Risks, Benefits, and Therapeutic Potential of Hematopoietic Stem Cell Transplantation for Autoimmune Diabetes

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Abstract Type 1 diabetes mellitus is a chronic disease that results from the autoimmune response against pancreatic insulin producing β cells. Apart of several insulin regimens, since the decade of 80s various immunomodulatory regimens were tested aiming at blocking some steps of the autoimmune process against β cell mass and at promoting β cell preservation. In the last years, some independent research groups tried to cure type 1 diabetes with an "immunologic reset" provided by autologous hematopoietic stem cell transplantation in newly diagnosed patients, and the majority of patients became free form insulin with increasing levels of C-peptide along the time. In this review, we discuss the biology of hematopoietic stem cells and the possible advantages and disadvantages related to the high dose immunosuppression followed by autologous hematopoietic stem cell transplantation.

Keywords Type 1 diabetes · Stem cell · Hematopoietic · Cure · Diabetes · Immunosuppression · Autologous transplantation · Autoimmune diabetes

Introduction

Type 1 diabetes mellitus (T1DM) is a chronic disease that results from the autoimmune response against pancreatic

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insulin producing β cells. This autoimmune response begins months or years before the first presentation of signs and symptoms of hyperglycemia but at the time of clinical diagnosis a small amount of β cell mass still remains [1].

In daily clinical practice, the main therapeutic option for T1DM mellitus is multiple subcutaneous insulin injections, which are shown to promote tighter glucose control and reduce much of diabetic chronic complications, especially microvascular complications [2].

Another important aspect related to long-term complications of type 1 diabetes is that in newly diagnosed patients the larger the residual β cell function, the lower the microvascular complications, and the lower the incidence of hypoglycemic events. In the face of this, β cell preservation is a potential target in the management of type 1 diabetes and its related complications [3].

Since the decade of 1980s, various immunomodulatory regimens were tested, aiming at blocking some steps of the autoimmune process against β cell mass and at promoting β cell preservation, mainly in secondary prevention trials [4].

In this review, we summarize the benefits, risks, and potential therapeutics of the most important studies involving β cell preservation by autologous nonmyeloablative hematopoietic stem cell transplantation in newly-diagnosed type 1 diabetes mellitus.

Overview about Stem Cells

Stem cells are identified in most tissues and contribute to repair and restoration of cells that are destroyed following damage or regular tissue turnover. They divide asymmetrically, generating two distinct daughter cells: one copy of the original stem cell and a second programmed to differentiate [5]. Therefore, stem cells are mostly known by two functional characteristics: self-renewal and multipotency. The

first is observed when cellular divisions preserve stem cell potential, important for the maintenance of the pool of stem cells within a tissue. The latter is implicated in differentiation into functionally distinct lineages. Embryonic stem cells are on one end of the stemness spectrum and are considered pluripotent, for their ability to differentiate into tissues from any of the 3 germ layers [6]. Although resistant, readily expandable, and easily manipulated in the laboratory, safety issues, ethical concerns, and religious divergences limit clinical studies using these cell types. Adult stem cells, on the other end, are undifferentiated cells found in differentiated tissues, which include cord blood, perinatal sources (amnion, chorion and Wharton's Jelly), and tissues from fully formed organisms, from fetuses to adults [7–9].

Despite little tolerance to in vitro manipulation, low proliferative potential, and restricted plasticity, these cells have been extensively investigated in regenerative research. Exceptions to these are mesenchymal stem cells, which are a subset of adult stem cells that have a more immature behavior, with better in vitro proliferation rates, higher differentiation potential, and immunomodulatory effects [9].

Bone marrow is the most used stem cell source for clinical research and practice. Although other tissues, such as the adipose tissue [10], umbilical cord blood [11], and umbilical cord tissue [12] may be considered better sources of stem cells, bone marrow is still preferred in the clinical setting due to its ease of access, abundance and previous knowledge concerning safety, derived from conventional bone marrow transplantation.

Autoimmunity in Type 1 Diabetes

Type 1 diabetes results from a cell-mediated autoimmune attack against pancreatic β cells. Since more than 70 % of β cell mass has been destroyed at the time of clinical diagnosis, the autoimmune process is markedly advanced when hyperglycemia appears [1]. The rate of β cell destruction is rapid in children associated with much less β cell mass at the time of diagnosis; in contrast, in adults the rate of autodestruction is slower with larger β cell mass at diagnosis [13]. In the later stage of the natural history of the disease, there is a reduction of β cell mass as manifested by low or undetected C-peptide plasma levels.

Autoimmune destruction of β cells is the result of the wide interplay between genetic susceptibility and environmental factors that are both still poorly defined [14]. The concordance for type 1 diabetes is only 50 % for monozygotic twins and the risk of first degree relative is approximately 5 % [9]. The most important, but not the only genetic determinant to T1DM is the major histocompatibility complex. More than 90 % of patients who develop type 1 diabetes have either DR3/DQ2 or DR4/DQ8 haplotypes, whereas less than 40 % of normal individuals have these haplotypes [15].

Many associations with various environmental triggers have been found in type 1 diabetes, but so far congenital rubella syndrome and infection with Coxsackie virus have been more associated with the disease [16]. Another hypothesis is the "hygiene hypothesis" (ie, environmental factors can also inhibit the development of autoimmunity). According to this theory, environment for young infants is far too clean, leading to a marked increase in the incidence of "Th1 diseases" such as type 1 diabetes and "Th2 diseases" such as asthma [17].

There is a large repertoire of cells and cytokines involved in the autoimmune process in type 1 diabetes. Based on studies in animal models and in humans, it is well recognized that there is an imbalance between effector cells (related to β cell destruction) and regulatory cells (related to immune tolerance). Effector cells and cytokines are basically pathogenic B cells, pathogenic CD4 and CD8 cells, and activated dendritic cells, inducible nitric oxide synthase, interleukin-12 etc. Cells and molecules related to immune tolerance are regulatory B and T cells modulated by interleukin-10, indoleamine-2,3,dioxygenase, transforming growth factor-β etc [18].

In the pathophysiology of autoimmune diabetes it is observed as an invasion of the islets by mononuclear cells leading to an inflammatory reaction termed insulitis. β cell death in the course of insulitis is probably caused by direct contact with activated macrophages and T-cells, and/or exposure to soluble mediators secreted by these cells, including cytokines, nitric oxide and oxygen free radicals [19]. However, accumulating evidence indicates that β cells die by apoptosis in early type 1 diabetes. Apoptosis is an active, gene directed process and depends on the parallel and/or sequential up- and down-regulation of hundreds of genes [20]. The picture emerging from these findings is that β cells are not passive bystanders of their own destruction. They respond to cytokine-mediated damage by triggering several genes involved in defense/repair and endoplasmic reticulum stress, by decreasing their most differentiated functions and their capacity for growth and regeneration, and by inducing expression of diverse cytokines and chemokines depending on activation or blocking of NF-κB and STAT-1 [21].

Hematopoietic Stem Cell Transplantation for Autoimmune Diseases

Hematopoietic stem cells are considered adult stem cells generally characterized by the expression of CD34 and Thy1 and the lack of expression of CD38, CD33, and HLA-DR [22]. Hematopoietic stem cells may be obtained through direct aspiration of bone marrow, a procedure that requires anesthesia and aspiration of large volumes of bone marrow. Alternatively, stem cells can be mobilized from

bone marrow into the peripheral blood after administration of growth factors, with or without additional chemotherapy, followed by harvesting through apheresis.

In 1974, Morton et al demonstrated transmission of a lupus-like syndrome from genetically predisposed mice to wild-type irradiated animals, through bone marrow transplants [23]. Several years later, a different research group was able to treat mice with autoimmune encephalomyelitis using bone marrow from healthy animals [24]. Numerous other experiments followed, exploring therapeutic applications for different autoimmune diseases [25, 26]. In the beginning, only allogeneic bone marrow cells were used for transplants and the animals, although remitted from the original autoimmune disease, presented a sometimes equally or more severe graft-vs-host disease, caused by an immunological aggression from the grafted lymphocytes against the host tissues. A turn in history happened in 1991, when Knaan-Shanzer et al were able to induce remission of mouse models of adjuvant arthritis using autologous bone marrow grafts [27]. This first and unexpected model of autologous transplantation for an autoimmune disease surprised the scientific community and began a new research line. It was now possible to avoid graft-vs-host disease and investigate the applications of the much less aggressive autologous transplantation.

A second push for stem cell transplantation towards the treatment of autoimmune diseases came from clinical observations of bone marrow transplantation, procedure that has been used for decades in the treatment of hematological disorders. Ablative or suppressive doses of chemo or radiotherapy are applied to the patient, followed by infusion of autologous (same donor), syngeneic (identical twin) or allogeneic (different donor) hematopoietic stem cells harvested from the bone marrow or peripheral blood. The stem cell infusion aims to repopulate the destroyed bone marrow, thereby reestablishing the hematopoietic tissue. Since 1997, sporadic publications have reported cases of patients bearing concomitant hematological and autoimmune diseases, which have presented remission of both disorders after bone marrow transplantation [28]. In 2004, Marmont analyzed 38 such cases from the literature and from his personal experience and demonstrated that both autologous and allogeneic transplants were effective in the control of autoimmune manifestations in humans [29]. Similarly to the observations in animals, allogeneic transplants in humans seemed to induce better control of the autoimmune diseases over time. A careful analysis, however, reveals that autologous transplants for hematological diseases do not usually involve any kind of T cell depletion, and auto reactive lymphocytes are injected with the graft, thus perpetuating the autoimmune disease. The same publication has addressed the impact of bone marrow transplantation on type 1 diabetes; 3 patients who had type 1 diabetes

for more than 3 years remained diabetic after transplantation, without any change in the metabolic profile [29].

Since 1996, hematopoietic stem cell transplants for autoimmune diseases have been reported [30, 31]. Initial publications established safety of the procedure, followed by evaluations of efficacy for different disease subsets. Today, over 1800 transplants for autoimmune diseases have been registered all over the world [32]. Most cases are of multiple sclerosis, followed by systemic sclerosis, systemic lupus erythematosus and several other diseases less frequently included. The main concerns are to maintain safety, detect disease subsets that may benefit most from the procedure, and improve long-term disease remission.

Different centers use slightly divergent procedures, but in most cases patients are initially mobilized with G-CSF and cyclophosphamide, followed by peripheral blood cell harvesting through apheresis. Cells may be manipulated for lymphocyte removal and are cryopreserved in dimethyl sulfoxide (DMSO). In a second phase, patients receive immunosuppressive or even myeloablative doses of chemo or radiotherapy associated to anti-thymocyte (ATG) or anti-lymphocyte globulin (ALG), followed by intravenous infusion of the previously cryopreserved unmanipulated hematopoietic stem cells. The use of ATG/ALG promotes an in vivo depletion of T-cells and some, but not all transplant centers, believe that additional graft selection can be waived. This is a point of controversy, still to be determined by future comparative studies.

Specific mechanisms of action of hematopoietic stem cell transplants for autoimmune diseases are still undetermined, and we do not exactly know if the above mechanisms are a consequence of high dose immunosuppression, hematopoietic stem cell infusion, or both. In general, the immune system becomes more tolerant to auto-antigens. Immunological reconstitution investigations evidence an increase in naive lymphocytes, recently emigrated from the thymus, an increase in the population of T-regulatory cells and a normalization of the T-cell receptor repertoire, all indicators of a renewed immune system. These changes are associated with disease remission and are reversed with disease reactivation, indicating persistence of the autoimmune clone [33, 34]. De Oliveira and colleagues showed that another effect of "immunologic reset" is up-regulation of fas and fasL proapoptotic genes expression in patients' peripheral blood mononuclear cells after autologous hematopoietic stem cell transplantation [35].

Although immunological resetting seems to be the main therapeutic mechanism of these transplants, some other effects of stem cells are questioned. The potential of the transplanted hematopoietic cells to directly differentiate into pancreatic β cells is one issue. In some diseases, repair is observed or at least suggested after transplantation, but whether it is promoted by the injected cells or through

paracrine effects upon the tissue resident cells has not yet been determined [36]. Another point of discussion concerns the use of autologous or allogeneic grafts. Autologous transplants are safer, but may implicate in higher rates of disease reactivation. Allogeneic transplants, on the other hand, bear the risk of graft-vs-host disease, but may induce longer remission, not only because of avoiding the infusion of auto reactive lymphocytes within the graft, but also because circulating donor lymphocytes may have a long-lasting surveillance function, eliminating possible host auto reactive lymphocytes that may have survived therapy. This is called the graft-vs-autoimmunity effect [29].

Most centers consider allogeneic transplants excessively aggressive for autoimmune diseases, whose mortality rates are relatively low compared with hematological malignancies [37]. Therefore, some researchers support the use of autologous transplants associated with maintenance drugs after transplantation or intensifying the conditioning regimen by addition of immunosuppressive or immunomodulatory agents [38].

Clinical Trials Analyzing the Effects of Nonmyeloablative Immunosuppression Followed by Autologous Hematopoietic Stem Cell Transplantation in Type 1 Diabetes

Based on the theory of possible reconstitution of immune tolerance after "immunologic reset" with autologous nonmyeloablative hematopoietic stem cell transplantation, the optimal time to apply this therapy is soon after the clinical diagnosis (Fig. 1) [39, 40]. As discussed above, the higher the residual β cell mass, the lower the incidence of chronic complications in type 1 diabetes. This approach is somewhat different from research protocols for other disabling autoimmune diseases such as systemic lupus erythematous, multiple sclerosis, and scleroderma that use stem cells transplantation only in cases of

failure of traditional immunologic treatments. Patients with long-term type 1 diabetes are less likely to achieve therapeutic success with autologous hematopoietic stem cells transplantation because they have much less functioning β cells and there is no evidence that hematopoietic stem cells are able to directly differentiate into β cells [36].

In the face of all discussed above, our research group of the Faculty of Medicine of Ribeirão Preto in Brazil started in 2003 a pioneer phase I/II study of autologous nonmyeloablative hematopoietic stem cell transplantation in patients with newly diagnosed type 1 diabetes [41••, 42••]. Patients were mobilized with 2 g/m2 cyclophosphamide plus a granulocyte-colony stimulating factor. Stem cells were then harvested from the peripheral blood through apheresis and cryopreserved in 10 % DMSO, unselected. After approximately 15 days from beginning of mobilization, patients were conditioned with 200 mg/kg cyclophosphamide plus 4.5 mg/kg rabbit anti-thymocyte globulin, followed by intravenous infusion of previously collected unmanipulated autologous hematopoietic stem cells. A subsequent neutropenic phase lasted about 6 days, during which fever and infections were closely monitored and treated preemptively with broad spectrum antibiotics. After leukocyte engraftment, patients were discharged from the hospital and transferred to an outpatient clinic, with regular monitoring of glycemic status, hematological recovery and infections. Since 2008, every patient who resumed insulin use started to use sitagliptin 100 mg/day.

A similar protocol was conducted at the University of Nanjing in China and was published in 2012 [43•]. The group of the University of Warsaw in Poland published an almost similar study in 2009. In the latter protocol, they included 2 or 3 plasmaphereses before the mobilization regimen with the theoretic objective to remove circulating antibodies and immunological complexes. As part of the endocrine control, all patients used acarbose after transplantation to help control glucose levels (up to 300 mg/day)

Fig. 1 Temporal evolution of β cell mass in type 1 diabetes mellitus; the dark area represents the optimal time when autologous hematopoietic stem cell transplantation is most appropriate to preserve residual β cell mass
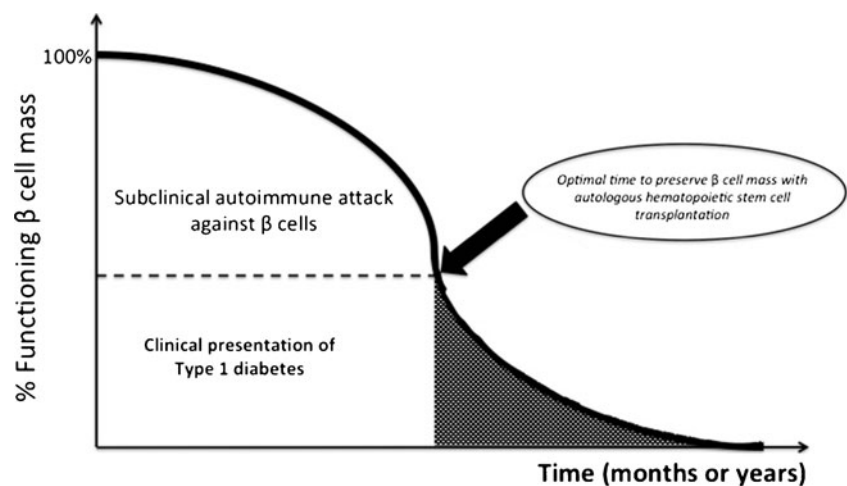
[44•, 45]. Some laboratory and demographic characteristics of patients included in the 3protocols are shown in Table 1.

In theory, factors associated to better β cell preservation are conditions known to be accompanied by larger β cell mass and higher probability of disease remission: older age at diagnosis, absence of previous diabetic ketoacidosis, shorter time of disease, higher C-peptide levels at diagnosis, and lower markers of autoimmunity [18]. However, since most trials included only a small number of patients, statistical significance was not achieved with many of these variables.

In these non-randomized, uncontrolled trials, 22 % to 84 % of the patients presented periods free from insulin varying from months to years and all these studies presented temporal increments in C-peptide levels compared with pretreatment values after 3 or 4 years. The smallest proportion of patients insulin-free occurred in the Chinese protocol and it maybe due to the fact that they enrolled more patients with diabetic ketoacidosis and with longer time of clinical diagnosis.

In the Chinese trial, the survival of remaining β cells was positively associated with the preexisting C-peptide levels but negatively with preexisting autoantibodies. The numbers of infused CD34+ cells were positively correlated with the concentrations of serum interleukin-10, interleukin-4, transforming growth factor-β and fasting C-peptide but negatively correlated with the levels of serum tumor necrosis factors -α and insulin doses after stem cell transplantation.

Some criticism has come because of the fact that these trials were not designed with a control group. However, it is almost impossible to include a consecutive number of patients who coincidently became insulin-free for months or years simply because of a honeymoon phase.

In the Brazilian trial, the vast majority of patients who resumed insulin needed only one injection of long-acting insulin per day at low doses; SF-36 score of quality of life improved significantly among enrolled patients in a medium-term period (data not published).

Recent trials using less severe immunointerventions such as teplizumab [46], daclizumab [47], etanercept [48], abatacept [49], and rapamycin + interleukin-2 [50] have failed in promoting insulin independence and in making an increase in C-peptide levels in a short follow-up. The possible explanation is that these drugs act only in limited steps of the autoimmune process of the disease. At the present time, "immunologic reset" provided by autologous nonmyeloablative hematopoietic stem cell transplantation remains the only treatment capable of reversing type 1 diabetes in humans.

In one hand, if the benefits of autologous hematopoietic stem cell transplantation are closely related to the wide immunosuppression, risks are also related to this procedure. We must take into account that newly diagnosed diabetic patients differ from patients with hematological malignancies, who have classic indications for this approach. From this point of view it is expected that immunoablation can be a safer procedure in this group of individuals. The vast majority of enrolled patients presented only mildly acute side effects such as chemotherapy-related fever, nausea, vomiting, headache, alopecia, urticaria, rash, and diarrhea. The only severe infectious complication was bilateral pneumonia that occurred in 2 Brazilian patients and was rapidly cured.

Concerns also exist about late complications. A large retrospective survey of fertility after stem cell transplantation involving 37,362 patients revealed that only 0.6 % of

Table 1 Laboratory and demographic characteristics of patients included in clinical trials of hematopoietic stem cell transplantation in type 1 diabetes

	Brazilian trial[28]	Polish trial [29]	Chinese trial [31]
Number of patients	23	8	13
Age at diagnosis, y (mean, range)	18.4 (13–31)	25.8 (19–32)	14.1 (8.3–20.8)
Mean number of unmanipulated infused CD34+ cells	10.5×10^6 /kg	4.1×10^6 /kg	4×10^6 /kg
Diabetes duration at enrollment, months (mean, range)	1.2 (0.8–1.8)	2 (1.8–2.4)	5 (2.2–10.6)
HbA1c at diagnosis, % (mean, range)	8.4 (5.4–11.6)	12.3 (8.4–16.7)	9.1 (5.3–15.5)
n (%) with DKA	3 (13)	0	10 (77)
Daily insulin dose before treatment, IU/Kg (mean, range)	0.41 (0.13–0.59)	0.39 (0.1–0.64)	Not available
Mean fasting C-peptide, ng/mL	1.02 (0–1.28)	0.71 (0.3–2.2)	0.69 (0.48–1.99)
Follow-up, months (mean, range)	29.8 (7–58)	7 (3–16)	42.3 (31–54)
Patients continuously free from insulin, n (%)	12 (52)	7 (87.5)	2 (15.2)
Mean period patients became continuously free from insulin, mo (range)	31 (14–52)	6 (2–15)	42 (36–48)
Patients transiently free from insulin, n (%)	8 (34.7)	1 (12.5)	1 (7.6)
Mean period patients became transiently free from insulin, mo (range)	17.7 (6–47)	Not available	7

DKA diabetic ketoacidosis.

patients conceived after autologous or allogeneic stem cell transplantation [51]. Several studies have demonstrated that a younger age (<25 years) at transplantation is an important predictor of gonadal function recovery [52–54]. However, it is important to point out that there is no long-term analysis exclusively in patients with benign diseases undergoing autologous hematopoietic stem cell transplantation.

Interestingly, in the Brazilian trial, none of the 13 analyzed males had normozoospermia before or after transplantation. A total of 4 patients had low seminal volume, 2 patients had low sperm count, 13 patients had low sperm motility, and 11 patients had morphological abnormalities. After treatment, mean sperm concentration and motility were low, and sperm concentration decreased significantly compared with pretreatment values. Two patients had low seminal volume and all of them had low sperm counts and low sperm motility. In patients with normally formed sperm, the count was initially low and decreased significantly after treatment. In addition, after treatment, the incidence of severe sperm damage (oligoasthenoteratozoospermia) increased from 15.4 % to 50 % of patients in comparison with pre-treatment sperm evaluation. Of note, one of the patients who declined to collect a semen sample 2 years after treatment had fathered a child 6 months earlier and another patient who provided a semen sample at 2 years fathered a child 3 years after the collection [55•]. Two patients fathered children 2 years after transplantation. Other patients are not interested in having children so far.

During long-term follow-up, there was 1 case each of Graves' disease, transient hyper gonadotropic hypogonadism, and autoimmune hypothyroidism, and these complications were not related to the status of insulin use. These late endocrine dysfunctions may be caused by autoimmune dysregulation associated with the transplant procedure per se [56], or by autoimmune polyendocrine syndrome frequently associated with type 1 diabetes [57].

Conclusions

Autologous hematopoietic stem cell transplantation seems to be effective in limited group of recent-diagnosed type 1 diabetic patients. In spite of the adverse effects, this procedure was able to increase C-peptide levels along with great reductions or even suspension of insulin use in the majority of patients.

The cohort of type 1 diabetic patients treated with autologous hematopoietic stem cell transplantation is still being followed for occurrence of long-term complications. Mechanistic analysis is being conducted to explore the immunologic effects of high dose immunosuppression and the causes of relapse presented by some patients. In our opinion, it is possible that even high dose immunosuppression with cyclophosphamide plus ATG is not able to completely deplete peripheral auto-reactive T lymphocytes.

To the best of our knowledge, high dose immunosuppression followed by hematopoietic stem cell transplantation is the only immunotherapy capable of restoring glucose homeostasis in type 1 diabetic patients. A multicenter controlled randomized phase I/II trial has been underway since 2011 to address some of the unsolved questions and concerns regarding the risks and possible benefits of the procedure [58]. It is expected that this ongoing research protocol definitely figures out why some patients have resumed insulin use and whether the period free from insulin is merely a honeymoon phase. Moreover, other research groups are also individually enrolling patients for similar protocols.

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