

Artigo / Article

The role of hematopoietic stem cell transplantation for type 1 diabetes mellitus

O papel do transplante de célula-tronco hematopoética no diabetes mellitus tipo 1

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In this review, we present 1) scientific basis for the use of high dose immunosuppression followed by autologous peripheral blood hematopoietic stem cell transplantation for newly diagnosed type 1 diabetes mellitus, 2) an update of clinical and laboratory outcomes in 21 patients transplanted at the University Hospital of the Ribeirão Preto Medical School, University of São Paulo, Brazil, including 6 relapses in patients without previous ketoacidosis and 3) a discussion of future perspectives of cellular therapy for type 1 diabetes mellitus. Rev. bras. hematol. hemoter. 2008; 30(Supl. 2):55-59.

Key words: Diabetes mellitus; cellular therapy; hematopoietic stem cell transplantation.

Introduction

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 disorders (reviewed in¹⁻⁴) in 1996 the first patients with isolated AID were treated with hematopoietic stem cell transplantation (HSCT). Since then, more than 1,000 patients with severe and refractory AID have been treated,⁵ 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 human type 1 diabetes mellitus

After more than ten years of clinical use of HSCT for severe and refractory AID, the evidence that this approach could be beneficial for human type 1 diabetes mellitus (T1DM) is derived from experimental studies with animal models of T1DM and from clinical studies using immunosuppression for early onset DM-1 or in hematopoietic stem cell transplantation for hematological diseases where donor or recipient had T1DM (Table 1).

Experimental studies

There are two types of experimental models of autoimmune diseases, one genetically determined, like the non-obese diabetic (NOD) and the NZB/W mice, in which AID develops spontaneously in most animals, and another one in, like adjuvant arthritis and experimental autoimmune encephalomyelitis, in which AID is induced by immunization with the relevant antigen plus 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 clinical overt T1DM is easily prevented by allogeneic stem cell transplantation, but

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Table 1. Scientific evidence for the possible benefit of high dose immunosuppression followed by hematopoietic stem cell infusion in newly diagnosed type 1 diabetes mellitus

Evidence	Ref
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

not by autologous HSCT, result that can be anticipated by the genetic nature of the disease in this model.⁸ On the other hand, overt T1DM in NOD mice cannot be reversed by allogeneic HSCT alone, requiring a source of pancreatic beta-cells.⁹ These findings indicate that allogeneic HSC can reinduce tolerance to pancreatic beta-cells in DM1 but cannot restore the pool of those cells once it was destroyed by the autoimmune process.

Immunosuppression for early onset disease

Immune-mediated islet cell destruction is not complete until sometime after onset of T1DM. This has lead, beginning in the 1980s, to immunosuppression trials for new onset T1DM (reviewed in ^{10,11}). Early diagnosed type I diabetes patients were treated with prednisone, cyclosporine and/or azathioprine. Several trials including French, Canadian-European, Australian, and American 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 with 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, indicate persistence of islet cells with low normal C-peptide levels 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 were 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 group.¹³

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 was maintained up to 2 years after diagnosis.¹⁴ 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 antibodies.¹⁰

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

Only one study, from Seattle, investigated the effect of high dose immunosuppression and HSCT on the metabolic control of three patients with T1DM transplanted for hematological diseases (Fanconi anemia, T cell acute lymphoblastic leukemia or acute myelomonocytic leukemia).¹⁵ 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 diseases,¹⁶ indicating that HSC may carry disease predisposition in the allogeneic transplantation setting.

Based on the above discussed evidence, HSCT for early onset T1DM was proposed in review articles in the literature in 2001¹⁷ and 2002¹⁸ 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) started in Brazil in the end of 2003 after approval by local and national Institutional Review Boards.

Results of autologous HSCT for newly diagnosed type 1 diabetes mellitus

Since 2003, our research group in Brazil is conducting an original study of nonmyeloablative autologous hematopoietic stem cell transplantation (AHSC) 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 cells.¹⁹ 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 β cells, therefore these cells are used solely to regenerate new immune system without autoreactive memory cells against pancreatic antigens (see below). 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 were 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 were in good health status before transplantation, which explains in part the low frequency and severity of adverse effects (see below). 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. His insulin requirements increased progressively until 12 months following transplantation (when he abandoned follow-up) reaching the dose 250% higher than his initial requirement. His hemoglobin A1c level was 11.1% at 12 months and his C-peptide concentration did not increase. The possible cause for his poor clinical response is the very low β cell reserve, predicted by the previous diagnosis of diabetic ketoacidosis, 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.

At April 2008, after a median follow-up of 28 months (range between 1 to 49 months) all but one of the subsequent 19 patients became insulin-free, most of them shortly after starting high dose immunosuppression, even before stem cell infusion. Six out of 19 patients resumed insulin use after transient periods free from insulin ranging from 7 to 48 months (mean 22.4 months) (Figure 1). 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. Two of these patients resumed insulin use after an upper respiratory tract infection. The other 13 patients are continuously insulin-free since insulin suspension: 3 patients for at least 3 years, 4 patients for at least 2 years, 3 patients for at least 1 year and 4 patients for at least 3 months

Patients transiently free from insulin along the follow-up

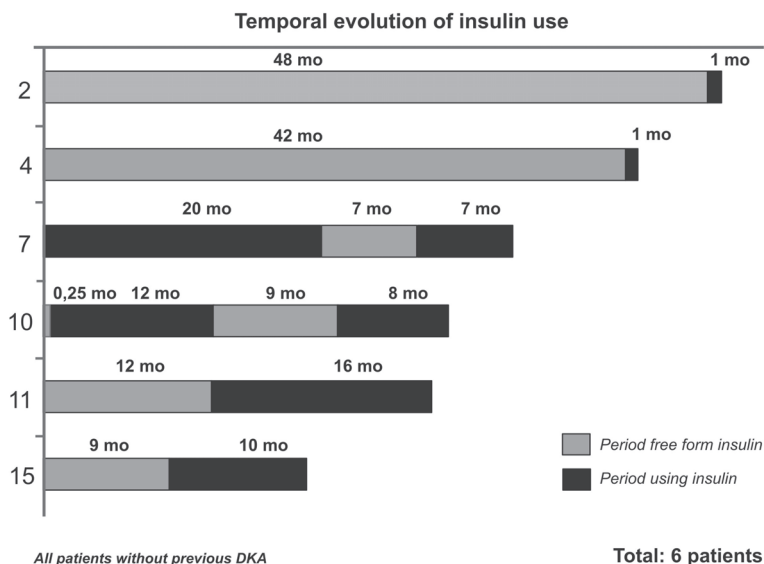


Figure 1. Insulin usage after autologous hematopoietic stem cell transplantation in 6 type 1 DM patients with transient response. No patient had diabetic ketoacidosis or used corticosteroids during the transplantation procedure.

Patients continuously insulin free since AHSCT

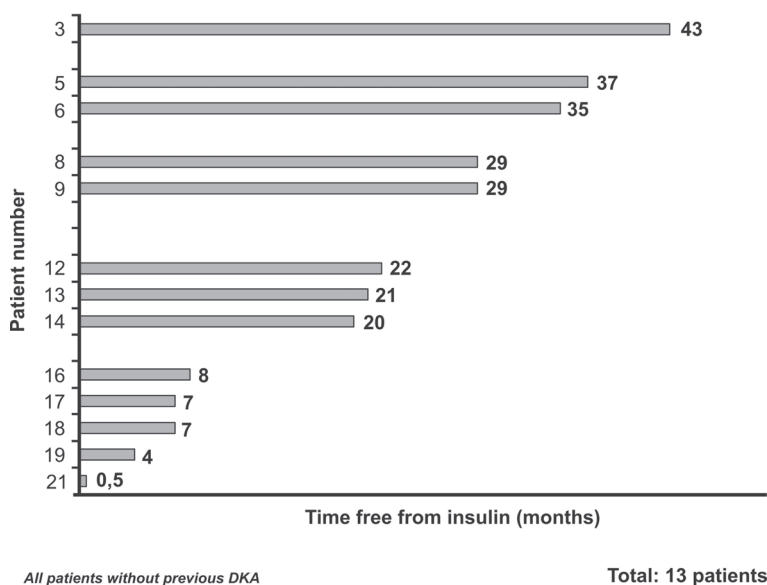


Figure 2. Insulin usage after autologous hematopoietic stem cell transplantation in 13 type 1 DM patients with sustained response. No patient had diabetic ketoacidosis or used corticosteroids during the transplantation procedure

(Figure 2). The 20th patient did not present any period free from insulin, but this patient received steroids (300 mg hydrocortisone) along with stem cell infusion to prevent reactions to DMSO.

There was a statistically significant reduction of mean hemoglobin A1c concentrations after transplantation. All but

2 patients (the 1st and the 11th) presented all measurements below 7% (upper limit of good glucose control) during follow-up. As noted above, soon after inclusion, the 1st patient did not achieve good glucose control. The 11th patient presented A1c levels < 7% until 12 months after transplantation when insulin use was restarted and hemoglobin A1c began to increase.

Regarding the time course of β cell function, of the first 14 patients who had C-peptide levels partially analyzed the majority (n= 11) presented increased values in comparison with pretreatment levels, indicating preservation and even improvement of β cell function. Analyzing C-peptide levels during a stimulus with mixed meal tolerance test, there was a statistically significant increase in mean area under the curve 6 months after transplantation and this increase was maintained until 24 months after stem cell transplantation.¹⁹

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. 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. There was no mortality.

In July 2007 we initiated a similar study of nomyeloablative autologous hematopoietic stem cell transplantation in newly diagnosed individuals with T1DM who presented previous diabetic ketoacidosis. By January 2008 only one patient had been enrolled in the study and insulin independence was not achieved, but insulin doses decreased by less than 40% of the initial requirements.

We are currently performing exhaustive studies of immunoreconstitution (phenotypic and functional) in the transplanted patients to unravel the mechanisms by which AHSCT produces clinical benefit in type 1 diabetic patients. Preliminary results show that after transplantation, there is an increase in the numbers of regulatory CD4+CD25+Foxp3+ T cells and Th2 cytokines-producing cells, compared to the pre-transplant status. In addition, we found after AHSCT profound qualitative and quantitative changes in TCR

repertoire, as well as alterations in the expression of pro- and anti-apoptotic genes.^{20,21} Anti-GAD autoantibodies decreased in most patients but did not correlate with clinical response. Our immune reconstitution results are underway and preliminary results resemble those observed in other autoimmune diseases after AHSCT.^{6,7} The results support the suggested hypothesis that a new and more tolerant immune system is generated after the treatment, explaining the reduction of autoimmune destruction and clinical improvement (Mechanism #2, Table 2). However, in the presence of anti-GAD autoantibodies and in the absence of specific immunologic reactivity tests to beta cell antigens, we cannot be sure that our treatment blocks autoimmune attack to endocrine pancreas more efficiently and for longer time than other immunosuppressive interventions, particularly anti-T cell therapies.

Table 2. Possible mechanisms of action of autologous hematopoietic stem cell transplantation for type 1 diabetes mellitus

1. Ablation of autoreactive immune system by high dose immunosuppression
2. Regeneration of a naïve immune system from autologous hematopoietic stem cells after lymphoablation by high dose immunosuppression
3. Regeneration of pancreatic beta-cells from autologous bone marrow stem cells mobilized to the peripheral blood

Conclusions and prospectives

Our preliminary study of autologous HSCT in a subset of non-ketoacidotic newly diagnosed type 1 DM patients yield unexpected positive results: 19/20 patients could stop insulin use after initiation of high dose immunosuppression and 16 patients maintained this status after a median follow-up of almost two years (maximum of 4 years). Six patients relapsed after stopping insulin use and one patient who received steroids during conditioning never discontinued insulin. Longer follow-up and controlled studies are certainly needed to evaluate the full potential of the procedure in the reversion of type 1 DM.

The underlying mechanism of action of the various components of the AHSCT (cyclophosphamide, ATG and stem cells) cannot be studied by direct methods in humans, but our immune reconstitution study which is currently underway and similar studies in other AIDs suggest that the immune system is reset towards a tolerant phenotype by increased regulatory T cell numbers and by regeneration of a different and more diverse TCR repertoire. We hypothesize that the combination of high dose immunosuppression and hematopoietic stem cell infusion act synergistically to downregulate the autoreactive cells, to renew the immune system and to improve the immune regulatory networks.

While our approach provides the proof of principle that high dose immunosuppression coupled with autologous hematopoietic stem cell boosting can revert clinical type 1 DM in humans, it will hardly solve the problem of the disease. Firstly, only a small subset of patients were successfully treated with AHSCT, millions of patients with long standing type 1 DM need another source of stem cells to regenerate pancreatic beta-cells and other damaged tissues. Secondly, AHSCT is an expensive, cumbersome and complex procedure performed in specialized bone marrow transplantation facilities and has the potential of life threatening short and long term complications. In the future, more simple approaches such as chemical, biological or cellular immunoregulatory interventions may accomplish the same therapeutic goal and may be applied to millions of type 1 diabetic patients who need a definitive treatment. In the meanwhile, AHSCT remains the only treatment to reverse the disease in humans and has to be tested in other groups of patients (with previous ketoacidosis, with longer duration of the disease and in young children).

Resumo

Nesta revisão, são apresentadas: 1) as bases científicas para o uso de imunossupressão em alta dose seguida de transplante autólogo de células-tronco hematopoéticas do sangue periférico no diabete melito do tipo 1 recém-diagnosticado; 2) uma atualização da evolução clínica e laboratorial de 21 pacientes transplantados no Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto, da Universidade de São Paulo, Brasil, incluindo recaídas em seis pacientes transplantados sem cetoacidose prévia; e 3) uma discussão das perspectivas futuras de terapia celular no diabete melito do tipo 1. Rev. bras. hematol. hemoter. 2008; 30(Supl. 2):55-59

Palavras-chave: Diabete melito; terapia celular; transplante de células-tronco hematopoéticas.

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