

Stem cell therapies for type 1 diabetes mellitus

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The present review discusses the use of autologous hematopoietic stem cell transplantation (HSCT) for the treatment of diabetes mellitus type 1 (DM 1). It has been observed that high dose immunosuppression followed by HSCT shows better results among other immunotherapeutic treatments for the disease as the patients with adequate beta cell reserve achieve insulin independence. However, this response is not maintained and reoccurrence of the disease is major a major challenge to use HSCT in future to prevent or control relapse of DM 1.

Keywords: Diabetes mellitus, HSCT, Immunosuppression, Stem cell

Worldwide increased incidence of autoimmune type 1 diabetes mellitus (DM1) and recent advances in stem cell (SC) biology led to a dramatic effort of the scientific community to find a curative approach for the disease using stem cell therapies. Main types of stem cells involved in this effort are adult SC (hematopoietic and mesenchymal), cord blood derived SC and pluripotent (embryonic and induced pluripotent SC).

Hematopoietic stem cells

Based on animal models of autoimmune diseases successfully treated with high-dose immunosuppression plus hematopoietic stem cells (autologous or allogeneic) and on remission of coincidental autoimmune diseases (AID) in patients treated for hematological disorders¹⁻⁴, in 1996 the first patient with isolated AID was treated with hematopoietic stem cell transplantation (HSCT). Since then, more than 1,500 patients with severe and refractory AID have been treated⁵, most with autologous HSCT, because of the lower risk of complications compared to allogeneic HSCT. Between one to two-thirds of patients experienced sustained remission of their disease. Relapses and mortality rates varied with type and status of the disease and with the intensity of immunosuppression

employed pre-transplant (myeloablative versus nonmyeloablative conditioning regimens).

Few mechanistic studies performed after HSCT for AID suggest that the regenerated immune system is more tolerant with a regulatory phenotype, marked by increased numbers of naïve and regulatory T cells and more diverse T cell receptor repertoire diversity^{6,7}.

Evidence for using HSCT in DM1

After more than ten years of clinical use of HSCT for severe and refractory AID, there is evidence that this approach may also be beneficial for treating human type 1 diabetes mellitus (T1DM). Experimental studies with animal models of T1DM and clinical studies using immunosuppression for early onset DM-1 or in hematopoietic stem cell transplantation for hematological diseases where donor or recipient had T1DM have been already been reported (Table 1).

Table 1—Reports of possible beneficial effect of high dose immunosuppression followed by hematopoietic stem cell infusion in newly diagnosed type 1 diabetes mellitus

Effect of HSCT	Refs
Beneficial effects of HSCT for human severe autoimmune diseases	2-5
Results of HSCT in experimental models of type 1 DM	1, 8-9
Beneficial effects of immunosuppression in human type 1 DM	10-14
Lack of benefit of HSCT in long term T1DM	15
Transfer of DM1 during HSCT in humans	16

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Experimental studies

There are two types of experimental models of autoimmune diseases, one genetically determined, like non-obese diabetic (NOD) and NZB/W mice, in which AID develops spontaneously in most animals, and another, like adjuvant arthritis and experimental autoimmune encephalomyelitis, in which AID is induced by immunization by relevant antigen with Freund adjuvant. The latter model is considered more relevant to human AID, but no such model exists for type 1 DM.

In NOD mice, development of clinically overt T1DM has been easily prevented by allogeneic stem cell transplantation, but not by autologous HSCT, result that can be anticipated by the genetic nature of the disease in this model⁸. However, overt T1DM in NOD mice cannot be reversed by allogeneic HSCT alone, requiring a source of pancreatic beta-cells^{1,9}. These findings indicate that allogeneic HSC can reinduce tolerance to pancreatic beta-cells in DM1 but cannot restore pool of those cells once it was destroyed by autoimmune process.

Immunosuppression for early onset disease

Immune-mediated islet cell destruction is not complete until sometime after onset of T1DM. This has lead, to immunosuppression trials for new onset T1DM^{10,11}. Early diagnosed type I diabetes patients were treated with prednisone, cyclosporine and/or azathioprine. Several trials in various countries indicated that cyclosporine and/or azathioprine preserved insulin secretion and/or increased the duration of insulin independence. The best results seemed to occur for patients within 8 weeks of T1DM onset. Despite preserving insulin secretion, long term immunosuppression was impractical due to chronic side effects. These studies indicate that islet cells persist, at least for a short time interval of weeks to months after T1DM onset. Measurements of C-peptide, a marker for endogenous insulin production, indicate persistence of islet cells with normal C-peptide production for 1 year after T1DM onset.

The most encouraging results have been observed after short-term courses of engineered anti-CD3 monoclonal antibodies. These studies are pioneered by Eisenbarth *et al.*¹² who induced transient remission (up to 8 months) in a small group of T1DM treated with prednisone plus antithymocyte globulin (ATGAM). In one recent study, 12 patients treated with the antibody showed better beta-cell function and lower insulin dosage after one-year, compared to the

placebo group¹³. In a subsequent study with larger number of patients and extended follow-up, the metabolic (increase in C-peptide levels) and clinical (decrease in insulin usage) benefits were maintained up to 2 years after diagnosis¹⁴. However, in neither study a significant number of patients became insulin free after immunointervention. In those studies, the long-term increase of regulatory T cells (Tregs) could be implicated in prolonged protection (18-24 months) of pancreatic beta-cells from autoimmune aggression. Currently, several trials of immunosuppression for early onset T1DM are being conducted, using polyclonal ATG, anti-IL2 receptor monoclonal antibody, mycophenolate mofetil, sirolimus, tacrolimus, anti-CD52 (Campath-1H) or anti-CD20 (Rituximab) monoclonal antibodies¹⁰.

HSCT for hematological diseases where donor or recipient had type 1 DM

Effect of high dose immunosuppression and HSCT has been investigated on metabolic control of three patients with T1DM transplanted for hematological diseases (Fanconi anemia, T cell acute lymphoblastic leukemia or acute myelomonocytic leukemia¹⁵). Two patients received HLA-identical bone marrow transplantation from family relatives (mother or sister) and one patient received syngeneic HSCT from an identical twin. In this study, the long term disease process (3 to 7 years) was not changed by HSCT, as evaluated by continuous use of insulin after transplantation. On the other hand, there are few reports of transference of T1DM from donor to recipient of allogeneic HSCT for hematological diseases¹⁶, indicating that HSC may carry disease predisposition in the allogeneic transplantation setting.

Based on the above discussed evidence, HSCT for early onset T1DM has been proposed^{17,18} and a cooperative protocol between Northwestern University in Chicago, USA (Richard Burt) and University of São Paulo in Ribeirão Preto, Brazil (Júlio Voltarelli and others) has been initiated in 2003 at Brazil after approval by local and national Institutional Review Boards.

Results of autologous HSCT for newly diagnosed DM1

Since 2003, our research group in Brazil is conducting an original study of nonmyeloablative autologous hematopoietic stem cell transplantation (AHSCT) in patients with newly diagnosed T1DM. The objective of the treatment was to stop

autoimmune destruction of β cells with high-dose immunosuppressive drugs (cyclophosphamide and rabbit antithymocyte globulin) and to “reset” the deleterious immunologic system with a reconstituted one originated from autologous hematopoietic stem cells¹⁹. The rationale is to preserve residual β cell mass and facilitate endogenous mechanisms of β cell regeneration. Hematopoietic stem cells probably do not have the capacity to differentiate into large numbers of β cells, therefore these cells are used solely to regenerate new immune system without autoreactive memory cells against pancreatic antigens. The exact mechanism of action operating in this treatment is still unclear. However, it has been suggested that AHSCT may shift the balance from destructive immunity to immune tolerance through clonal exhaustion, regulatory cells, cytokine alterations and changes in T- or B-cell repertoires^{1,2}.

The procedure of AHSCT comprises several steps from patient selection through long-term follow-up. Most patients interested in the study have been excluded for not fulfilling protocol criteria, especially the short time period (6 weeks) from diagnosis, occasionally positivity for anti-GAD antibodies or fully understanding and complying with the study protocol. Apart from the diabetic status, all treated patients have been considered in good health status before transplantation, which explains in part the low frequency and severity of adverse effects. This is also explained by the rapid engraftment of neutrophils (mean of 9 days) and platelets (mean of 11.4 days)¹⁹.

The first patient enrolled in December/2003 presented discouraging response. The patient's insulin requirements increased progressively until 12 months following transplantation (when the patient abandoned follow-up) reaching the dose 250% higher than the initial requirement. The hemoglobin A1c level was 11.1% at 12 months and C-peptide concentration did not increase. Possible cause for the poor clinical response was due to very low β cell reserve, predicted by the previous diagnosis of diabetic ketoacidosis (DKA), and further jeopardized by the β cell apoptotic effect of glucocorticoids used to prevent rabbit antithymocyte globulin reactions. Considering these possibilities, we decided not use glucocorticoids in the conditioning regimen in the following patients and did not include those with previous diabetic ketoacidosis.

Results of this trial have been published with 15 patients¹⁹ and updated in 2009 with 23 patients²⁰. At this latter point, after a median follow-up of 29.8

months, 20 patients without previous DKA became insulin-free, most of them shortly after starting high dose immunosuppression, even before stem cell infusion (mean D+2, range D-6 to D+34). Eight patients had resumed insulin use after transient periods free from insulin ranging from 6 to 47 months (mean 17.7 months). Five of them are receiving 30-50% of insulin doses compared to the doses used before transplantation and one patient is using higher doses than pre-transplantation. Four of these patients resumed insulin use after an upper respiratory tract infection. The other 12 patients were continuously insulin-free since insulin suspension (for a mean of 31 months, range 14-52 months): 1 patient for more than 4 years, 4 patients for at least 3 years, 3 patients for at least 2 years and 4 patients for at least 1 year. Two other patients did not experience any insulin free period, one had previous ketoacidosis and other received steroids to prevent reaction to DMSO during stem cell infusion.

There was a statistically significant reduction of mean hemoglobin A1c concentrations after transplantation in the insulin-free group of patients, but not in the patients that resumed insulin use. Regarding the time course of β cell function, in both groups of patients (continuously insulin-free and transiently insulin free) there was an increase in stimulated C-peptide levels in posttransplant period compared to pretransplantation, but the increase was bigger in the continuous insulin free group (from 225 to 785 ng/ml) than in the transient insulin free group (from 148 to 546 ng/ml) at 48 months post-transplantation. Two patients who resumed insulin use after 47 and 43 months after transplantation were treated with sitagliptin (a drug that stimulates pancreatic secretion of insulin and have immunomodulatory activity on T cells) and both achieved again insulin independence, which was associated with increased C-peptide levels²⁰.

In the last update of the results (November 2010), all 21 patients without previous DKA and/or steroid use submitted to autologous HSCT became insulin independent, but most (15) patients resumed insulin use and 6 are continuously insulin free. All relapsed patients are receiving sitagliptin, most of them need low dose of insulin (0.1 to 0.3 IU/kg) to maintain metabolic control and 3 discontinued insulin for a second time.

In face of the good metabolic results presented, the adverse effects were acceptable. With respect to acute complications, most patients had febrile neutropenia,

nausea, vomiting and alopecia due to the immunosuppressive agents used in the mobilization and conditioning phases of the protocol. Bilateral pneumonia of unidentified etiology that required supplementary oxygen therapy and responded completely to broad-spectrum antibiotics occurred in patients #2 and #21 and they were the only severe acute complications of the transplantation procedure. During long-term follow-up, patient #2 presented Graves disease identified 3.5 years after transplantation, patient #3 developed autoimmune hypothyroidism and transient renal dysfunction associated with rhabdomyolysis, a complication that was successfully treated with levothyroxine. Patient #10 presented mild transient hypergonadotropic hypogonadism 12 months after transplantation and subsequently mild systemic lupus erythematosus. These late onset endocrine dysfunctions presented by these 3 patients can be related to the transplant procedure itself or to an autoimmune polyendocrine syndrome frequently associated with T1DM. Nine patients presented oligospermia and functional sperm abnormalities and 3 patients fathered children after transplantation. There was no mortality.

Mesenchymal stem cells

Mesenchymal stem (or stromal) cells (MSC) can be isolated from the bone marrow, adipose tissue and a variety of other organs, since they appear to localize inside the blood vessel wall in the form of pericytes²¹. They can differentiate to mesodermal tissues (adipocytes, chondrocytes and osteocytes) but also to other tissues including insulin-producing cells²². In addition, they have remarkable immunomodulatory properties inhibiting various components of the immune system *in vitro*²³ and successfully treating severe refractory graft-versus-host disease in humans²⁴. These properties stimulated several groups of investigators to study the effect of MSC in autoimmune diseases, including DM1. In animal models of DM1, MSC showed beneficial effect in the glycemic control, either isolated^{25,26} or combined to HSC²⁷. This combination, associated to high-dose immunosuppression, was tested 5 patients with disease duration from 6 months to 10 years, and showed reduction of insulin dose and increase in C-peptide levels after a mean follow-up of 29 months²⁸. Presently, two protocols of allogeneic MSC and one of autologous MSC are underway worldwide (Table 1). In our own protocol, MSC are obtained and expanded from the bone marrow of a first degree

relative and we are starting to see positive metabolic results when we increased the dose and number of infusions compared to the GVHD protocols (unpublished).

Umbilical cord cells

Umbilical cord contains several types of stem cells that could be used for cellular therapy of diabetes, such as HSC, MSC, endothelial progenitor cells and embryonic-like cells²⁹. These cells, in general, are more immature with higher regenerative potential and less prone to be rejected than BM cells after allogeneic transplantation. In animal models of DM1, cord blood (CB) mononuclear cells³⁰, mesenchymal stem cells³¹ and T regulatory cells cocultivated with CB-SC³² prevented or reversed the disease. CB-MSC are obtained with higher efficiency from the cord wall (vein or Wharton jelly) than from the cord blood and those cells also have the potential to treat DM1³³. The group of Florida University in Gainesville published the results of a phase II study where autologous mononuclear CB cells were injected in 15 DM1 patients and followed for one year³⁴. No significant improvement has been observed in insulin usage or C-peptide levels compared to the control group. Similar study is being conducted in Germany and the Gainesville group is trying to improve their results combining CB cells with Vitamin D and Omega-3 fatty acids in another clinical trial.

Embryonic stem cells and pancreatic stem cells

Embryonic stem cells (ES) are pluripotent stem cells usually derived from the inner cell layer of blastocysts and can differentiate into every tissue of the organism, including insulin producing cells³⁵. However, the clinical application of ES or their progeny in the treatment of diabetes depends on several issues, ethical and scientific, such as immunologic rejection and tumor formation. Promising approaches to avoid rejection are the induction of pluripotent stem cells (iPS) from differentiated tissues of the patient or reprogramming these tissues directly to insulin producing cells without reversion to a pluripotent stem cell state³⁶. The presence of truly stem cells in the pancreatic ducts and in other locations of the organ has been postulated but its application to diabetes treatment needs further studies³⁷.

The types of stem cells that have potential to be used in the treatment of type 1 diabetes mellitus are given in Table 2.

Table 2—Types of stem cells with potential to treat type 1 diabetes mellitus

Source	Type of stem cells
Adult stem cells	Hematopoietic stem cells Mesenchymal stem cells
Umbilical cord stem cells	Cord blood hematopoietic stem cells Cord blood or cord wall mesenchymal stem cells Embryonic-like stem cells
Embryonic stem cells	Blastocyst-derived embryonic stem cells Induced pluripotential stem cells
Pancreatic cells	Pancreatic stem cells Reprogrammed adult pancreatic exocrine cells

Conclusions and prospectives

Many types of stem cells are candidates to be used in the future for the treatment of DM1. The ideal SC would be one, like MSC, with strong regenerative and immunomodulatory properties, which would have to deal with the active process of autoimmunity that probably target the newly formed insulin-producing cells. Most of these stem cells are being tested in pre-clinical models of DM1, very few reached clinical trials and some are being prepared to be tested in humans.

Our approach of high dose immunosuppression followed by autologous HSCT achieved the best results so far among all immunotherapeutic trials for the disease, since every patient with adequate beta cell reserve achieved insulin independence. However, this response was not maintained in most patients, relapse probably represented reemergence of the autoimmune process. The biggest challenge for the future trials using this approach is to prevent or treat relapses and maintain complete or very good partial responses for long time.

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