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Autologous Umbilical Cord Blood Infusion for Type 1 Diabetes

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Abstract

Objective—The physical, emotional, and economic costs of type 1 diabetes (T1D) mandate continued efforts to develop effective strategies to prevent or reverse the disease. Herein, we describe the scientific and therapeutic rationale underlying efforts utilizing umbilical cord blood (UCB) as a therapy for ameliorating the progression of this autoimmune disease.

Patients and Methods—We recently embarked on a pilot study to document the safety and potential efficacy of autologous UCB infusion in subjects with T1D. Under this protocol, patients recently diagnosed with the disease and for whom autologous cord blood is stored, undergo infusion. Studies are performed before infusion and every 3–6 months post-infusion for immunologic and metabolic assessment. To date 15 autologous infusions have been performed.

Results—Preliminary observations suggest that autologous cord blood transfusion is safe and provides some slowing of the loss of endogenous insulin production in children with T1D. Mechanistic studies demonstrate that umbilical cord blood contains highly functional populations of regulatory T cells (Treg) and that increased Treg populations may be found in the peripheral blood of subjects more than 6 months after cord blood infusion. We provide the rationale for cord blood based therapies, a summary of our initial protocol, and plans for future studies designed to explore the potential of cord blood derived regulatory T cells to treat T1D.

Conclusions—Prolonged follow up and additional mechanistic efforts are urgently needed to determine if umbilical cord blood derived stem cells can be used as part of safe and effective therapies for T1D.

Keywords

Type 1 Diabetes; Cord Blood; Stem Cells; Islets

Introduction - The Need to Prevent or Reverse Type 1 Diabetes

Type 1 diabetes (T1D) is an autoimmune disease characterized by T-cell mediated destruction of insulin producing beta cells and lifelong dependence on exogenous insulin administration. T1D affects nearly 1 in 300 within the United States and the incidence of the disease continues to rise at approximately 3% per year [1,2]. On an international level, the incidence of T1D varies dramatically; as much as 500-fold [3].

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Since the discovery of insulin by Banting, Best, Collip, and McCleod in 1921, T1D has evolved from a uniformly fatal disease to a chronic disease; one with a continuously evolving armamentarium of devices and insulin analogs that have greatly improved care. Nevertheless, the overwhelming majority of patients with T1D are still unable or unwilling to utilize currently available resources that would minimize their risk of diabetes related complications [4]. With this, the tremendous physical, emotional, and economic costs of T1D demand that we explore novel strategies for the prevention and reversal of T1D.

Over the last 25 years, a majority of efforts seeking to ameliorate the autoimmune process and reverse disease in those recently diagnosed have focused on the use of immunosuppressive or immunomodulatory drugs [5,6]. While several of these agents have shown and continue to show promise (e.g., anti-CD3), no single agent has succeeded in demonstrating long term success in preventing or reversing T1D. More recently, additional efforts have focused on the use of both autologous and allogeneic stem cells as sources of new islets, and perhaps more intriguingly, as potential sources of safe and effective immunomodulation [7–9]. Among the broad array of potential cell based therapies, the use of autologous umbilical cord blood (UCB) as a source of immunomodulatory cells for the treatment of autoimmune diseases has become an increasingly popular concept [10–13]; this, based on the potential for UCB to potentially restore proper immune regulation.

Type 1 Diabetes - A Disease Characterized by Loss of Tolerance

The pathophysiology of T1D in both humans and non-obese diabetic (NOD) mice appears largely related to an innate defect in the immune system culminating in a loss of self tolerance and destruction of the insulin producing beta cells [14,15]. NOD mice, the prototypic animal model for human T1D, have obvious defects in central and peripheral tolerance [16] and exhibit a variety of abnormalities in immune function (e.g., reduced IL-2 production, proliferative hyporesponsiveness, etc.) [17]. In terms of the cellular basis for this immunoregulatory failure, it is key to note (albeit somewhat controversial) that both NOD mice and patients with T1D have potential deficiencies in at least two T cell populations intimately involved in immune regulation; NKT cells and CD4+CD25+ or so called “regulatory” T cells (Treg) [18–22]. In addition to defects in T cell based immune regulation, developmental and functional defects have also been reported in B-lymphocytes as well as antigen presenting cells (APC) of both NOD mice and human’s with T1D; including those of differentiation and function of macrophages and DC [23–29].

Because of this, an intense degree of research interest has recently been generated towards an improved understanding of the mechanisms that regulate the immune response and form a state of immunological tolerance. Indeed, the ability to develop a means for imparting tolerance may have a dramatic impact not only on the way autoimmune disorders such as T1D are treated, but in addition, towards therapeutic applications related to allergy, transplantation, and oncology.

Why Stem Cell Therapy in Type 1 Diabetes?

Currently, the only means allowing for the reversal of T1D involves whole pancreas or islet cell transplantation in association with non-specific immunosuppressive therapy [30]. Unfortunately, immunosuppressive therapy not only results in compromised immune function, but the potential exists for additional complications with the long-term use of this form of intervention. This facet, combined with limitations afforded by the paucity of available cadaveric organs, have resulted in ever increasing attention towards the potential use of embryonic stem cells, cord blood derived stem cells, adult stem cells, pancreatic progenitor cells, and the transdifferentiation of other non-pancreatic cells as a means to cure this disease [31].

Indeed, recent advances in stem cell research provide an exciting and potentially new approach towards finding a cure for T1D and many other clinical disorders. Stem cells possess the capacity to multiply and to differentiate into a variety of cell populations. As evidence of the potential for these cells to treat T1D, bone marrow transplantation has been shown to prevent autoimmune insulinitis and diabetes in NOD mice [32]. In addition, recent research in immunodeficient mice with chemically-induced pancreatic damage has shown that bone marrow-derived stem cells may have the capacity to initiate beta cell regeneration [33]. However the mechanism involved in pancreatic regeneration may be somewhat contrary to the classic concept of direct stem cell differentiation into cells of the desired target tissue. In their chemically induced diabetes model, Hess et al. determined that transplanted bone marrow derived stem cells travel preferentially to damaged organs and initiate tissue regeneration via the organ's own stem cell population [33]. As such, it may be that the role of stem cells in ameliorating T1D is to protect remaining beta cells from further destruction or stimulate remaining tissue to regenerate rather than participating directly in the production of de-novo stem cell derived islets. The question remains, do similar processes occur at the level of the human pancreas?

Autologous Transplantation in Humans with Type 1 Diabetes

In terms of human application, autologous stem cell transplantation, in which the transplant recipient is the stem cell donor, is the most common and potentially safest form of stem cell transplantation. Autologous bone marrow transplants have been used successfully for patients undergoing high dose chemotherapy, and for the treatment of many forms of cancer [34]. More recently, stem cell transplants have also been used as a treatment option for autoimmune disorders including multiple sclerosis (MS), Evans syndrome, lupus, and rheumatic disorders [35–37]. While initial attempts at using transplantation to treat autoimmune diseases involved traditional myeloablative protocols, non-myeloablative or “lymphoablative” protocols have recently demonstrated remarkable success in treating autoimmune disease. Non-myeloablative approaches are clearly less risky than traditional myeloablative regimens. Nevertheless, the risk of serious morbidity or even mortality with non-myeloablative transplants may still be unacceptably high. In perhaps the largest series of non-myeloblative transplants for autoimmune disease performed to date (180 patients) the overall mortality rate is approximately 1.7% [38]. As mortality was only observed in patients with longstanding, severe autoimmune diseases treated for many years with immunosuppressive regimens, the question remains what the morbidity and mortality rates would be in otherwise healthy subjects with new onset autoimmune disease?

Voltarelli et al recently published the first attempts to determine the safety and efficacy of non-myeloablative autologous transplantation in new onset T1D patients [13]. In this study of 15 new onset T1D subjects (mean age 19.2 years) undergoing non-myeloablative auto-transplantation, subjects underwent autologous stem cell mobilization with cyclophosphamide ($2\text{g}/\text{m}^2$) and daily granulocyte colony stimulating factor ($10\mu\text{g}/\text{kg}/\text{d}$) followed by leukapheresis and cryopreservation of stem cells. Following conditioning with rabbit anti-thymocyte globulin ($4.5\text{mg}/\text{kg}$) and cyclophosphamide ($200\text{mg}/\text{kg}$), the previously mobilized cells were re-infused intravenously. Fourteen of the 15 subjects were able to discontinue insulin injections for at least one month post therapy, with the majority being able to remain off insulin for over 6 months. Fortunately, no mortality was observed in their small study. Nevertheless, morbidity was still an issue. Male patients had semen samples stored prior to treatment to preserve fertility, all patients received antimicrobial and antifungal prophylaxis, mean hospital stay was 19.2 days (range 15–24), and nearly all patients experienced the common transplantation-related complications of febrile neutropenia, nausea, vomiting, and alopecia.

These results force us to continue the discussion as to what level of risk physicians, patients, and parents of children with T1D are or should be willing to take to achieve a potential cure. While T1D is undoubtedly a terrible life long disease, the success of contemporary treatment modalities make the use of any therapy with a significant mortality risk unacceptable. Whether a mortality risk of 1%, 0.1%, or even 0.01% is justifiable for a potential cure is a difficult question to answer but one we must grapple with as we explore new therapies. As such, we remain cautiously optimistic that stem cell therapies can be safely modified and applied to the treatment of T1D. Because our priority is to develop effective strategies that minimize and preferably eliminate the risk for treatment related severe morbidity or mortality, our group's recent focus has been the potential use of non-pretreated autologous UCB transfusion in children with T1D.

Why Cord Blood? - Properties and Therapeutic Potential

In an era where the mere mention of "stem cell therapies" stirs controversy, the use of UCB is attractive as it avoids much of the debate surrounding this delicate issue. In addition, UCB offers additional major advantages over other ethically acceptable stem cell sources. When compared to bone marrow and peripherally mobilized stem cells, UCB is preferable because of its immediate availability, absence of risk to the donor (and if autologous to the recipient as well) low risk of graft-versus-host disease, and increased capacity for expansion [39]. As such, UCB has been used successfully in transplantation for a variety of diseases, including acute lymphocytic and myeloid leukemia, lymphoma, Fanconi anemia, and sickle cell disease [34]. While the use of UCB transplantation has been hampered somewhat by the relatively small number of stored cord blood samples, the fixed number of cells available in single UCB unit, and the overall lack of experience with UCB transplantation, UCB transplantation could replace bone marrow transplantation as the standard of care in the near future [40].

From a research perspective, UCB has shown great promise as a source for deriving HLA matched hematopoietic stem cell populations. The ability of UCB-derived stem cells to differentiate into a variety of non-blood cell types, including hepatocytes, neural cells, and endothelial cells has already been documented [41]. As further evidence of the potential use of UCB in T1D therapies, UCB stem cells have successfully been directed in vitro to differentiate into insulin and c-peptide producing cells [42].

Since cord blood contains a large population of immature unprimed highly functional regulatory T lymphocytes, this may be the most important reason or exploring therapeutic applications of UCB in T1D. The population of highly functional regulatory T cells in UCB may function to decrease the inflammatory cytokine response and anergize the effector T cells which play a key role in the cellular-mediated autoimmune process [39,43]. As protocols for expanding Treg from UCB continue to evolve, the limitations of cord blood as a limited resource begin to diminish and the therapeutic potential of UCB Tregs continues to expand [44,45]. As such, the role of the cord blood Treg has become the focus of our work in designing UCB based therapies for T1D (FIGURE 1).

Practically, the lack of disease reversal trials for children with T1D under the age of 8 years of age (due to safety concerns with the immunosuppressive regimens being tested) also makes the use of UCB particularly appealing. As the rates of UCB storage continue to increase exponentially, the number of potential subjects for autologous UCB based clinical trials will continue to grow. The fact that UCB is stored at birth without the need for any additional intervention (i.e. bone marrow biopsy or stem cell mobilization and aphaeresis) is an additional advantage in considering an UCB based therapy for children. As UCB storage facilities continue to rethink storage methods that would allow for multiple potential "withdrawals", potential exists for protocols that involve cell expansion or multiple cell infusions.

Autologous Cord Blood Infusion in T1D

Based on available pre-clinical data and the agreement that infusion of minimally manipulated autologous cord blood cells was likely to be extremely safe, we began the process of designing and implementing an unblinded observational pilot study to determine if autologous UCB infusion could ameliorate the T1D autoimmune process and provide patients with preservation of remaining endogenous insulin production.

We hypothesized that autologous UCB transfusion in the setting of T1D may help mitigate the autoimmune process by a number of potential mechanisms: (1) UCB stem cells may migrate to the damaged pancreas where they will differentiate into insulin producing beta cells, (2) UCB stem cells may act as nurse cells to foster the proliferation of new islets from remaining viable tissue, and/or (3) UCB regulatory T cells may facilitate direct or bystander suppression of effector T cells or allow for the restoration of tolerance by their inhibitory effects on multiple cell types [46].

The study has been designed as a 2 year, unblinded, observational study with peak c-peptide following a standard mixed meal tolerance test, HbA1c, and daily insulin requirement set as the primary outcome variables. Subjects undergo mixed meal tolerance testing immediately before cord blood infusion and then every 3 months during the 1st post infusion year and every 6 months in the 2nd post infusion year (FIGURE 2). In addition to measures of insulin production and metabolic control, we study the immunological effects of autologous cord blood infusion by obtaining blood for peripheral blood mononuclear cell (PBMC) analysis at each visit. PBMCs are analyzed by flow cytometry with staining for the cell surface markers CD3, CD4, CD8, CD25, and the intracellular marker FOXP3. In addition, suppression assays are performed to measure the function of peripherally collected Tregs.

Our study has been designed with broad inclusion criteria as little preclinical data are available to guide selection of specific subjects. As such, we allow any child over 1 year of age with T1D, stored autologous cord blood, normal screening labs (complete blood count and basic metabolic profile), and no other significant past medical history to participate in the study. Similarly, no specific criteria as to length of time since diagnosis or baseline insulin production are included. To be usable, the cord blood cell viability must be at least 50%, and both the unit and the maternal sample at time of collection must be free of any infectious disease markers. After a potential subject is identified and consented, an aliquot of the cord blood unit and the child's blood is shipped to our stem cell lab where infectious disease testing and HLA confirmation are performed. Once these screening tests are confirmed, the cord blood unit is shipped to our stem cell lab for storage and the child is scheduled for admission to our general clinical research center. Upon arrival in our research center, the subject undergoes a standard mixed meal tolerance test and has blood drawn for baseline metabolic and immunologic studies. The cord blood unit is then thawed and washed per the standard operating procedures of our stem cell laboratory. An aliquot of the cells is analyzed for viability at infusion, CD34 percentage, and Treg frequency.

Following the preparation of the unit, the subjects receive pretreatment with diphenhydramine and acetaminophen. No chemotherapy or other preparative therapy is given. The thawed cord blood cells (typically in a volume of less than 100 mL) are then infused through a peripheral IV over 10–20 minutes. Following the infusion, subjects are observed closely for at least 6 hours prior to being discharged home. Subjects then return for follow testing as previously described every 3 months in the first post infusion year and every 6 months in the second post infusion year.

Recruitment for our cord blood studies officially began in late 2005 after an investigational new drug permit was obtained from the federal drug administration. Despite initial concerns

that we would have great difficulty in identifying T1D patients with stored autologous UCB, we were pleasantly surprised with the large number of referrals we received after simply posting our study on the clinicaltrials.gov and Children with Diabetes websites. In just over six months, we were contacted by more than 50 families with eligible children. A large majority of eligible subjects declined to participate due to parental concerns over using up the cord blood. Many parents felt inclined to continue to store the cord blood until a more definitive therapy had been developed. This issue brings up an interesting Catch-22 with regards to the use of cord blood for T1D. If, in fact, the main action of UCB cells in our subjects is the amelioration of the autoimmune process, the use of UCB infusion shortly after diagnosis or during the honeymoon phase of the disease may be much more effective than if the cells are used later on in the disease process. As such, saving the cells for potential future use may turn out to be less advantageous unless manipulation of the cells can provide for both stem cell mediated islet regeneration and restoration of immune tolerance.

In June of 2007 we reported preliminary data from our first 8 subjects to reach the 6-month post UCB infusion visit [11,12]. At enrollment, the average age of our infused subjects at that time was 5.29 ± 1.8 years (range 2.4–7.3), the average duration of T1D was 0.84 ± 0.8 years, and the average HbA1c was $6.3 \pm 0.7\%$. We compared the daily insulin requirements (units/kg/day) and the HbA1c values of our infused cord blood subjects with an age matched and disease duration matched group of contemporary intensively treated “controls” from our diabetes clinic (n=13, age 4.5 ± 2.2 years, duration of diabetes 0.77 ± 0.6 years). While this comparison has significant flaws in that the control subjects were not part of the initial study and that the study subjects were highly motivated to maintain the best possible control, we did observe significant differences between the groups which suggested benefit in the UCB infusion group [11,12].

In terms of providing evidence of a mechanism for the action of UCB, our group has focused on the frequency and function of Treg in the peripheral blood of our UCB infused subjects. While poor access to the pancreas limits our ability to dissect the importance of the potential mechanisms involved in humans, we hope to employ both our clinical trial data and novel humanized mouse models of T1D to elucidate the potential efficacy and mechanisms involved in UCB therapies for T1D. Undoubtedly, these intriguing data support further efforts to characterize these critical immunoregulatory cells.

Perhaps the most important finding to report at this time is that we had absolutely no significant adverse events associated with the study. As mentioned previously, our group feels that interventional efforts in T1D must continue to put the highest value on patient safety. To date, we have safely infused a total of 15 T1D subjects with autologous UCB. Our FDA approval allows for a total of 23 subjects to be treated under this protocol and as such, we are continuing to recruit eligible subjects. We expect to formally report data once 10 subjects have reached the one year post infusion visit with the final report coming after all subjects have reached the two year post infusion visit. While preliminary data remain supportive of the concept that UCB infusion provides benefit, we would caution readers to patiently await the reporting of more robust data before making conclusions. Though the potential of UCB to participate in the future of T1D interventional therapies is immense, the reality remains that multiple therapeutic avenues will need to be explored and that several modalities will likely need to be combined in order to achieve the dream of safely and permanently reversing or perhaps even preventing T1D.

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CORD BLOOD MEDIATED RESTORATION OF IMMUNE TOLERANCE IN T1D

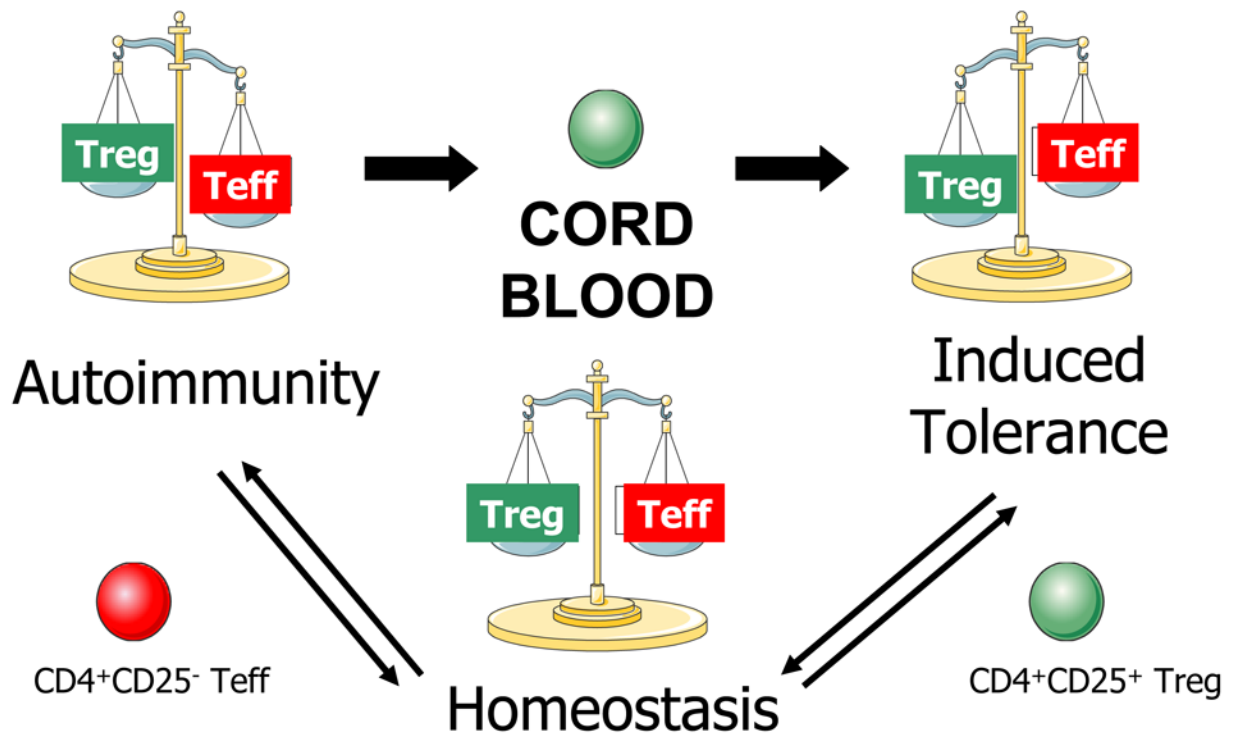


FIGURE 1. Cord Blood Mediated Restoration of Immune Tolerance In T1D

This theoretical model of the basic imbalance between Treg number or function seen in autoimmune disease demonstrates the simple concept that a rich source of Treg such as cord blood may have the potential to tip the scales back in favor of immune tolerance. If true, this concept could be applied to the treatment of many autoimmune diseases. (Figure produced, with permission, using software from www.servier.com).

CORD BLOOD IN T1D: STUDY TIMELINE

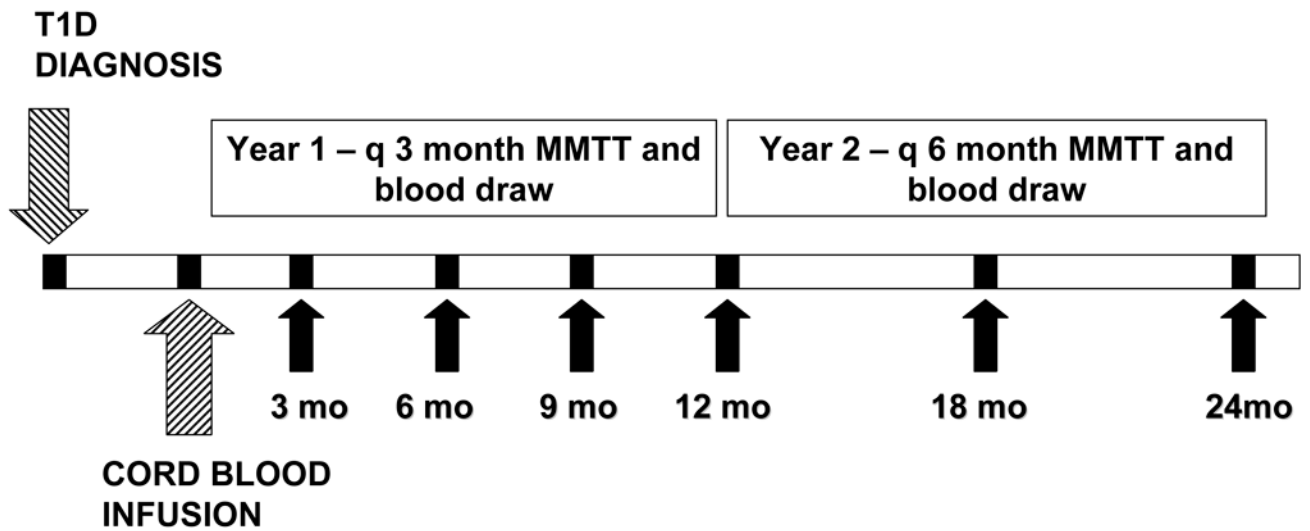


FIGURE 2. Cord Blood in T1D Study Timeline

Our study was designed to be a 2 year observational study of the effects of autologous cord blood infusion in children with T1D. We follow each child every 3 months during the first year post infusion and every 6 months during the second year post infusion. Blood is obtained for metabolic and immunologic studies at each visit.