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Review

Human cord blood stem cells and the journey to a cure for type 1 diabetes

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Umbilical cord blood contains several types of stem cells that are of interest to a wide range of disciplines in regenerative medicine. The translational potential to the clinical applications of cord blood stem cells has increased enormously in recent years, mainly because of its advantages including no risk to the donor, no ethical issues, low risk of graft-versus-host disease (GVHD) and rapid availability. Type 1 diabetes (T1D) is an autoimmune disease caused by an autoimmune destruction of pancreatic islet β cells. Understanding the nature and function of cord blood stem cells is an exciting challenge that might set the stage for new approaches to the treatment of T1D. Here, we review progress in this field and draw conclusions for the development of future therapeutics in T1D. New insights are provided on a unique type of cord blood-derived multipotent stem cells (CB-SC), including the molecular mechanisms underlying immune modulation by CB-SC, protection of β-cell mass, and promotion of islet β-cell neogenesis.

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Contents

1. Introduction . . . 103
2. Introduction of stem cells in cord blood . . . 104
3. Control of autoimmunity and related molecular mechanisms . . . 104
    3.1. Suppression of the proliferation of T1D patient-derived pancreatic islet β-cell specific diabetic T cell clone . . . 104
    3.2. Prevention and reversal of type 1 diabetes by correcting the functional defects of Tregs . . . 104
4. Protection of pancreatic islet β cell mass and related molecular mechanisms . . . 104
    4.1. Restoration of islet basement membrane . . . 104
    4.2. Formation of a TGF-β1 ring . . . 105
    4.3. Promoting vascularization . . . 105
5. Promotion of β cell regeneration (neogenesis) . . . 106
    5.1. Promoting β cell replication . . . 106
    5.2. Differentiation of cord blood stem cells into insulin-producing cells . . . 106
    5.3. Induced pluripotent stem cells (iPS) . . . 106
6. Conclusions . . . 106
Take-home messages . . . 107
Acknowledgments . . . 107
References . . . 107

1. Introduction

Stem cells have been characterized in most human tissues. They contribute to tissue differentiation, development, regeneration, remodeling, and replenishing of aged and/or diseased tissues. These tissue-specific stem cells usually display considerable heterogeneity and are difficult to isolate for cell-based therapy. As a special tissue, cord blood circulates in the umbilical cord that connects the developing embryo or fetus with the placenta. The formation of the umbilical cord starts as early as the third week of embryonic

Abbreviations: CB-SC, cord blood-derived multipotent stem cells; EPC, endothelial progenitor cells; GVHD, graft-versus-host disease; iPS, induced pluripotent stem cells; MHC, major histocompatibility complex; MSC, mesenchymal stem cells; NOD, nonobese diabetic; TGF β1, transforming growth factor β1; T1D, type 1 diabetes; Tregs, regulatory T cells.

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development; the embryo is attached to the placenta via a connecting stalk (the beginning of umbilical cord). During embryonic development, oxygen and nutrition are transported through the umbilical cord blood between the placenta and the fetus. After delivery, umbilical cord blood and/or their stem cells can be easily collected and banked for clinical applications. This is supported by extensive clinical evidence with no risk to the donor, no ethical issues, low risk of graft-versus-host disease (GVHD) and rapid availability [1]. The unique nature of human umbilical cord blood provides an alternative source to generate stem cells for regenerative medicine.

Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease that results in a shortage of pancreatic islet β cells. Millions of T1D patients worldwide must have daily insulin injections to stay alive. However, it is not a cure. To date, many approaches have been investigated to find a cure for T1D [2–9]. All in all, control of autoimmunity is the top priority for the prevention and treatment of T1D, followed by the provision of β-cell surrogates and reconstruction of functional islets (e.g., transplanted islets and stem cell-derived insulin-producing cells). If unaddressed, the autoimmune cells can destroy those β-cell surrogates and limit their therapeutic potential. Due to the polyclonal nature of the autoimmune response and the global problem of immune regulation in T1D, approaches that individually targeting different arms of the immune system have been uniformly unsuccessful in T1D prevention trials [3]. Thus, combinations of individual therapies have been proposed for immune interventions. Stem cells hold great promise for the treatment of T1D in overcoming the shortage of insulin-producing cells. For a long time, most work on stem cells has focused on regulating their differentiation to replace a specific physiologic function. Recently, increasing evidence has demonstrated that stem cells function as an immune modulator that can lead to control of the immune responses, which could in turn be used as a new approach to overcome the autoimmunity of T1D [4,7,10]. The multiple aspects of immune modulations can be achieved via co-culture with stem cells. During co-culture, T1D-derived effector T cells and/or Tregs can be educated by the favorable microenvironment created by CB-SC or MSC through cell to cell contact and soluble factors [4,10,11]. This review emphasizes the recent progress in work using cord blood stem cells in T1D research. Focusing on use of these cells addresses three key issues — control of the autoimmunity, protection of islet β-cell mass, and overcoming the shortage of insulin-producing cells.

2. Introduction of stem cells in cord blood

Human cord blood contains several types of stem cells including hematopoietic stem cells (HSC), multipotent stem cells that have been designated cord blood-derived stem cells (CB-SC) [12], mesenchymal stem cells (MSC), endothelial progenitor cells (EPC), and monocyte-derived stem cells [13]. Phenotypic characterizations of HSC, MSC and EPC have been reviewed recently [14]. Here, we focus on the novel type of stem cells — CB-SC. We identified CB-SC as a unique type of stem cell by virtue of their capability to attach to a plastic surface of non-tissue cultured-treated Petri dishes [10,12] (Fig. 1). Phenotypic characterization demonstrates that CB-SC display embryonic cell markers (e.g., transcription factors OCT-4 and Nanog, stage-specific embryonic antigen (SSEA)-3, and SSEA-4) and leukocyte common antigen CD45, but they are negative for blood cell lineage markers (e.g., CD1a, CD3, CD4, CD8, CD11b, CD11c, CD13, CD14, CD19, CD20, CD34, CD41a, CD41b, CD83, and CD133). Additionally, CB-SC displayed very low immunogenicity as indicated by expression of a very low level of major histocompatibility complex (MHC) antigens and failure to stimulate the proliferation of allogeneic lymphocytes [11,12]. They can give rise to three embryonic layer-derived cells in the presence of different inducers (Fig. 1). More specifically, CB-SC tightly adhere to culture dishes with a large rounded morphology and are resistant to common detaching methods (trypsin/EDTA), making it easy to collect suspended lymphocytes after co-culture [10–12]. *In vitro* and *in vivo* experiments have demonstrated the therapeutic potential of CB-SC in T1D.

3. Control of autoimmunity and related molecular mechanisms

3.1. Suppression of the proliferation of T1D patient-derived pancreatic islet β-cell specific diabetic T cell clone

In healthy donor-derived T cells, CB-SC display an immune modulation on $CD4^+$ T cells and $CD8^+$ T cells via surface molecular program death ligand-1 (PD-L1) and secreted nitric oxide (NO) [11], with similar mechanisms as the immune modulation of MSC [4,15]. CB-SC can markedly suppress the proliferation of mitogen phytohemagglutinin (PHA)-stimulated lymphocytes [11]. To further determine the therapeutic potential of CB-SC in T1D, we explored the direct modulation of CB-SC on islet β-cell glutamic acid decarboxylase (GAD)-specific $CD4^+$ T cell clones generated from T1D patients. Results demonstrated that the proliferation of this T cell clone stimulated with antigen-presenting cells (APC) and different doses of GAD peptide were markedly and specifically decreased in the presence of CB-SC compared to control group in the absence of CB-SC (Fig. 2). This suppression was further confirmed by flow cytometry on the carboxyfluorescein succinimidyl ester (CFSE)-labeled T cells (data not shown). Thus, these data indicate that CB-SC have a potential to suppress the GAD-specific T cells.

3.2. Prevention and reversal of type 1 diabetes by correcting the functional defects of Tregs

Regulatory T cells (Tregs) represent the specialized T cell population that plays a crucial role in maintaining homeostasis and self-tolerance through their inhibitory impact on autoreactive effector T cells [16]. Although defects of effector T cells or antigen-presenting cells could play a role, increasing evidence demonstrates that abnormalities of Tregs, either in cell number or in function, are associated with initiation and progression of T1D, both in diabetic patients and animal models [16,17]. Manipulation of Tregs is becoming the most attractive research focus for developing a successful immunotherapy to prevent and treat T1D. We are the first group using CB-SC to correct functional defects of $CD4^+CD62L^+$Tregs, leading to prevention of diabetes onset and the reversal of overt diabetes in an autoimmune-caused diabetic nonobese diabetic (NOD) mouse model [7,10]. Mechanistic studies demonstrated that control of diabetes was correlated with systemic immune alterations including restoration of Th1/Th2 cytokine balance in blood, as well as local regulation in pancreatic islets through a unique distributional pattern of TGF-β1 ('a TGF-β1 ring') that may protect islet β cells against the infiltrated lymphocytes. In addition, clinical trials in T1D patients also demonstrate that Tregs play an essential role in the control of autoimmunity. Haller et al. observed that autologous cord blood transfusion is safe and provides some slowing of the loss of endogenous insulin production in children with T1D due to the highly functional populations of Treg in cord blood [18].

4. Protection of pancreatic islet β cell mass and related molecular mechanisms

4.1. Restoration of islet basement membrane

Healthy pancreatic islets physiologically display a dense capillary network with a specialized basement membrane located around the pancreatic islets. Laminin 511 is the major component of islet basement membrane in humans [19]. As a natural barrier, these basement membranes play a key role in the protection of pancreatic islet architecture, the maintenance of homeostasis of pancreatic

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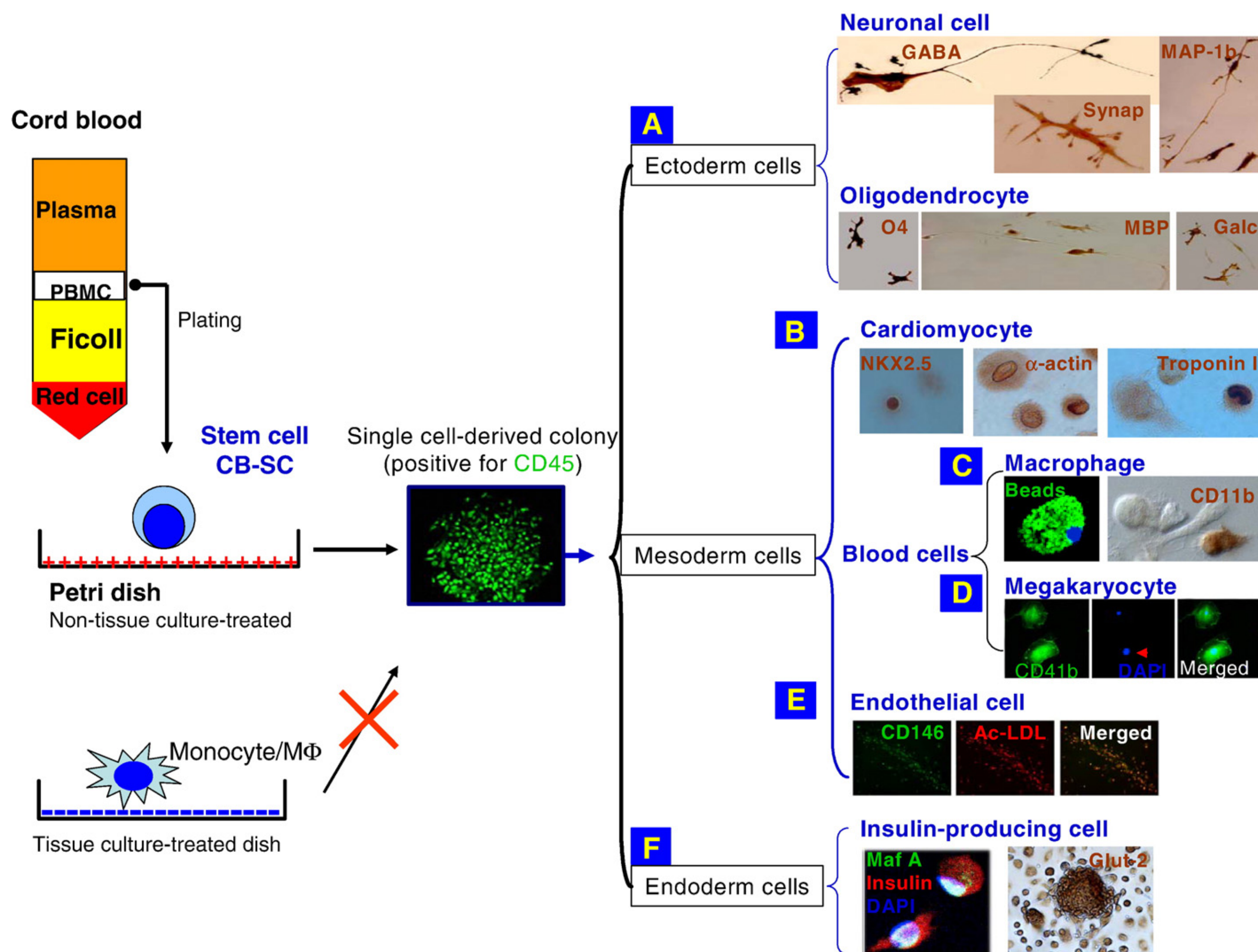

Fig. 1. Human cord blood-derived stem cells (CB-SC) give rise to multiple lineages in response to different physiological growth factors and inducers. CB-SC were isolated from human umbilical cord blood using Ficoll-Hypaque (γ = 1.077) and plated in non-tissue culture-treated Petri dishes in RPMI 1640 medium supplemented with 7% fetal bovine serum (left panels), with a formation of single cell-derived colony positive for leukocyte common antigen CD45 (middle panel). (A) Differentiation to neuronal cell and oligodendrocyte. CB-SC were treated with 100 ng/ml NGF for 10–14 days and characterized with lineage markers including γ-aminobutyric acid (GABA), microtubule associated protein (MAP) 1B, synaptophysin (Synap), sulfatide O4, myelin basic protein (MBP), galactocerebroside (Galc). (B) Differentiation to cardiomyocytes. CB-SC were treated with a chemical 3 μM 5-aza-2′ deoxycytidine for 24 h, followed by testing with cardiomyocyte markers including nuclear transcription factor Nkx2.5, cardiomyocyte specific α-actin and troponin I. (C) Differentiation to macrophages. CB-SC were treated with 50 ng/ml M-CSF for 7–10 days and then characterized with phagocytosis of fluorescence beads and surface marker CD11b/Mac-1. (D) Differentiation to megakaryocytes. CB-SC were treated with 10 ng/ml TPO for 10–14 days and then characterized with specific marker CD41b and polyploidy nuclear (red arrow). (E) Differentiation to endothelial cells. CB-SC were treated with 50 ng/ml VEGF for 10–14 days, and then characterized with specific marker CD146 and incorporation of the acetylated low density lipoprotein (Ac_LDL). (F) Differentiation to insulin-producing cells. CB-SC were treated with 10 nM exendin-4 + 25 mM glucose for 5–8 days and characterized with markers for β cell marker insulin and Glut2. Isotype-matched IgG served as negative controls for every experiment.

microenvironment, and optimizing islet function (e.g., insulin expression, β-cell function and proliferation) [19,20]. Different from rodent islets, Virtanen et al. revealed a unique feature in the human islets; the capillaries are surrounded by a double basement membrane [19]. These natural barriers form the front lines against autoimmune destruction in T1D. Using a NOD mouse model, Irving-Rodgers et al. found that degradation of the islet basement membrane marks the onset of destructive autoimmune insulitis and T1D development [21]. Restoration of the integrated islet basement membrane will be critical to a cure for T1D.

4.2. Formation of a TGF-β1 ring

During the pathogenesis of T1D, islet architecture is destroyed by the infiltration of autoimmune cells [10]. Importantly, we found that treatment with mCD4CD62L Tregs can reconstitute the islet cell architecture, as demonstrated by the fact that pancreatic islets in diabetic NOD mice treated with mCD4CD62L Tregs displayed a similar pattern of α- and β-cell distribution as that noted in normal islets of non-diabetic NOD mice [10]. Transforming-growth factor-beta (TGF-beta) is a pleiotropic growth factor that is produced by most cells in the human body. It is known that TGF-β1 is a key regulator of the production and re-modeling of the extracellular matrix through its effect on stromal cells. Due to the multiple function of TGF-β1 on endocrine β-cell differentiation [22] and rearrangement of stromal cells [22–24], the formation of "TGF-β1 ring" may play a key role in this reconstitution of islet architecture.

4.3. Promoting vascularization

Pancreatic islets are highly vascularized tissue receiving 5–10% of total pancreatic blood flow. Due to the production of angiogenic factors such as vascular endothelial growth factor-A (VEGF-A) and angiopoietin-1 in islets, blood vessels within pancreatic islets are of a greater density than those in surrounding exocrine tissue and are lined with fenestrated endothelial cells [25,26]. This enriched blood

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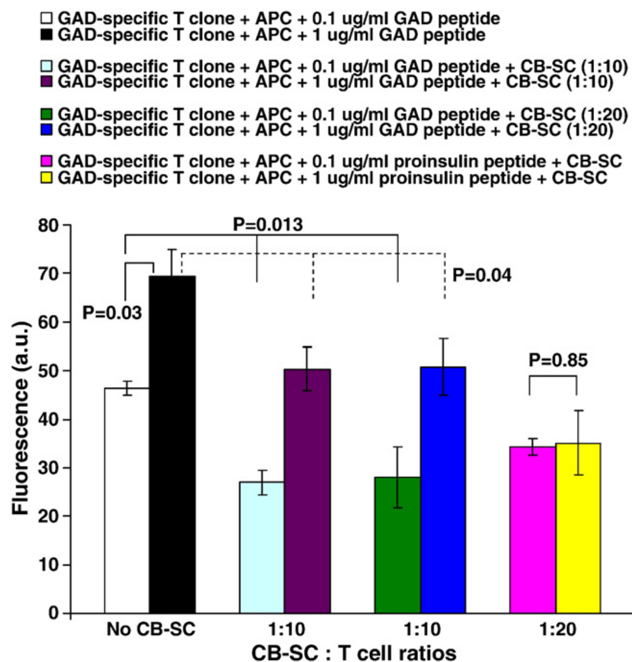

Fig. 2. Cell proliferation assay.T1D patient-derived GAD-specific CD4$^+$ T cell clone was co-cultured for 3 days with CB-SC in the presence of antigen-presenting cells (APC) and specific GAD peptide or non-specific control pro-insulin peptide. Data are shown as mean ± s.d. from three experiments.

circulation provides the islet cells with nutrients and oxygen, and in turn transfers the secreted islet hormones to target tissues. This physiological feature is destroyed by autoimmune cells in T1D and needs to be addressed for the treatment of T1D.

Human cord blood contains several potential endothelial-generating cells that may contribute to the angiogenesis and revascularization of damaged islets. For example, Au et al. investigated the potential of umbilical cord blood-derived EPCs for the *in vivo* vasculogenesis. They found that umbilical cord blood EPCs form normal-functioning blood vessels that last for more than 4 months. These vessels exhibit normal blood flow, perm-selectivity to macromolecules, and induction of leukocyte–endothelial interactions in response to cytokine activation similar to normal vessels [27]. We and others have demonstrated that CD14$^+$ monocytes can de-differentiate into stem cells that give rise to endothelial cells [6,13,28]. Human MSC possess considerable potential for vascular repair and regeneration therapies via their differentiation toward endothelial cells. Using a co-culture model, Park et al. observed that cord blood-derived MSC can improve the survival, function, and angiogenesis/revascularization of islets via secretion of trophic molecules including interleukin-6 (IL-6), vascular endothelial growth factor-A (VEGF-A), hepatocyte growth factor (HGF), and transforming growth factor-beta (TGF-beta) [29]. Finally, our *in vitro* data demonstrate that CB-SC can turn into endothelial-like cells in the presence of VEGF treatment [12].

5. Promotion of β cell regeneration (neogenesis)

5.1. Promoting β cell replication

Our previous work confirmed that treatment with mCD4CD62L Tregs not only diminishes the autoimmunity, but also eliminates the hyperglycemia via promotion of islet β-cell regeneration and restoration of islet normal architecture (β and α cell proportion) [10]. The *de novo* proliferation of β cells, with a high expression of a cell proliferation nuclear marker Ki67, accounts for the noted increase in total β cell mass leading to the euglycemia in diabetic NOD mice after treatment with mCD4CD62L Tregs. Molecular mechanistic studies revealed that TGF-β signaling may contribute to β-cell regeneration after treatment with mCD4CD62L Tregs in overt diabetic NOD mice [7].

5.2. Differentiation of cord blood stem cells into insulin-producing cells

Stem cells in cord blood can differentiate into functional insulin-producing cells in response to different inducers, as supported by following studies. *In vivo* differentiation demonstrated that CB-SC can give rise to functional insulin-producing cells after administration to the chemical streptozotocin (STZ)-induced diabetic NOD-scid mice, as indicated by the production of human C-peptide in mouse plasma and reduction of hyperglycemia [12]. Another *in vivo* experiment demonstrated that approximately 25% of the grafts exhibit human insulin production after 9 weeks of transplantation; interestingly, the number and abundance of pro-insulin transcript-containing cells increased when the animals underwent partial pancreatectomy, 15 days after transplantation [30]. Additionally, treatment with different inducers can turn cord blood-derived MSC into insulin-producing islet-like structures, which co-express insulin and C-peptide and other β cell-related markers [31,32]. This is in contrast to the bone marrow-derived MSC [33]. Using an established protocol that differentiates mouse embryonic stem cells (ESCs) toward the pancreatic phenotype, Denner et al. showed that cord blood-derived lineage negative, CD133$^+$ and CD34$^+$ cells can be differentiated into insulin-producing cells [34]. These data therefore prove the concept that stem cells in cord blood can produce insulin. However, *in vivo* evaluations are required to further prove that these *in vitro* differentiated insulin-producing cells are really functional in glycemic control.

5.3. Induced pluripotent stem cells (iPS)

Generation of iPS can circumvent the shortage of donors and alloimmunity entirely. Giorgetti et al. generated iPS from human umbilical cord blood using retroviral transduction with only two factors (OCT4 and SOX2) in 2 weeks and without the need for additional chemical compounds [35]. Maehr et al. showed that iPS cells can be generated from patients with T1D by reprogramming their adult fibroblasts with three transcription factors (OCT4, SOX2, KLF4). T1D-specific iPS cells have the hallmarks of 12 pluripotency and can be differentiated into insulin-producing cells [36]. The clinical applications of iPS are a very long way off due to the possibility that transplanted cells could conceivably change their phenotype and functions *in vivo* and have harmful effects on patients. Such alterations in phenotype or effect could depend on a patient's genotype or exposure to environmental factors, such as infection [36,37]. Importantly, it is vital to make iPS that are tolerated to the autoimmunity of T1D patients; otherwise, they will be will be rapidly recognized and destroyed by the autoimmune cells [37]. These primary hurdles need to be solved before iPS become a real cure for T1D.

6. Conclusions

Since the first sibling-donor cord blood transplant was performed by Rubinstein in 1988 [38], cord blood transplant has been widely applied as an alternative safe and rapid source of blood transfusion in clinics. There are different types of stem cells in cord blood that hold great promise dealing in with many facets of T1D treatment. Successful cell-based therapies depend on addressing those three key issues simultaneously in T1D. For most cell-based therapies, HLA compatibility and cell dose are major concerns that impose constraints and affect their introduction into the clinic. However, using current banking technology, autologous cord blood stem cells can be used to treat T1D patients [18,39], without the need for HLA matching.

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Importantly, emerging clinical evidence [40] demonstrates that cord blood stem cells (e.g., $CD34^{+}$ hematopoietic stem cells) can be transplanted into unrelated recipients due to their low immunogenicity. The major disadvantage of cord blood transplantation is the low number of stem cells that limit their practical application. Therefore, an ex-vivo expansion is required to generate sufficient numbers of cells while retaining stem cell properties. Cord blood stem cells can "educate" the patient's immune cells to reduce autoimmunity. The challenge now is how to use these stem cells that target the autoimmunity of T1D, systemically or locally? In humans, due to the possibly complex triggers for the autoimmunity of T1D, including physical, psychological, social, and environmental factors, a comprehensive approach to treatment is necessary for a true cure of T1D. Precise understanding of the molecular mechanisms of cord blood stem cells in immune modulation and β-cell differentiation can potentially open up an exciting area for the development of new therapeutics for human T1D.

Take-home messages

- CB-SC is a unique type of stem cell in cord blood.
- Cord blood stem cells cannot only differentiate into the insulin-producing cells for islet β surrogates, but also function as an immune modulator that can lead to the control of the autoimmune responses in T1D.
- The survival and protection of residual β cells are crucial for the treatment of T1D as earlier as possible after onset.
- Promotion of β-cell neogenesis must be part of any therapy aimed at T1D.
- The "ring of TGF-β1" in pancreatic islets creates a tolerogenic barrier against the autoimmune cells; this, in turn, leads to the regeneration of β cells and reconstitution of islet architecture after treatment with mCD4CD62L Tregs.

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