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Research Article

Modulation of Autoimmune T-Cell Memory by Stem Cell Educator Therapy: Phase 1/2 Clinical Trial

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ABSTRACT

Background: Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease that causes a deficit of pancreatic islet β cells. The complexities of overcoming autoimmunity in T1D have contributed to the challenges the research community faces when devising successful treatments with conventional immune therapies. Overcoming autoimmune T cell memory represents one of the key hurdles.

Methods: In this open-label, phase 1/phase 2 study, Caucasian T1D patients (N = 15) received two treatments with the Stem Cell Educator (SCE) therapy, an approach that uses human multipotent cord blood-derived multipotent stem cells (CB-SCs). SCE therapy involves a closed-loop system that briefly treats the patient's lymphocytes with CB-SCs in vitro and returns the "educated" lymphocytes (but not the CB-SCs) into the patient's blood circulation. This study is registered with ClinicalTrials.gov, NCT01350219.

Findings: Clinical data demonstrated that SCE therapy was well tolerated in all subjects. The percentage of naïve $CD4^+$ T cells was significantly increased at 26 weeks and maintained through the final follow-up at 56 weeks. The percentage of $CD4^+$ central memory T cells (T_{CM}) was markedly and constantly increased at 18 weeks. Both $CD4^+$ effector memory T cells (T_{EM}) and $CD8^+$ T_{EM} cells were considerably decreased at 18 weeks and 26 weeks respectively. Additional clinical data demonstrated the modulation of C–C chemokine receptor 7 (CCR7) expressions on naïve T, T_{CM}, and T_{EM} cells. Following two treatments with SCE therapy, islet β-cell function was improved and maintained in individuals with residual β-cell function, but not in those without residual β-cell function.

Interpretation: Current clinical data demonstrated the safety and efficacy of SCE therapy in immune modulation. SCE therapy provides lasting reversal of autoimmune memory that could improve islet β-cell function in Caucasian subjects.

Abbreviations: AIRE, autoimmune regulator; CB-SCs, human cord blood-derived multipotent stem cells; CCR7, C–C chemokine receptor 7; HbA_1C, glycated hemoglobin; HLA, human leukocyte antigen; IL, interleukin; MLR, mixed leukocyte reactions; MNC, mononuclear cells; M2, muscarinic acetylcholine receptor 2; OGTT, oral glucose tolerance test; PBMC, peripheral blood mononuclear cells; R, responder; SCE, Stem Cell Educator; S, stimulator; T_{CM}, central memory T cells; TCR, T-cell receptor; T_{EM}, effector memory T cells; TGF-β1, transforming growth factor-β1; Th, helper T cell; T1D, type 1 diabetes; Tregs, regulatory T cells.

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1. Introduction

Type 1 diabetes (T1D) is a major global health issue, and its incidence is increasing. T1D is a T cell-mediated autoimmune disease that reduces the population of pancreatic islet β cells, which limits insulin production and interferes with glucose homeostasis. The immune dysfunction in T1D is complicated, with effects both in pancreatic islets and outside the pancreas. Different components of the immune system [e.g., $CD4^+$, $CD8^+$ T cells, Tregs, B cells, dendritic cells (DCs), monocyte/macrophages (Mo/Mϕs), natural killer T cells (NKTs)] contribute to autoimmune responses in T1D, complicating efforts to develop successful treatments or a cure that will work across most or all individuals with the disease. Several recent clinical trials (Bach, 2011; Wherrett et al., 2011) highlight the challenges in conquering T1D, but their failures provide some valuable lessons about the limitations of conventional immune therapy and the future direction of the quest. Specifically, they point to the need for an approach that produces comprehensive immune modulation at both the local pancreatic and systematic levels rather than targeting the pancreatic effects of one or a few components of the immune system. The Stem Cell Educator therapy takes this broader approach (Zhao and Mazzone, 2010; Zhao et al., 2012; Zhao, 2012; Zhao et al., 2013; Li et al., 2015).

Physiologically, the human immune system constantly protects the body against a variety of pathogens that may be encountered. Following the recognition and eradication of pathogens through adaptive immune responses, the majority (90–95%) of T cells undergo apoptosis with the remaining cells forming a pool of memory T cells, designated central memory T cells (T_{CM}), effector memory T cells (T_{EM}), and resident memory T cells (T_{RM}) (Clark, 2015). In comparison to conventional T cells, these memory T cells are long-lived with distinct phenotypes, such as expression of specific surface markers, rapid production of different cytokine profiles, capability of direct effector cell function, a different potential for proliferation, and unique homing distribution patterns. As a group, memory T cells display quick reactions upon re-exposure to their cognate antigens in order to eliminate the reinfection of pathogens and restore balance and harmony of the immune system. Nevertheless, increasing evidence establishes that autoimmune memory T cells become the "stumbling blocks" and hinder most attempts to treat or cure autoimmune diseases, including T1D, multiple sclerosis (MS), rheumatoid arthritis (RA), and system lupus erythematosus (SLE) (Ehlers and Rigby, 2015; Clark, 2015; Devarajan and Chen, 2013). Therefore, novel and more comprehensive approaches are needed to fundamentally correct the inordinate dominance of autoimmune T cell memory and overcome the complexities of autoimmune responses.

We previously characterized a new type of stem cell from human cord blood, designated a cord blood-derived multipotent stem cell (CB-SCs) (Zhao et al., 2006; Zhao and Mazzone, 2010). CB-SCs display a unique phenotype with both embryonic and hematopoietic markers that distinguish them from other known stem cell types, including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and monocytes/macrophages (Mo/Mϕ) (Zhao et al., 2003). SCE therapy functions as an "artificial thymus" that circulates a patient's blood through a blood cell separator, briefly treats the patient's lymphocytes with CB-SCs in vitro, induces immune tolerance through the action of autoimmune regulator (AIRE, expressed by CB-SCs), returns the educated autologous lymphocytes to the patient's circulation, and restores immune balance and homeostasis (Zhao and Mazzone, 2010; Zhao et al., 2012; Zhao, 2012). This approach was piloted in clinical studies for the treatment of diabetes and other autoimmune diseases in China with patients of Chinese origin (Zhao et al., 2012; Zhao, 2012; Zhao et al., 2013; Li et al., 2015). Our clinical data demonstrates that the SCE therapy provides long-lasting reversal of autoimmunity that induces the regeneration of pancreatic islet β cells and improvement of metabolic control in individuals with longstanding T1D (Zhao et al., 2012). Findings from recent autoimmune-caused Alopecia Areata (AA) trial provide visible evidence that SCE therapy can control autoimmunity and lead to the regeneration of tissues like hair regrowth (Li et al., 2015). Here, we explored the expansion of the therapeutic potential of the SCE therapy to the treatment of Caucasian T1D subjects in Spain.

2. Methods

2.1. Patients

T1D patients receiving care at the Endocrinology and Nutrition Service, Hospital Universitario Central de Asturias (Oviedo, Spain) were enrolled in this phase 1/phase 2, open-label clinical trial conducted from November 27, 2012 through October 1, 2014. The principal investigator designed the clinical trial and received ethical approval for the clinical treatment protocol and consent form from Regional Committee for Clinical Research Ethics and the Comisión Permanente de Trasplantes del Consejo Interterritorial del Sistema Nacional de Salud. The signed informed consent was obtained from each participant. The clinical trial was conducted in 15 subjects with established T1D (Table 1). Subjects were qualified for recruitment if they met the 2012 diagnosis standards of the American Diabetes Association (ADA) and if a blood test indicated the presence of at least one autoantibody to pancreatic islet β cells (Fig. 1). Key exclusion criteria included clinically significant liver (AST or ALT 2 ≥ x upper limit of normal), kidney (creatinine ≥ 2.0 mg/dl), or heart disease; pregnancy or breastfeeding mothers; immunosuppressive medication; known active infection with viral diseases; or diseases associated with immunodeficiency; or hemoglobin <10 g/dl or platelets <100 k/ml; use of immunosuppressive medication within one month.

2.2. SCE Therapy and Follow-up

All the participants received two treatments with the SCE (Tianhe Stem Cell Biotechnology®, USA). The preparation of CB-SC cultures and SCEs was performed as previously described (Zhao et al., 2012). Briefly, human cord blood units derived from healthy allogeneic donors were obtained from Centro Comunitario de Sangre y Tejidos de Asturias (CCST, Oviedo, Spain). All cord blood samples were screened for HIV I&II, HBsAg, HBcAg, HCV, HIVNAT, STS, HBVNAT, HCVNAT, HTLV I/II, West Nile, Chagas, and CMV, and only pathogen-free cord blood units were used for clinical treatment. Human CB-SCs were produced as previously described (Zhao et al., 2006, 2009) with the following modifications. Cord blood mononuclear cells were plated in SCE devices in serum-free culture medium (Lonza, Walkersville, MD) and incubated at 37 °C, in 8% CO_2. After 2–3 weeks, CB-SCs growing at 90% confluence (about 10^7 cells/device) were prepared for clinical trial. CB-SCs were characterized by flow cytometry, using markers such as the leukocyte common antigen CD45 and embryonic stem (ES) cell-specific transcription factor OCT3/4 (Fig. S1). The measured endotoxin level was <0.05 EU/ml. One Educator device was generated from one cord blood unit, and used for one subject at one treatment.

For the SCE therapy (Zhao et al., 2012, 2013), a 16-gauge IV needle was placed in the left (or right) median cubital vein, and the patient's