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Clinical Outcomes of Fetal Stem Cell Transplantation in Type 1 Diabetes Are Related to Alternations to Different Lymphocyte Populations

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Abstract

Background: In patients with diabetes, transplantation of stem cells increases C-peptide levels and induces insulin independence for some period. Today, this positive therapeutic outcome is widely attributed to the well-documented immunomodulatory properties of stem cells. The aim of this study was to report alternations (the trend of increase or decrease) in different lymphocyte populations in a stem cell clinical trial performed in our institute.

Methods: Recorded data of a clinical trial conducted on 72 patients with type 1 diabetes who had received fetal stem cell transplantation several years ago and were regularly monitored before and after the procedure in 1, 3, 6, 12, 24 months were analyzed. In these regular follow-up visits, insulin demand, HbA1c, C-peptide, and alternation to B cell and T cell populations were analyzed and recorded. For the purpose of the current study, patients were retrospectively divided into 2 groups, namely, those with the positive response to treatment and patients without such response. Temporary positive therapeutic response was defined by 2 different indicators, namely, plasma C-peptide levels and insulin dose-adjusted A1C (IDAA1c), which was calculated as A1C (percent) + (4 × insulin dose (units per kilogram per 24 h). Data analysis was performed by means of SPSS Version 18.

Results: Besides the short-term therapeutic effect, we observed remarkably significant alternations to the populations of B and T lymphocytes in the recipients. When patients were retrospectively assigned to 2 different groups of patients with a positive therapeutic response (based on C-peptide changes) and those without it, it was observed that alternations to different populations of B-cells and T-cells were significantly different in these 2 groups.

Conclusion: Our results demonstrated that transplantation of stem cells leads to significant positive therapeutic outcomes in one group of patients who showed totally distinct patterns of alternation to different groups of lymphocytes.

Keywords: Stem Cells, Type 1 Diabetes, Immune System, Lymphocytes

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Introduction

Type 1 diabetes (T1D) is caused by T cell-mediated autoimmune destruction of β-cells of the pancreatic islets, which leads to insulitis, insulin deficie ncy, and ultimately, hyperglycemia (1). In T1D, the process of beta-cell

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↑What is "already known" in this topic:
Stem cell interventions lead to significant therapeutic response in patients with type 1 diabetes, but this effect is temporary and short-lived, and the underlying mechanisms remain obscure.

→What this article adds:
Findings of this study demonstrate that therapeutic response is related to alternations to different lymphocyte populations.

destruction commences several months before the clinical presentation, and, on diagnosis, there still remains a considerable number of functional β-cells (2). Considering this, in the recent decades, several immunomodulation-based therapeutic approaches have emerged aiming at preservation (and ideally proliferation) of the surviving β-cells through mitigation of the underlying immune-mediated insulitis (3). Nonetheless, despite remarkable temporary success confirmed by means of both laboratory and clinical indicators, most such studies have failed to address insulin deficiency in a clinically effective and permanent manner (4).

Recently, stem cell transplantation has emerged as a potentially-curative treatment option for different autoimmune diseases, including T1D (5). However, despite remarkable positive short-term achievements in this field, the precise underlying mechanisms remain obscure (6). In this regard, whereas some researchers have attributed positive clinical outcomes to regenerative properties of stem cells, today, most believe them to be caused by the well-documented immune-modulating properties of these cells (5). It should be noted, however, that although several in vitro studies have demonstrated that murine embryonic stem cells (ESC) can differentiate into cells with β-cell-like phenotypes (7-12), evolvement of efficiently-functional cells possessing all aspects of beta cells has not yet been realized (13). Furthermore, stem cells, which are most commonly administered via intravenous infusion, are known to be captured in the microvasculature of the lungs where the majority of them are cleared within 24 hours (14). Therefore, it appears that stem cell-based interventions alleviate type 1 diabetes through some hitherto-obscure alternations to the recipients' immune system.

In the current study, we intend to describe the reanalysis of the recorded data and updated clinical outcomes and immune-monitoring results of 72 patients with type 1 diabetes who received allogenic fetal stem cells. All patients were regularly monitored for a period of 24 months. We then retrospectively divided the patients into 2 groups, namely, those with positive response to the intervention and those without such response. Response to treatment was assessed through measurement of fasting plasma C-peptide concentrations. Subsequently, IDAA1c confirmed C-peptide results. As described in details elsewhere, calculation of insulin dose-adjusted A1C (IDAA1c) is considered as one of the most reliable representative indicators of preservation of β cells in interventions aiming at reversing treatment of type 1 diabetes (15). Subsequently, we analyzed these 2 groups in terms of alternations made to different lymphocyte populations and compared the outcomes.

Methods

For the purpose of the current study, recorded data of the laboratory investigation of a phase 2 single-arm clinical trial designed for assessment of efficacy of allotransplantation of fetal liver-derived stem-cells in 72 diabetic patients were analyzed. Details of this procedure are elaborated elsewhere (16). This clinical trial which is registered on the national clinical trial registry of Islamic Republic of Iran (IRCT) (identifier code: IRCT201103171414N23; at http://clinicaltrials.ir) was conducted in accordance with good clinical practice guidelines, and was approved by the organizational ethics board of Tehran University of Medical Sciences (Code: E-0089). Before the study, written informed consent was obtained from all participants in full compliance with the Declaration of Helsinki. Besides, a data safety and monitoring board supervised all stages of the procedure and subsequent follow-up sessions.

Patient Selection

All participants were newly-diagnosed cases of T1D from Tehran (Iran) who were referred to Shariati hospital affiliated to Tehran University of Medical Sciences in Tehran from 2010 to 2013. The population included 72 patients (33 men and 39 women) aged between 5 and 53 and 19.84±9.14 years with diabetes duration of 26±6.2 months. All patients fulfilled the clinical diagnostic criteria set for type 1 diabetes, but due to some constraints, which are elaborated as the limitation of the study in the discussion section, we failed to confirm the diagnosis through measurement of diabetes antibodies. Exclusion criteria were acute vascular inflammation, acute thrombosis, recent retinal hemorrhage, pulmonary hypertension, corpulmonale, bone marrow malignancies, end-stage diseases, infections, and any sign of progressed stages of the disease, such as diabetic ketoacidosis.

In order for the procedure to proceed, all patients were admitted to an affiliated hospital. On the admission day, primary clinical examinations were performed, and laboratory data (including FBS, HbA1c, fasting serum c-peptide, CBC, liver function tests, lipid profile tests, and U/A) were carefully collected and recorded. These data were again collected on regular follow-up visits on the 1st, 3rd, 6th, 12th, and 24th months after the procedure. Each follow-up visit included a complete history taking, physical examination, and laboratory tests. To optimize the diabetes care, it was ensured that each participant had access to a physician through a designated 24-hour phone line during the first year of the follow-up period.

Stem Cell Preparation

Having obtained informed consent from either or both of the parents, fetal liver-derived hematopoietic stem cells (HSCs) were harvested and isolated from legally-aborted human fetuses aged 6 to 12 weeks. Subsequently, to detect any possible chromosomal abnormality and to identify the sex of the aborted fetuses, karyotyping was performed in all samples.

Whole fetal livers were stored in Hank balanced salt solution without calcium and magnesium (HBSS, Sigma, USA) and mechanically dissociated and homogenized. The cell suspension was then filtered through a nylon mesh so that the cells could be collected for transplantation. Then, the isolated cells were cryopreserved with the use of 5% dimethyl sulfoxide (DMSO) in HBSS, (Wak Chemie) by means of a programmable freezer and were transferred to liquid nitrogen containers for long-term storage. Before infusion, samples were thawed at 37°C

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Table 1. Bacterial and viral particles the stem cells suspensions were tested for before administration

Microorganisms
Rubella
Herpes Simplex Virus
Cytomegalovirus
Mycoplasma Homonis
Toxoplasma Gondii
Chlamydia
Treponema Pallidum
HBV
HCV
HIV

and cryoprotectant was diluted by 5-m normal saline. Total cell count in the prepared suspension was approximately 35-55×10^6 and at least 20% of them were identified as hematopoietic (CD34+) stem cells.

The suspension was monitored before, during, and after processing for any possible aerobic, anaerobic, fungal, or viral contamination. Rubella, Herpes Simplex Virus, Cytomegalovirus, Chlamydia, Mycoplasma Homonis, Toxoplasma Gondii, and Treponema Pallidum were specifically tested. DNA/RNA extraction and polymerase chain reaction (real-time PCR) were performed for investigation of any potential viral contamination (HBV, HCV, and HIV). In the end, having passed several safety tests, all prepared cells were declared contamination-free and suitable for transplantation (Table 1).

Intervention

On the day of transplantation, each participant in the intervention group intravenously received fetal liver-derived cell suspension at the dosage of approximately 35-55×10^6 cells (7-11×106 CD34+ HSCs) in 5 m of normal saline (It should be noted that we did not adjust the doses according to the body mass index of the individual patients and all received the same mentioned dose) (15).

Calculation of IDAA1c

We deliberately reduced daily insulin doses to presumably "stimulate" beta cells to produce more insulin. Consequently, in most patients, HbA1c levels significantly rose, and neither of the aforementioned parameters could be relied on for assessment of the therapeutic response. Therefore, we measured and reported IDAA1c as a surrogate indicator of β cell function. This parameter is widely used for the assessment of the therapeutic response after interventions for treatment of type 1 diabetes. The related equation was calculated according to the following formula: HbA1c (%) + [4 × insulin dose (units per kilogram per day) (15).

Analysis of Lymphocyte Subsets

Isolated cells were analyzed for several markers using flow cytometry. Antibodies against CD8, CD4, CD19, CD20, and HLA-DR (all from Abcam, USA) were used in flow cytometry. The cell cycle analysis was performed by flow cytometry using specific staining buffer, including PBS with RNase A (Sigma), and Propidium Iodide (Sigma, USA).

Data Analyses

Normality of quantitative variables was assessed by the Kolmogorov-Smirnov test, and quantitative variables with normal distribution were reported as mean and standard deviation. Variables without normal distribution were reported as median (interquartile range). To compare quantitative variables without normal distribution between groups, the Friedman test was applied. Statistical analyses were performed using SPSS-16 and significance was set at $P < 0.05$.

Results

Short-term and Long-term Safety of the Procedure

Stem cell infusion was generally tolerated well and no serious adverse events occurred (apart from one case of hypersensitivity reaction, which was successfully managed at the hospital). Nonetheless, local erythematous skin reactions (without swelling) were observed in a few patients immediately after the procedure, which subsided spontaneously soon afterwards. We did not detect any incident of death, and no lymphoproliferative disease, malignancy, or infection were observed which could be attributed to the procedure. As for possible side effects, the outcomes of all clinical examinations and laboratory investigations did not indicate any side effects after the procedure. Even in a long-term follow-up study 10 years after the intervention, no side effects attributable to the procedure was detected (results to be published soon).

Transplantation of Stem Cells Leads to Temporary Increase in c-Peptide Levels

In the current study, insulin doses were progressively reduced after the procedure, and in some patients they were completely suspended. This was performed as it was believed that low insulin levels and high blood sugar concentrations may stimulate remaining beta cells to produce and release more insulin. As published and discussed in details elsewhere (16), fasting C-peptide levels significantly increased during the first 3 months of the study, from which point of time onwards, they gradually decreased. Due to some technical constraints, we compensated to the measurement of the fasting C-peptide instead of stimulated C-peptide (17). However, fasting C-peptide levels are closely correlated to glucagon-stimulated C-peptide in diabetic patients and many contend that it can substitute measurement of stimulated C-peptide levels for the assessment of the function of the remaining β-cell (18).

Transplantation of stem cells leads to temporary Increase in IDAA1c

IDAA1c is widely used for the assessment of success of different immunomodulatory interventions for treatment of type 1 diabetes. In our study, it was demonstrated that IDDA1c only decreased in those whose C-peptide levels had demonstrated significant increase during the first few months of the intervention. This clearly indicates that the intervention had led to a prominent therapeutic response which could be measured through both C-peptide levels

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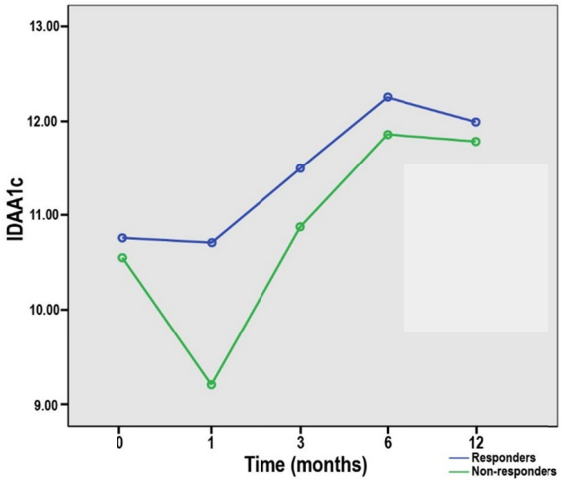

Fig. 1. Comparison of IDAA1c between those whose C-peptide levels was temporarily increased and those who did not. As can be seen, IDDA1c only decreased in those patients who had increased C-peptide levels.

and IDAA1c (Fig. 1).

Intravenous Infusion of Fetal Stem Cells Alters Populations of CD4+ and CD8+ T Cells

In this study, we aimed to analyze possible alternations to different lymphocyte populations after transplantation of stem cells in the 24 months period after the intervention. As published elsewhere (19), our findings demonstrated that there were significant alternations to different populations of lymphocytes after the procedure, and the frequencies of CD4+ T-helper cells, CD8+ cytotoxic T cells, and CD19+ and CD20+ B lymphocytes were significantly altered in the recipients.

As discussed in detail elsewhere (19), after the intervention, the number of CD4+ cells showed an increase in the first month ($p=0.003$). However, their frequencies decreased during the next 2 months insignificantly ($p=0.269$). At this point, the number of CD4+ lymphocytes demonstrated a sharp increase ($p<0.001$), which reached to its peak in the nineth month when started to decrease to higher levels in comparison with the baseline at the end of the 24 months period of the study (19).

The number of CD8+ leucocytes demonstrated an increasing trend in the third month, which returned to the initial levels afterwards to peak again at the point of the 12th month. With regard to the CD4/CD8 ratio, the changes were significant throughout the whole period of the study. This ratio was significantly decreased during the first month after the intervention, and steadily increased up to the sixth months of the study. Then, it again decreased to levels lower than the initial records.

Therapeutic Outcome Is Correlated With Alternations to T Lymphocyte Populations

One objective of this study was to compare alternations made to T lymphocytes between those who responded to the intervention and those who did not. As mentioned, response to treatment was defined through increase in fasting C-peptide levels. We observed that after the intervention, both those who responded and those who did not showed a significant increase in the number of CD4+ T cells (Table 2). This was followed by a decrease in the initial levels 3 months after the procedure. Subsequently, those who responded to the treatment demonstrated a sharp increase in the number of CD4+ cells 6 months after the intervention, while those who did not respond did not show any such increase. Both groups finished the 24 months of follow-up with CD4 levels similar to the beginning levels (Fig. 2 A).

With regard to CD8+ cells, both groups showed a significant increase in the first month after the intervention (Fig. 2 B). However, at this point, those who responded demonstrated a significant decrease, which continued until the sixth month. On the contrary, in those who did not respond, the frequencies of these cells sharply increased up to the third month, when they sharply decreased until the 6 months after the intervention. At this point, in both groups of patients, this number sharply increased up to the 24th month, which marked the end of the study period. (Fig. 2 B)

In both groups, the CD4+/CD8+ ratio slightly increased during the first month after the intervention (Table 3). Then, they continued to increase in both groups up to the third month when totally different patterns were observed in the 2 groups. While there was a sharp increase in the index in those who responded until the 6th month, only a slight increase was observed in those who did not demonstrate any therapeutic response. At this point of time, the responders showed a significant decrease, and in those who did not respond, only a slight decrease was observed. From the 12th to the 24th month, both groups demonstrated a sharp decrease in the CD4+/CD8+ ratio (Fig. 2 C).

Table 2. Alternations to different lymphocyte populations following transplantation of stem cells in two groups of patients

CD-19	1 months	3 months	6 months	12 months	24 months	p-value
Positive	12.0000 (7.1)	8.9000 (7.4)	11.4000 (6.3)	14.5000 (6.65)	13.7500 (8.1)	0.000
Negative	12.0000 (6.9)	11.7000 (6.7)	14.2000 (6.25)	15.3000 (8.2)	14.2000 (8.1)	0.05
CD20						
Positive	12.0000 (5)	9.5000 (6.6)	11.3000 (4.2)	14.4000 (5.275)	14.0500 (8.725)	0.000
Negative	12.0500 (5.45)	11.1000 (7.425)	13.5500 (6.6)	14.6000 (9)	13.2000 (7.25)	0.000
CD8						
Positive	24.0000 (4)	20.2000 (7)	19.6000 (6.7)	24.4500 (6.65)	30.3500 (8.45)	0.000
Negative	23.0000 (5.75)	20.5500 (7.525)	21.5500 (8.575)	23.9000 (8.45)	31.0000 (8.5)	0.000
CD4						
Positive	44.0000 (7.4)	43.7000 (9)	46.3000 (6.7)	40.7000 (4.2)	43.3500 (10.35)	0.004
Negative	42.1000 (6.55)	40.6000 (10.3)	41.7500 (12)	42.4000 (8.8)	40.4000 (13.5)	0.337

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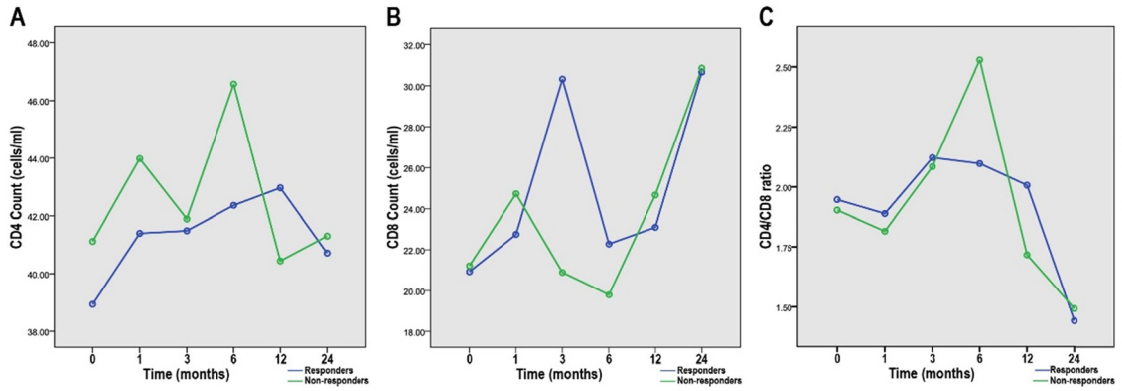

Fig. 2. Comparison of alternations made to CD4+ and CD8+ lymphocytes. A) Changes in the populations of CD4+ cells demonstrated that alternations in CD4+ T helper lymphocytes was different between those who responded to the intervention and those who did not B) Analysis of populations of CD8+ cytotoxic T cells demonstrated that there were significant differences between responders and non-responders C) Comparison between CD4+/CD8+ ratio in those who responded to the intervention and those who did not showed that six months after the intervention there was a sharp increase in this ratio only in those who clinically responded to the stem-cell intervention.

Table 3. Alternations to CD4/CD8 ratio following transplantation of stem cells in patients with type 1 diabetes according to their response

CD4/CD8 ratio	1 month	3 months	6 months	12 months	24 months	P-value
Positive	1.8000	2.1000	2.3500	1.8400	1.4000	0.000
	(0.205)	(0.55)	(0.9925)	(0.5)	(0.95)	
Negative	1.8100	1.8700	1.9750	1.8000	1.8000	0.000
	(0.445)	(0.69)	(0.925)	(0.6)	(0.85)	

Therapeutic Outcome Is Correlated With The Alternations To B Lymphocytes

Following the intervention, in those who responded, B cells demonstrated a sharp decline, which continued until the third month afterwards. In those who did not, on the contrary, a very slight decrease was observed up to the third month after the procedure. At this stage, both respondents and nonrespondents demonstrated a sharp increase, which continued until 12 months after the intervention. At this stage, both groups showed a significant decrease in the population of these cells up to 24 months after the treatment (Fig. 3 A). It should be noted that this pattern was quite similar when both CD19+ and CD20+ markers were measured as indicators of the frequencies of B-cell populations (Fig. 3 B).

Discussion

In this study, indicators of preservation of pancreatic beta cells, namely, fasting C-peptide levels and IDAA1c, showed remarkable improvements during the first few

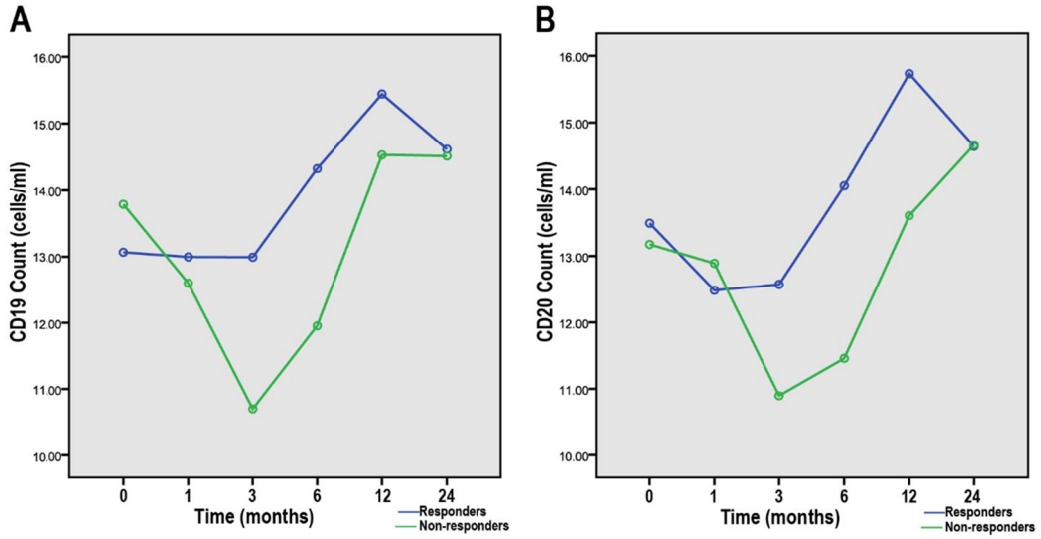

Fig. 3. Alternations in frequencies of B-cells following stem-cell transplantation. A) Analysis in the frequencies of CD19+ lymphocytes showed a sharp decrease during the first three months after the intervention only in those who responded to the intervention B) The temporary decrease in the B lymphocytes was also confirmed by a similar pattern in the frequencies of CD20+ lymphocytes.

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months of the intervention, indicating that the stem cell procedure did halt (or possibly reversed) the process of destruction of the pancreatic beta cells for a short period of time. The C-peptide has an increasing trend during the first years following presentation of the clinical manifestations of T1D, and our findings were quite contrary to what would be expected from the progress of the natural course of the disease (20). Moreover, we observed that following the procedure, significant alternations to different cellular elements of the immune system occurred. Remarkably, we observed that these immune system alternations were closely correlated to the therapeutic response of the patients, and those with positive response demonstrated strikingly different patterns of immune cell alternations in comparison with those who did not respond. Our outcomes may be of considerable application and assistance for design and implementation of future studies aiming at reversing the progress of T1D by means of alternating the immune system.

In 2006 and 2007, a series of clinical trials designed for assessment of effectiveness of stem cell transplantation for treatment of patients with new onset T1D using different stem cell sources was performed and remarkable (though temporary) outcomes were achieved. In one clinical trial using allogenic fetal stem cells, significant therapeutic outcomes (measured based on insulin demand, HbA1c levels, and C-peptide concentrations) were achieved in a group of patients (21). In another trial using a more homogenously-selected young patients with new-onset T1D, C-peptide levels significantly increased in a group (approximately half) of participants (16), and the residual β -cell function, which was estimated by means of calculation of insulin dose-adjusted A1C (IDAA1c) as HbA1c (%) + [4 × insulin dose (units per kilogram per day)], demonstrated significant improvements during the first months after the intervention (15).

Although our primary clinical trial was not designed as a controlled trial, the longitudinal analysis and long-term follow-up of the patients provided excellent opportunity for us to monitor diabetes control indicators and alternations made to the immune system simultaneously. Indeed, as each patient was followed regularly for as long as 12 months, we were able to monitor all related outcome indicators in each individual patient and compare them with those of other patients. Although the intervention was generally safe, a few years after the intervention, 1 case of meningioma was detected, which has been reported and published in details elsewhere (17).

Our measure choices, namely, C-peptide and IDAA1c, can be considered as a relatively accurate approach, which can reflect β cell preservation and diabetes control in an acceptable manner. As the location of the inflammation in T1D is not practically accessible, alternative endpoints, such as stimulated C-peptide secretion and insulin-free periods, have been widely used for measurement of the decline of β cell mass in T1D (3). For instance, Malmegrim et al used the period of insulin independency (in months) as an indicator of positive response to treatment, and accordingly divided their patients into 2 categories of short- and prolonged-remission groups (22). It should be noted, however, that although they did measure C-peptide levels (which verified the general positive outcomes), they did not choose to use it to divide the patients into 2 groups with favorable and unfavorable outcomes (22). Instead, they have used the periods of insulin independence for discriminating patients who responded to the intervention and those who did not. Nonetheless, it should not be overlooked that daily insulin dose and HbA1c levels are closely interrelated. Therefore, one may argue that neither of them can reasonably represent response to treatment. Considering this, we decided that simultaneous measurement of C-peptide levels and IDAA1c can more accurately reflect this response, particularly considering the fact that the latter indicator includes both HbA1c and daily insulin dose (15). In our study, this was confirmed by the finding that only those with increased C-peptide levels demonstrated decreased IDAA1c measures in the initial months of the trial (Fig. 1). This clearly verifies that the stem cell intervention indeed did lead to positive therapeutic response for a short period of time, and suggests that it may be possible to extend this outcome through different methods, such as manipulation of stem cells or application of booster stem cell doses. In fact, as idealistic outcomes, such as definite cure in stem cell interventions seem elusive, recent research recommends that future studies should primarily rely on patient-proximal outcomes, such as temporary improvements in C-peptide levels for months or years in a proportion of patients rather than cure (23).

In 2017, a study by Malmegrim et al, with a design similar to our study, was conducted based on a clinical trial conducted by Voltarelli et al approximately 10 years earlier. Following this trial, they monitored all patients and assessed them for insulin independence, C-peptide levels, frequencies of islet-specific autoreactive CD8+ T cells (CTL), regulatory lymphocyte subsets, thymic function, and T-cell repertoire diversity. As mentioned, they retrospectively divided the patients into 2 groups of short-term and long-term respondents based on the insulin-free periods in months. In the end, they concluded that what they described as “improved immunoregulation” may balance autoreactivity and lead to better metabolic outcomes in a subgroup of patients (22).

It should not be overlooked, however, that the clinical trial that we used as the primary study was quite different in design and implementation from that of Voltarelli et al that provided the data for Malmegrim et al study. In a clinical trial in 2007, Voltarelli et al included patients with less than 6 weeks of clinically and antibody-based diagnosis of T1D. Subsequently, they mobilized their patients’ hematopoietic stem cells with cyclophosphamide and G-CSF, and harvested the cells. The patients were then treated with cyclophosphamide plus rabbit anti-thymocyte globulin, and intravenously received cryopreserved autologous hematopoietic stem cells (24, 25). In our study, on the contrary, no immune suppression was introduced and instead of autologous hematopoietic stem cells, we used allogenic fetal stem cells. It should be emphasized that in contrast to the Voltarelli et al trial, we did not manipulate our patients’ immune systems anyhow. Therefore, unlike

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Malmegrim et al study, we can confidently assert that all changes we observed in the immune systems of our patients were exclusively caused by the injected allogenic fetal stem cells. Therefore, our result can be of superb application in illumination of underlying mechanisms through which stem cells induce their therapeutic effects.

It is documented that C-peptide levels are inversely correlated with the incidence and severity of a wide range of diabetes complications including diabetic nephropathy, neuropathy, and severe hypoglycemia (26). The outcome of our study in terms of decreasing C-peptide levels, even though short-lived, can clearly verify the therapeutic effects of stem cell therapy in type 1 diabetes provided that this effect can be maintained for a longer period. This could be of immense importance for the design and the implementation of future studies investigating therapeutic effects of stem cells or other immunomodulatory approaches for treatment in T1D.

Hitherto, the mechanism of action of stem cells in T1D and other autoimmune diseases has remained obscure.(5, 14) Nonetheless, the prime objective of stem cell-based approach to different autoimmune diseases has generally been induction of immunological tolerance with the main objective of preservation of the remaining pancreatic beta cells (22, 27). Our study provided encouraging evidence that therapeutic effects of stem cells are closely correlated to the alternations made to the immune system lymphocytes. Moreover, we observed similar alternations to immune systems of those who responded to the intervention, and those who did not demonstrated a similar pattern that was quite different. Therefore, it may be hypothesized that stem cell therapy can be effective in a subtype patient whose immune system would respond to this immunomodulatory intervention in a certain manner. Recently, it has been even suggested that the immunopathology of type 1 diabetes is extremely complicated and might be different in different individuals (28). In this regard, our findings may shed some light on the specific subclass of type 1 diabetes in whom stem cell therapy would be effective.

T cells are considered of great importance in pathogenesis of T1D, as they are demonstrated to dominate the islet immune infiltrate. Moreover, transplantation of non-T-cell depleted bone marrow from a patient can intensify diabetes manifestations in the recipient. Besides, anti-T-cell therapies are demonstrated to halt or slow the progress of the destruction of β cells (29). Accordingly, several studies have attempted to measure and compare frequencies of pancreatic or circulating CD4+, CD8+ T, and other T lymphocyte subsets among patients with T1D in comparison with healthy people (30). The results have been encouraging, and some authors have suggested that measurement of the relative frequencies of T cell subsets may be of some clinical application in monitoring and predicting T1D progression, and might even assist in illuminating the pathogenesis of T1D (29). Our findings demonstrated significant differences between alternations in the lymphocyte frequencies of those who respond to the intervention and those who did not. Further analysis for identifying background differences between those 2 groups of patents can be of great value in this regard. Fortunately, we have stored frozen samples of all patients and there are plans to thaw and analyze these samples at some stage in the future.

B lymphocytes play a significant role in the development of T1D. It is documented that B cells exist in a considerable amount in the immune infiltration of islets in the course of disease. Moreover, B lymphocyte depletion by means of monoclonal antibodies temporarily hinders destruction of β cells and slows progression of the disease. (31). In the current study, among respondents and nonrespondents, we measured and compared 2 B lymphocyte markers, namely, CD 19+ and CD 20+ cells. Strikingly, we observed that in the respondents, but not in the other group, B lymphocytes showed a steep decrease during the first 3 months of the intervention (Fig. 3). Comparing with the overall decrease in B lymphocytes in all participants, it is clear that the decrease in the B cell frequencies only occurred in those who responded. This may demonstrate, for the first time, that B cells are effectively involved in the underlying mechanisms of stem cell therapy in preservation of the remaining mass of β cells. This can be of even more significance when one considers that circulatory B cells and those infiltrated into islets are demonstrated to have a close relationship (32).

One of the main limitations of our study was that we failed to measure the area under the curve of the stimulated C-peptide, which many believe that may provide a more reliable indicator for the response to treatment. Moreover, the absence of a healthy control group can be observed as a weakness for our study.

Conclusion

The results of our study demonstrated that transplantation of fetal stem cells can lead to temporary therapeutic response in a subgroup of patient whose lymphocyte frequencies alternate in a particular manner.

Conflict of Interests

The authors declare that they have no competing interests.

References

1. Zhou H, Sun L, Zhang S, Zhao X, Gang X, Wang G. The crucial role of early-life gut microbiota in the development of type 1 diabetes. Acta Diabetol. 2020:1-17.
2. Petrov MS. Panorama of mediators in postpancreatitis diabetes mellitus. Curr. Opin. Gastroenterol. 2020;36(5):443-51.
3. Warshauer JT, Bluestone JA, Anderson MS. New frontiers in the treatment of type 1 diabetes. Cell Metab. 2020;31(1):46-61.
4. Loretelli C, Assi E, Seelam AJ, Ben Nasr M, Fiorina P. Cell therapy for type 1 diabetes. Expert Opin Biol Ther. 2020:1-11.
5. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell Prolif. 2020;53(1):e12712.
6. Helman A, Melton DA. A stem cell approach to cure type 1 diabetes. Cold Spring Harb Perspect Biol. 2020:a035741.
7. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science. 2001;292(5520):1389-94.
8. Millman JR, Xie C, Van Dervort A, Gürtler M, Pagliuca FW, Melton DA. Generation of stem cell-derived β-cells from patients with type 1 diabetes. Nat Commun. 2016;7:ncomms11463.

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9. Pagliuca FW, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, et al. Generation of functional human pancreatic β cells in vitro. Cell. 2014;159(2):428-39.
10. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol. 2014;32(11):1121.
11. Russ HA, Parent AV, Ringler JJ, Hennings TG, Nair GG, Shveygert M, et al. Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. EMBO J. 2015:e201591058.
12. Zhu S, Russ HA, Wang X, Zhang M, Ma T, Xu T, et al. Human pancreatic beta-like cells converted from fibroblasts. Nat Commun. 2016;7:10080.
13. Sneddon JB, Tang Q, Stock P, Bluestone JA, Roy S, Desai T, et al. Stem Cell Therapies for Treating Diabetes: Progress and Remaining Challenges. Cell Stem Cell. 2018;22(6):810-23.
14. de Witte SF, Luk F, Sierra Parraga JM, Gargesha M, Merino A, Korevaar SS, et al. Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells. Stem Cells. 2018;36(4):602-15.
15. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, et al. New Definition for the Partial Remission Period in Children and Adolescents With Type 1 Diabetes. Diabetes Care. 2009;32(8):1384-90.
16. Tootee A, Esfahani EN, Ghodsi M, Farideh R, Amini M, Larijani B, et al. Application of Allotransplantation of Fetal Liver-derived Stem-Cells for Treatment of Type 1 Diabetes: a Single-arm, Phase 3 Clinical Trial. Iran J Public Health. 2015;44(2):36-41.
17. Nasli-Esfahani E, Ghadami M, Amini P, Amiri S, Ghodsi M, Rambod C, et al. Transitional Meningioma After Fetal Liver-Derived Cell Suspension Allotransplant: A Case Report. ECT (Official journal of MESOT) 2017;15(2):231-4.
18. Gjessing HJ, Matzen LE, Frøland A, Faber OK. Correlations between fasting plasma C-peptide, glucagon-stimulated plasma C-peptide, and urinary C-peptide in insulin-treated diabetics. Diabetes Care. 1987;10(4):487-90.
19. Tootee A, Esfahani EN, Ghodsi M, Razi F, Adibi H, Heshmat R, et al. Flowcytometric Assessment of Lymphocyte Subsets in Type-1 Diabetic Patients following Allotransplantation of Liver-derived Fetal Stem-cells. Iran J Public Health. 2015;44(2):48.
20. Klocperk A, Petruzelkova L, Pavlikova M, Rataj M, Kayserova J, Pruhova S, et al. Changes in innate and adaptive immunity over the first year after the onset of type 1 diabetes. Acta Diabetol. 2020;57(3):297-307.
21. Ghodsi M, Heshmat R, Amoli M, Keshtkar A-A, Arjmand B, Aghayan H, et al. The effect of fetal liver-derived cell suspension allotransplantation on patients with diabetes: first year of follow-up. Acta Med Iran. 2012;50(8):541-6.
22. Malmegrim KC, de Azevedo JT, Arruda L, Abreu JR, Couri CE, de Oliveira GL, et al. Immunological balance is associated with clinical outcome after autologous hematopoietic stem cell transplantation in type 1 diabetes. Front Immunol. 2017;8:167.
23. Atkinson MA, Roep BO, Posgai A, Wheeler DC, Peakman M. The challenge of modulating β-cell autoimmunity in type 1 diabetes. Lancet Diabetes Endocrinol. 2019;7(1):52-64.
24. Voltarelli JC, Couri CE, Stracieri AB, Oliveira MC, Moraes DA, Pieroni F, et al. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA. 2007;297.
25. Couri CE, Oliveira MC, Stracieri AB, Moraes DA, Pieroni F, Barros GM, et al. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA. 2009;301(15):1573-9.
26. Huurman VA, Hilbrands R, Pinkse GG, Gillard P, Duinkerken G, Van de Linde P, et al. Cellular islet autoimmunity associates with clinical outcome of islet cell transplantation. PloS One. 2008;3(6):e2435.
27. Nikolic T, Zwaginga JJ, Uitbeijerse BS, Woittiez NJ, de Koning EJ, Aanstoot H-J, et al. Safety and feasibility of intradermal injection with tolerogenic dendritic cells pulsed with proinsulin peptide—for type 1 diabetes. Lancet Diabetes Endocrinol. 2020;8(6):470-2.
28. Congress D. Type 1 diabetes research: poised for progress. 2018.
29. Narsale A, Davies JD. Memory T cells in type 1 diabetes: the devil is in the detail. Curr Diab Rep. 2017;17(8):61.
30. Moya R, Robertson HK, Payne D, Narsale A, Koziol J, Davies JD, et al. A pilot study showing associations between frequency of CD4+ memory cell subsets at diagnosis and duration of partial remission in type 1 diabetes. J Clin Immunol. 2016;166:72-80.
31. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med. 2009;361(22):2143-52.
32. Hinman RM, Smith MJ, Cambier JC. B cells and type 1 diabetes in mice and men. Immunol Lett. 2014;160(2):128-32.

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