



## Autologous umbilical cord blood infusion for type 1 diabetes

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**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.

**Materials 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 to 6 months postinfusion 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. © 2008 ISEH - Society for Hematology and Stem Cells. Published by Elsevier Inc.

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, incidence of T1D varies dramatically; as much as 500-fold [3].

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.

During 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, use of autologous umbilical cord blood (UCB) as a source of immunomodulatory cells for treatment of autoimmune

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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 nonobese 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 interleukin-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 of both NOD mice and humans with T1D; including those of differentiation and function of macrophages and dendritic cells [23–29].

Because of this, an intense degree of research interest has recently been generated toward 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, therapeutic applications related to allergy, transplantation, and oncology.

### **Why stem cell therapy in T1D?**

Currently, the only means allowing for reversal of T1D involves whole pancreas or islet cell transplantation in association with nonspecific immunosuppressive therapy [30]. Unfortunately, immunosuppressive therapy not only results in compromised immune function, but the potential exists for additional complications with 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 toward the potential use of embryonic stem cells, cord blood-derived stem cells, adult stem cells, pancreatic progenitor cells, and transdifferentiation of other nonpancreatic cells as a means to cure this disease [31].

Indeed, recent advances in stem cell research provide an exciting and potentially new approach to finding a cure for T1D and many other clinical disorders. Stem cells possess the capacity to multiply and 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. [33] 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. 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 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 T1D**

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 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, Evans syndrome, lupus, and rheumatic disorders [35–37]. While initial attempts at using transplantation to treat autoimmune diseases involved traditional myeloablative protocols, nonmyeloablative or “lymphoablative” protocols have recently demonstrated remarkable success in treating autoimmune disease. Nonmyeloablative approaches are clearly less risky than traditional myeloablative regimens. Nevertheless, risk of serious morbidity or even mortality with nonmyeloablative transplants may still be unacceptably high. In perhaps the largest series of nonmyeloablative transplants for autoimmune disease performed to date (180 patients), the overall mortality rate is approximately 1.7% (Personal Communication From Dr. Richard Burt, September 25, 2007). 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. [13] recently published the first attempts to determine the safety and efficacy of nonmyeloablative autologous transplantation in new onset T1D patients. In this study of 15 new onset T1D subjects (mean age: 19.2 years) undergoing nonmyeloablative autotransplantation,

subjects underwent autologous stem cell mobilization with cyclophosphamide (2 g/m<sup>2</sup>) and daily granulocyte colony-stimulating factor (10 µg/kg/day), followed by leukapheresis and cryopreservation of stem cells. Following conditioning with rabbit anti-thymocyte globulin (4.5 mg/kg) and cyclophosphamide (200 mg/kg), the previously mobilized cells were reinfused intravenously. Fourteen of 15 subjects were able to discontinue insulin injections for at least 1 month posttherapy, with the majority being able to remain off insulin for >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 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 lifelong disease, the success of contemporary treatment modalities makes 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 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 nonpre-treated 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, use of UCB is attractive because 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-vs-host disease, and increased capacity for expansion [38]. 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 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 a 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 [39].

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 nonblood cell types, including hepatocytes, neural cells, and endothelial cells has already been documented [40]. 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 [41].

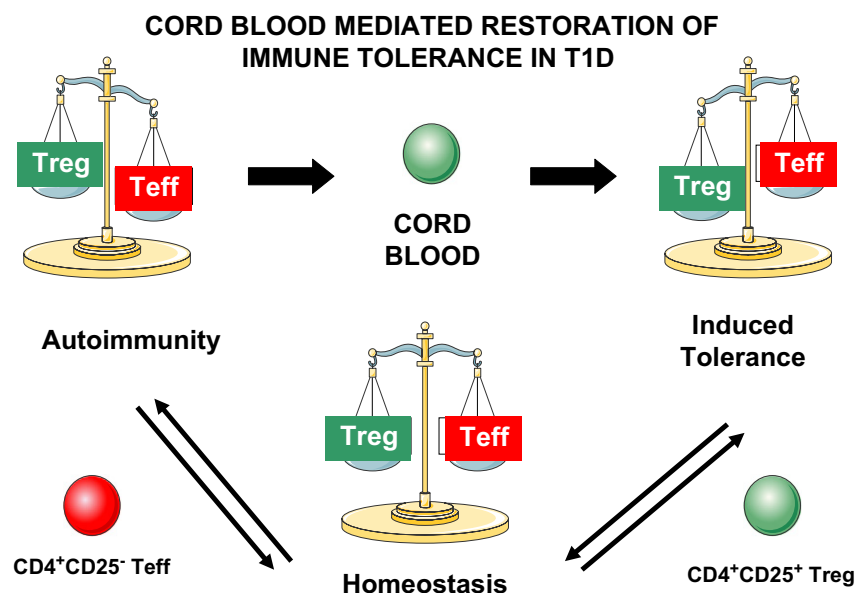
Because cord blood contains a large population of immature unprimed highly functional regulatory T lymphocytes, this may be the most important reason for exploring therapeutic applications of UCB in T1D. The population of highly functional regulatory T cells in UCB may function to decrease the inflammatory cytokine response and anergize the effector T cells, which play a key role in the cellular-mediated autoimmune process [38,42]. 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 [43,44]. As such, the role of the cord blood Treg has become the focus of our work in designing UCB-based therapies for T1D (Fig. 1).

Practically, the lack of disease-reversal trials for children with T1D younger than the age of 8 years (due to safety concerns with the immunosuppressive regimens being tested), also makes use of UCB particularly appealing. As 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 apheresis) is an additional advantage in considering a 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 preclinical 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 β cells; 2) UCB stem cells may act as nurse cells to foster proliferation of new islets from remaining viable tissue; and/or 3) UCB regulatory



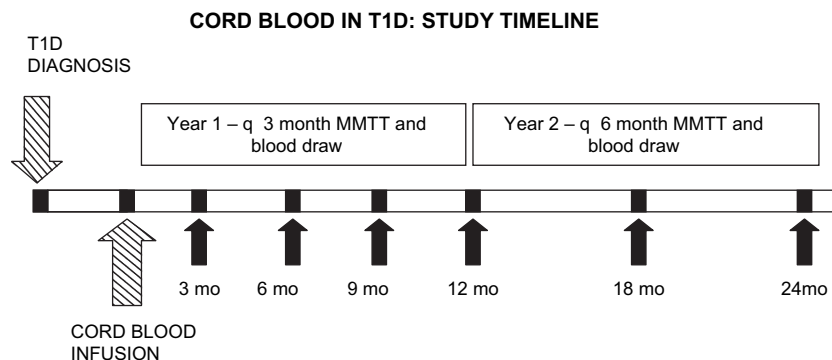
**Figure 1.** Cord blood-mediated restoration of immune tolerance in type 1 diabetes (T1D). This theoretical model of the basic imbalance between regulatory T cell (Treg) number or function seen in autoimmune disease demonstrates the simple concept that a rich source of Treg, such as cord blood, may have the potential to tip the scales back in favor of immune tolerance. If true, this concept could be applied in the treatment of many autoimmune diseases. (Figure produced, with permission, using software from [www.servier.com](http://www.servier.com)).

T cells may facilitate direct or bystander suppression of effector T cells or allow for restoration of tolerance by their inhibitory effects on multiple cell types [45].

The study has been designed as a 2-year, unblinded observational study with peak c-peptide following a standard mixed-meal tolerance test, hemoglobin A1c (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 first postinfusion year and every 6 months in the second postinfusion year (Fig. 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 older than 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



**Figure 2.** Cord blood in type 1 diabetes (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 postinfusion and every 6 months during the second year postinfusion. Blood is obtained for metabolic and immunologic studies at each visit. MMTT = mixed-meal tolerance testing.

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

Recruitment for our cord blood studies officially began in late 2005 after an investigational new drug permit was obtained from the US Food and 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](http://clinicaltrials.gov) and Children with Diabetes Websites. In just over 6 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 regard 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, 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 eight 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  $5.29 \pm 1.8$  years (range, 2.4–7.3), the average duration of T1D was  $0.84 \pm 0.8$  years, and the average HbA1c was  $6.3\% \pm 0.7\%$ . We compared daily

insulin requirements (units/kg/day) and 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  $4.5 \pm 2.2$  years, duration of diabetes  $0.77 \pm 0.6$  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 a 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 US Food and Drug Administration 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 1-year postinfusion visit, with the final report coming after all subjects have reached the 2-year postinfusion 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. Although 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.

## References

1. DIAMOND Project Group. Incidence and trends of childhood Type 1 diabetes worldwide 1990–1999. *Diabet Med.* 2006;23:857–866.
2. Vehik K, Hamman RF, Lezotte D, et al. Increasing incidence of type 1 diabetes in 0- to 17-year-old Colorado youth. *Diabetes Care.* 2007;30:503–509.
3. Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, Tuomilehto J. Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. *Diabetes Care.* 2000; 23:1516–1526.
4. Eeg-Olofsson K, Cederholm J, Nilsson PM, Gudbjornsdottir S, Eliasson B for the Steering Committee of the Swedish National Diabetes R.

- Glycemic and risk factor control in type 1 diabetes: results from 13,612 patients in a national diabetes register. *Diabetes Care*. 2007;30:496–502.
5. Haller MJ, Gottlieb PA, Schatz DA. Type 1 diabetes intervention trials 2007: where are we and where are we going? *Curr Opin Endocrinol Diabetes Obes*. 2007;14:283–287.
  6. Schatz D, Gale EAM, Atkinson MA. Why can't we prevent type 1 diabetes?: maybe it's time to try a different combination. *Diabetes Care*. 2003;26:3326–3328.
  7. Limbert C, Path G, Jakob F, Seufert J. Beta-cell replacement and regeneration: strategies of cell-based therapy for type 1 diabetes mellitus. *Diabetes Res Clin Pract*. 2008;79:389–399.
  8. Hussain MA, Theise ND. Stem-cell therapy for diabetes mellitus. *Lancet*. 2004;364:203–205.
  9. Couri CE, Foss MC, Voltarelli JC. Secondary prevention of type 1 diabetes mellitus: stopping immune destruction and promoting beta-cell regeneration. *Braz J Med Biol Res*. 2006;39:1271–1280.
  10. Ende N, Chen R, Reddi AS. Effect of human umbilical cord blood cells on glycemia and insulinitis in type 1 diabetic mice. *Biochem Biophys Res Commun*. 2004;325:665–669.
  11. Haller M, Viener H, Brusko T, et al. Insulin requirements, HbA1c, and stimulated C-peptide following autologous umbilical cord blood transfusion in children with type 1 diabetes. In: 2007 American Diabetes Association Scientific Sessions. Chicago, IL: American Diabetes Association; 2007. p. A82.
  12. Viener H, Brusko T, Wasserfall C, et al. Changes in regulatory T cells following autologous umbilical cord blood transfusion in children with type 1 diabetes. In: 67th American Diabetes Association Scientific Sessions. Chicago, IL: American Diabetes Association; 2007. p. A82.
  13. Voltarelli JC, Couri CEB, Stracieri ABPL, et al. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*. 2007;297:1568–1576.
  14. Bach JF, Chatenoud L. Tolerance to islet autoantigens in type 1 diabetes. *Annu Rev Immunol*. 2001;19:131–161.
  15. Atkinson MA, Leiter EH. The NOD mouse model of type 1 diabetes: as good as it gets? *Nat Med*. 1999;5:601–604.
  16. Rapoport MJ, Lazarus AH, Jaramillo A, Speck E, Delovitch TL. Thymic T cell anergy in autoimmune nonobese diabetic mice is mediated by deficient T cell receptor regulation of the pathway of p21ras activation. *J Exp Med*. 1993;177:1221–1226.
  17. Gombert JM, Herbelin A, Tancrede-Bohin E, Dy M, Carnaud C, Bach JF. Early quantitative and functional deficiency of NK1+-like thymocytes in the NOD mouse. *Eur J Immunol*. 1996;26:2989–2998.
  18. Lederman MM, Ellner JJ, Rodman HM. Defective suppressor cell generation in juvenile onset diabetes. *J Immunol*. 1981;127:2051–2055.
  19. Asano M, Toda M, Sakaguchi N, Sakaguchi S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med*. 1996;184:387–396.
  20. Salomon B, Lenschow DJ, Rhee L, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity*. 2000;12:431–440.
  21. Wu AJ, Hua H, Munson SH, McDevitt HO. Tumor necrosis factor- $\alpha$  regulation of CD4+CD25+ T cell levels in NOD mice. *Proc Natl Acad Sci U S A*. 2002;99:12287–12292.
  22. Liu J, Beller D. Aberrant production of IL-12 by macrophages from several autoimmune-prone mouse strains is characterized by intrinsic and unique patterns of NF- $\kappa$ B expression and binding to the IL-12 p40 promoter. *J Immunol*. 2002;169:581–586.
  23. Chatenoud L, Salomon B, Bluestone JA. Suppressor T cells—they're back and critical for regulation of autoimmunity!. *Immunol Rev*. 2001;182:149–163.
  24. Serreze DV, Gaskins HR, Leiter EH. Defects in the differentiation and function of antigen presenting cells in NOD/Lt mice. *J Immunol*. 1993;150:2534–2543.
  25. Alleva DG, Pavlovich RP, Grant C, Kaser SB, Beller DI. Aberrant macrophage cytokine production is a conserved feature among autoimmune-prone mouse strains: elevated interleukin (IL)-12 and an imbalance in tumor necrosis factor- $\alpha$  and IL-10 define a unique cytokine profile in macrophages from young nonobese diabetic mice. *Diabetes*. 2000;49:1106–1115.
  26. Dahlen E, Hedlund G, Dawe K. Low CD86 expression in the nonobese diabetic mouse results in the impairment of both T cell activation and CTLA-4 up-regulation. *J Immunol*. 2000;164:2444–2456.
  27. Kukreja A, Cost G, Marker J, et al. Multiple immunoregulatory defects in type-1 diabetes. *J Clin Invest*. 2002;109:131–140.
  28. Weaver DJ Jr, Poligone B, Bui T, Abdel-Motal UM, Baldwin AS Jr, Tisch R. Dendritic cells from nonobese diabetic mice exhibit a defect in NF- $\kappa$ B regulation due to a hyperactive I  $\kappa$ B kinase. *J Immunol*. 2001;167:1461–1468.
  29. Yan G, Shi L, Penforinis A, Faustman DL. Impaired processing and presentation by MHC class II proteins in human diabetic cells. *J Immunol*. 2003;170:620–627.
  30. Pavlakis M, Khwaja K. Pancreas and islet cell transplantation in diabetes. *Curr Opin Endocrinol Diabetes Obes*. 2007;14:146–150.
  31. Bottino R, Lemarchand P, Trucco M, Giannoukakis N. Gene- and cell-based therapeutics for type I diabetes mellitus. *Gene Ther*. 2003;10:875–889.
  32. Beilhack GF, Scheffold YC, Weissman IL, et al. Purified allogeneic hematopoietic stem cell transplantation blocks diabetes pathogenesis in NOD mice. *Diabetes*. 2003;52:59–68.
  33. Hess D, Li L, Martin M, et al. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol*. 2003;21:763–770.
  34. Lennard AL, Jackson GH. Stem cell transplantation. *BMJ*. 2000;321:433–437.
  35. Marmont AM. Stem cell transplantation for severe autoimmune disorders, with special reference to rheumatic diseases. *J Rheumatol Suppl*. 1997;48:13–18.
  36. Raetz E, Beatty PG, Adams RH. Treatment of severe Evans syndrome with an allogeneic cord blood transplant. *Bone Marrow Transplant*. 1997;20:427–429.
  37. Comi G, Kappos L, Clanet M, et al. Guidelines for autologous blood and marrow stem cell transplantation in multiple sclerosis: a consensus report written on behalf of the European Group for Blood and Marrow Transplantation and the European Charcot Foundation. BMT-MS Study Group. *J Neurol*. 2000;247:376–382.
  38. Fruchtman S. Stem cell transplantation. *Mt Sinai J Med*. 2003;70:166–170.
  39. Brunstein CG, Setubal DC, Wagner JE. Expanding the role of umbilical cord blood transplantation. *Br J Haematol*. 2007;137:20–35.
  40. Harris DT, Badowski M, Ahmad N, Gaballa MA. The potential of cord blood stem cells for use in regenerative medicine. *Expert Opin Biol Ther*. 2007;7:1311–1322.
  41. Denner L, Bodenbun Y, Zhao JG, et al. Directed engineering of umbilical cord blood stem cells to produce C-peptide and insulin. *Cell Prolif*. 2007;40:367–380.
  42. Han P, Hodge G, Story C, Xu X. Phenotypic analysis of functional T-lymphocyte subtypes and natural killer cells in human cord blood: relevance to umbilical cord blood transplantation. *Br J Haematol*. 1995;89:733–740.
  43. Porter SB, Liu B, Rogosheske J, et al. Suppressor function of umbilical cord blood-derived CD4+CD25+ T-regulatory cells exposed to graft-versus-host disease drugs. *Transplantation*. 2006;82:23–29.
  44. Li L, Godfrey WR, Porter SB, et al. CD4+CD25+ regulatory T-cell lines from human cord blood have functional and molecular properties of T-cell anergy. *Blood*. 2005;106:3068–3073.
  45. Brusko T, Atkinson M. Treg in type 1 diabetes. *Cell Biochem Biophys*. 2007;48:165–175.