16] (Fig. 2). To determine whether AIRE contributes to immune modulation by CB-SC, Zhao et al. used three pairs of human AIRE-specific small interfering RNAs (siRNAs) to knock down AIRE expression in CB-SC. Western blots confirmed that AIRE-specific siRNAs caused a reduction in the level of AIRE protein itself, as well as in the levels of programmed death ligand-1 (PD-L1) [16], which contributes to the immuno-modulatory activities by CB-SC [26], and carboxypetidase M (CPM), which contributes to nitric oxide (NO) production by CB-SC [66]. Knockdown of AIRE also reduced the percentage of Tregs in co-cultured phytohaemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) [16]. These results support the conclusion that AIRE contributes to CB-SC immuno-modulatory activities. Collectively, these findings show that, in contrast to conventional immune therapies that target the entire T cell compartment and thus have multiple undesired side effects, Educator therapy specifically targeted effector memory cells and activated $CD4^+$ and $CD8^+$ T cells, but did not interfere with total T-cell numbers [17]. Educator therapy targets T cells for modulation, rather than destruction, restoring populations of naïve T cells and eliminating those responsible for autoimmune responses. Thus, for T1D patients, Educator therapy provides an optimal solution to correct autoimmune memory and restore immune balance.

3.2.4. *Immune modulation of platelet-derived mitochondria on T cells*

Previous clinical studies confirmed the long-lasting clinical efficacy of Educator therapy in T1D [16,17], T2D [19] and AA patients [18], even lasting 4 years post one treatment with Educator therapy in some subjects [29]. Since the life span of most T cells is 3 months, this suggests that the parent CB-SC-educated T cells might transfer their properties to the daughter cells, leading to the stabilized and lasting clinical outcomes. To explore the molecular mechanisms underlying Educator therapy, we found that platelets numbers were elevated in T1D patients following Educator therapy [29], prompting further investigation of the possibility that platelets may contribute to the long-lasting clinical effects observed following Educator therapy.

To determine the expression of immune tolerance-related markers in platelets, flow cytometry demonstrated that both cord blood (CB)- and peripheral blood (PB)-derived platelets displayed high levels of several co-inhibitory surface molecules including the programmed death ligand 1 (PD-L1, CD274) and CD270 (a herpes virus entry mediator, HVEM), along with expression of the cytokine transforming growth factor β 1 (TGF-β1) [29]. Platelets are anucleate cells without genomic DNA. To identify the origin of these immune marker-associated genes, mitochondria were purified from CB- and PB-platelets respectively to be explored for the gene transcriptions of mitochondria DNA (MitoDNA). Real time PCR Array revealed expressions of human T cell anergy and immune tolerance-related genes in platelets-derived mitochondria [29].

Flow cytometry further proved that platelet-releasing mitochondria displayed immune tolerance-related markers CD270 and CD274 [29]. Notably, Song et al. found the circulating mitochondria in human and animal blood, as confirmed by electron microscopy and flow cytometry analyses [67]. Platelets and red blood cells (RBC) are the largest components of blood and however platelets have functional mitochondria, mature RBC do not have them [68]. Therefore, circulating mitochondria might be released from platelets. These circulating mitochondria may function as a novel mediator, leading to the energy balance and cross-talk among cells, tissues, and organs, and maintenance of homeostasis.

To further explore the immune modulation of platelet-derived mitochondria, the purified human $CD4^+$ T cells were treated with the isolated platelet-derived mitochondria. Confocal microscopy demonstrated that MitoTracker Deep Red-labeled platelet-derived mitochondria could directly target $CD4^+$ T cells through C-X-C motif chemokine receptor 4 (CXCR4) and its ligand stromal cell-derived factor-1 (SDF-1), with some of MitoTracker Deep Red-positive platelet-derived mitochondria entering into $CD4^+$ T cells after the co-incubation for 2 h at room temperature [69]. After treating the anti-CD3/CD28 bead-activated $CD4^+$ T cells with platelet-derived mitochondria, Yu et al. showed an up-regulation of Naïve and central memory (T_{CM}) $CD4^+$ T cells, the down-regulation of effector memory (T_{EM}) $CD4^+$ T cells, and modulations of cytokine productions and gene expressions, as proved by flow cytometry and RNA-seq analysis [69]. Thus, platelet-derived mitochondria may function as novel immune modulators to treat T1D and other autoimmune diseases.

3.3. B cells

Even though T cells are generally considered the major pathogenic effector cells contributing to the destruction of islet β cells, increasing evidence indicates that B cells are important effector cells involved in the pathogenesis of autoimmune-caused T1D through the production of autoantibodies, the promotion of $CD4^+$ T cell responses through antigen presentation, and the release of inflammatory cytokines (e.g., TNF-α and IL-6) [70,71]. Researchers have found that blocking B cells or impairing B cell function will significantly decrease the incidence of diabetes in NOD mice [72,73]. Thus, it is needed to correct B cell-associated immune dysfunctions for the treatment of T1D and other autoimmune diseases. Pescovitz et al. reported that the depletion of B cells with anti-human CD20 antibody (Rituximab) markedly preserved islet β-cell function and improved C-peptide levels after 1 year follow-up in recent-onset T1D patients [74]. Recently, our study demonstrated that CB-SC markedly suppressed the proliferation of activated B cells and reduced the antibody productions in these activated B cells through galectin-9-mediated cell-cell contacting mechanism. Notably, further phenotypic analysis revealed that treatment with CB-SC increased the percentage of IgD^+CD27^- naïve B cells, but markedly decreased the percentage of IgD^-CD27^+ switched memory B cells [75]. Therefore, these data clearly advance our understanding about the molecular mechanism of Educator therapy for the treatment of T1D and other autoimmune diseases. Due to the essential role of B cells in the pathogenesis of younger T1D patients ($\leq$ 7 years old) [[6][7]], it is preferred to utilize the open-loop system of Educator therapy to treat this group of patients, which have a longer incubation time for direct modulation between CB-SC and patient's B cells via the galeptin-9/IgM BCR or CD45 signaling pathways (Fig. 2) [75–78].

4. Improve the islet β-cell function and metabolic control by Stem Cell Educator Therapy

4.1. Educator therapy reverses T1D via islet β cell regeneration

The shortage of islet β cells is a crucial issue that must be addressed in any cure for T1D. By the time T1D patient is diagnosed, 70–80% of total islet β-cell mass has been wiped out. It is essential, therefore, to protect these residual β cells and promote β-cell regeneration. Our phase 1/2 clinical trial [16] in adults with long-standing T1D revealed that a single treatment with Educator therapy led to lasting reversal of autoimmunity, regeneration of islet β cells, and improvement of metabolic control. Participants in Group A (those with moderate T1D and some residual β cell function) exhibited improved fasting C-peptide (a by-product of insulin production) levels at 12 and 24 weeks post-treatment, and participants in Group B (those with severe T1D and no residual islet β cell function) displayed successive improvement in fasting C-peptide levels at each follow-up [16]. Notably, participants in Group B exhibited essentially no C-peptide production following glucose challenge at baseline, but proved a marked improvement at 12 weeks, which was maintained through the final follow-up (40 weeks posttreatment). Consistent with the improved β-cell function, their median daily doses of insulin were reduced 38% for Group A and 25% for Group B subjects at 12 weeks post treatment with Educator therapy [16]. In contrast, participants in the Control Group did not exhibit significant change at any follow-ups [16]. After control subjects received the real treatment with Educator therapy, their fasting C-peptide values were markedly increased to the normal range with significant reduction in the need for exogenous insulin (unpublished data). This was the first clinical study that demonstrated the regeneration of islet β cells in long-standing T1D patients after receiving Educator therapy.

4.2. Improve islet β-cell functions by platelet-derived mitochondria

Our clinical studies have demonstrated long-lasting β-cell functional improvement in T1D patients after Educator therapy, supporting the hypothesis that Educator therapy promotes lasting reversal of autoimmunity, allowing for regeneration of residual islet β cells. However, the detailed mechanisms underlying β-cell recovery remain unclear. Recently, Zhao et al. found that human pancreatic islet β cells can be reprogrammed to proliferate while maintaining good cell viability and restoring normal β-cell function by taking up platelet-releasing mitochondria via the β-cell expressions of CD29 and TLR4 molecules [29]. Using the insulin byproduct C-peptide as indicator for β cells, flow cytometry demonstrated that the percentage of C-peptide$^+$Ki67$^+$ islet β cells was markedly enriched following co-culture of freshly-isolated human islets with the purified platelet-derived mitochondria, and functional analysis demonstrated that the islet β cells exhibited improved C-peptide release in response to insulin secretagogues (e.g., 16.7 mM high glucose and tolbutamide) [29]. These data indicated the improvement of islet β-cell function after treatment with platelet-derived mitochondria. Platelets are the 2nd largest population of blood and have functional mitochondria. Clinical data established that both platelets' numbers and function were improved in T1D patients after receiving Educator therapy [29], which may release more mitochondria into the blood circulation [67] and then migrate into pancreatic islets through their expressions of chemokines and receptors [29], leading to the stimulation of residual β-cell regeneration. Thus, these innovative approaches may open up new avenues to protect and enrich islet β cells by using platelet-derived mitochondria.

5. Conclusions

Stem Cell Educator therapy offers a principally new therapeutic approach for T1D because it can modulate multiple immune cells (Fig. 2) and reverse the destruction of islet β cells, both of which are critical for effective treatment of T1D. Preclinical studies in NOD mice revealed the restoration of islet architectures after receiving the treatment with CB-SC-modulated Tregs, leading to the prevention and reversal of autoimmune-caused T1D [27,28]. International multicenter clinical trials confirmed the long-lasting clinical efficacy of Educator therapy for the treatment of T1D [16,17,29] and other inflammation-associated diseases [18,19]. Importantly, by using a patient's own immune cells that are "educated" by CB-SC, Educator therapy avoids the

safety and ethical concerns associated with other conventional immunological approaches. Additional pilot studies demonstrated the therapeutic potentials of Educator therapy to heal patients with other autoimmune or inflammation-associated diseases such as lupus, psoriasis, Hashimoto's disease, and Sjogren syndrome. Educator therapy has gained phase 2 clinical trial approval by the FDA for T1D treatment (IND19247, NCT04011020). As a global-leading technology in the field of T1D treatment (Juvenile Diabetes Cure Alliance Report, 2021, New York), it is highly expected that Educator therapy will achieve the expedited FDA approval under the designation of Regenerative Medicine Advanced Therapy (RMAT), due to an unmet medical need for the life-threating T1D patients. Further mechanistic studies in clnical trials will provide an in-depth understanding of the molecular mechanisms underlying Educator therapy, which is necessary to enhance treatment efficacy and islet β-cell recovery. Collectively, Educator therapy has the potential to revolutionize the treatment of T1D and eliminate the need for lifelong insulin therapy, without the safety and ethical concerns associated with conventional immune and/or stem cell-based approaches.

Authors contribution

Y.Z.: contributed to concepts, draft review article, and final approval of article; C.K., Z.J., and E.D.: clinical principal investigators, review the article and provide materials. A.V. H., S. G., Z.Z., H.Z., H.Y., W.H., H.L., X. L., M. P., L.Z., Ye. Z., J. G., R. W., and T. M. editing the review article.

Declaration of Competing Interest

Dr. Yong Zhao is an inventor for Stem Cell Educator and CB-SC technologies and has fiduciary roles at Throne Biotechnologies (Throne). Dr. Elias Delgado is a clinical advisor at Throne. Dr. Rona Weinberg is a scientific advisor at Throne. Dr. Theodore Mazzone is one of the inventors for CB-SC technology and a chief medical officer at Throne. All other authors have no financial interests that may be relevant to the submitted work.

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