A Possible Role in Immune Response Modulation after Autologous Bone Marrow Stem Cell Transplantation in Type1 DM

Mohamed F. Abd El Aziz [1], Abdel Sattar El Deeb[1], Alaa El Din Esmail[2], Hala Ahmed Talkan[2], Khaled Mahmoud Makboul[3], Hanan Mahmoud Ali [1].

[1] Internal Medicine Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[2] General Surgery, Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[3] Clinical Pathology, Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
dr_pharos2000@yahoo.com

Abstract: Background: Autologous hematopoietic stem cell transplantation (AHSCT) has been tested for the treatment of patients with new onset type 1 diabetes. T helper 1 (Th-1) cells secrete Interleukin (IL)-2, Gamma interferon (IFN-γ) and Tumor necrosis factor-β which may cause DM. On the other hand, T helper 2 (Th-2) cells secrete IL-4 and IL-10 which might be of protective value for beta cells. These findings have led to the development of the hypothesis that prevention of type 1 diabetes can beachieved by inhibition of Th-1 reactions and stimulation of Th-2 reactions. **Objective:** To evaluate the possible role of AHSCT done for cases of type 1 DM in modulating the immune response from Th-1 to Th-2. **Study design:** Pilot exploratory study conducted on cases of auto immune type 1 DM within the first 5 year of diagnosis, age above 16 years with positive antibodies against glutamic acid decarboxylase. Before AHSCT all patients were subjected to full history taking, clinical examination, and laboratory investigations: Fasting and post prandial blood sugar, Hemoglobin A_1C (HBA1C), C-peptide, Serum IL-4 and Serum IFN-γ. These laboratory investigations and insulin doses were re-evaluated during follow up periods after AHSCT.**Results:**After AHSCTthere was; a significant increase in the means of C-peptide and IL4, significant decrease in fasting and post prandialblood sugar, HBA1C and the means of IFN-γ, 70% of patients decreased their daily insulin requirement while all patients did not experience any time free from insulin.**Conclusion:** AHSCT intype 1 DM can modulate the immune response from Th-1 to Th-2.
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Introduction

Type 1 Diabetes Mellitus (DM) comprises only 5% to 10% of all diabetic etiologies but it is associated with a high frequency of vascular complications and compromises quality and expectancy of life ***(1)***. Combination of genetic, immunologic, and non-genetic factors contributes to the onset and progression of Type 1 DM ***(2)***.

Pathologically autoimmune diabetes is characterized by mononuclear cell infiltration into the pancreatic islets, termed insulinitis. These mononuclear cells consists of CD4 + and CD8+ T cells, B cells, NK cells, and macrophages ***(3)***. B-cells are among the earliest cells to infiltrate the pancreatic islets of NOD mice, and auto antibodies against islet antigens indicate disease onset in humans and mice. Despite this, autoantibody production is not sufficient to initiate disease and is disconnected from the occurrence of diabetes and insulitis***(4)***. Rather, B-cells are multifunctional and are crucial antigen-presenting cells (APCs) for priming pro- inflammatory T-cell responses to β-cell antigens ***(5).***

T lymphocytes play the most pivotal role in initiating the disease process ***(6).*** In 1986, ***Mosmann et al. (7)*** reported that upon activation, CD4+ T cells will differentiate into two distinct T helper (Th) cell clones expressing distinct cytokine profiles and effector functions, thus giving rise to a unifying Th1/Th2 paradigm. Th1 cells produce IL-2 and gamma interferon (IFN- γ), while Th2 cells produce IL-4, IL-5, IL-10, and IL-13 ***(8)***. Th0 cells, which produce both Th1 and Th2 cytokines, are generally regarded as precursors for Th1 and Th2 cells, being swayed into differentiating into either pathway in response to external stimuli and also in response to Th1 and Th2 cytokines ***(9, 10)***. Th1 cytokines induce Th1 activity and block Th2 activity ***(11)***, whereas Th2 cytokines promote Th2 activity while inhibiting Th1 activity ***(12).*** This indicates that induction of one Th program is accompanied by a corresponding decline in the activation of the other Th program. It was postulated that Th1 cytokines exacerbate, while Th2 cytokines protect from, IDDM ***(13, 14).*** Expression of IFN-{gamma} by Th-1 was not directly toxic to {beta}

cells, but rather affects diabetogenesis by promoting the recruitment and activation of cytotoxic T cells and macrophages***(15)***. Apparently signals induced by IFN-γ do play an important diabetogenic role, since disease development was inhibited in NOD mice congenic for a functionally inactivated IFN-γ receptor ***(16)***.

It is generally believed that effective therapy for autoimmune diabetes will require two scientific advances first restoration of insulin production by providing or eliciting new beta cells, secondly repair of the breakdown in immunological tolerance that precipitated the disease in the first place ***(17)***. Significant advances in the transplantation of human primary islets of Langerhans into individuals with Type 1 diabetes has largely removed this insulin dependency ***(18, 19)***. However, the application of this treatment is restricted by the very limited availability of primary human islets from heart beating donors, and what is now required is an essentially limitless supply of a physiologically competent substitute for primary human islets of Langerhans ***(20***). One alternative for islet transplantation would involve the use of a renewable source of stem cells capable of self-renewal and differentiation, as well as that of insulin production. Indeed, the development of a simple, reliable procedure to obtain autologous stem cells having the ability to differentiate into functional insulin producing cells would provide a potentially unlimited source of islet cells for transplantation and alleviate the major limitations of availability and allogeneic rejection ***(21)***.

A variety of tissues harbor progenitor or stem cell, and it is possible to isolate and expand these cells in vitro and then differentiate them to adopt a ß-cell phenotype, they would be a potential source of substitute tissue for transplantation. It has been reported that stem cells derived from bone marrow can be differentiated in vitro ***(22)*** and in vivo ***(23)*** into insulin-expressing cells. A report of high-dose immunosuppression followed by autologous nonmyelo-ablative hematopoietic stem cell transplantation for human type 1 DM done by ***Voltarelli et al. (24)*** recorded very encouraging results in a small number of patients with early-onset disease. Surprisingly, a number of patients undergoing HSCT for an autoimmune disease have been reported ***(25, 26)***, all were transplanted using autologous hematopoietic stem cells and actually, these data demonstrate the feasibility of stem cells transplantation in giving patients with these autoimmune diseases intense immunosuppressive state.

The autologous transplantation may shift the scales of balance between immunity and tolerance through as yet undefined mechanisms. Theoretically, this may include clonal exhaustion, veto cells, suppressor cells, other auto-regulatory cells, immune indifference, cytokine alterations, infectious agents, changes in T- or B-cell (oligo) clonality, or changes in immune-dominant autoantigens***(27)***.

Bingham et al. (28); stated that autologous stem cell transplantation is starting to be examined as a potential therapy for severe, refractory autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, Celiac disease, Crohn's disease, Dermatomyositis, diabetes mellitus (type 1), Juvenile rheumatoid arthritis, Polymyositis and Systemic vasculitis.

Alexander and colleagues. (29); set up an experimental autologous stem cell transplantation (ASCT) protocol for patients with life-threatening SLE, who were refractory to standard treatment with at least 2 immunosuppressive drugs, they concluded that ASCT induced stable, long-term, clinical and serologic remission in SLE patients with refractory disease as ASCT, autologous progenitor cells give rise to differentiated cells that modulate immune functions with correction of the autoreactive responses.

The aim of our study was to evaluate the possible role of autologous bone marrow transplantation done for cases of type 1 DM in modulating the immune response from Th-1 to Th-2.

2. Patients and Methods

Study design

This study was conducted on 10 cases of auto immune type 1 DM in Ain Shams University Hospitals in The Pancreatic Islet Transplantation and diabetes Research Unit, who were selected according to inclusion and exclusion criteria as follows:

Inclusion criteria; Diabetic cases type 1 within the first 5 years of diagnosis, age above 16 years and both male or female sex .

Exclusion criteria;

Receiving medications that may depress patient's immune system, patients with recurrent diabetic ketoacidosis, pregnancy or lactation, presence of other autoimmune disorder, presence of allergic conditions and patients having congestive heart failure, chronic liver disease or renal impairment.

Study procedures:

Laboratory preparation of stem cells from bone marrow of the patient: after obtaining an informed consent, the patient is given local anesthesia and 10-15 ml aspirates from his iliac crest is obtained.

a) Pre-Transplantation assessment: complete medical history and clinical examination, fasting and post prandial blood sugar, HBA_1C, C-peptide levels, antibodies against glutamic acid decarboxylase (Anti GAD), CBC, Liver function tests, kidney function tests, serum level of IL-4 (as a marker for T helper 2

cell activity) and serum level of INF-γ (as a marker for T helper1 cell activity).

b) Post-injection follow up of: temporal changes in exogenous insulin requirements (dailydose) in addition to assessment of serum levels of HbA_{1c}, C-peptide levels after the 3rd month and by the end of the 6th month post injection, serum level of IL4 and INF γ every 2 months following the procedure for 6 months.

Biochemical methods:

Glycatedhaemoglobin (HbA1c) level was determined using reagent kits while serum C-peptide, serum Interferon-γ and serum Interleukin-4 were measured by ELISA kit.

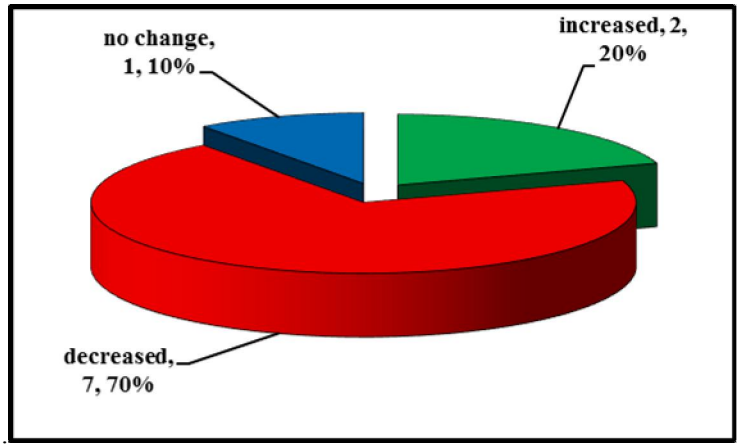
Statistical analysis:

The data was collected, revised, verified and analyzed statistically using SPSS V17.

3. Results:

Table (1): Descriptive data of studied cases as regard: age, weight, BMI, systolic and diastolic blood pressure, duration of type 1 DM, total insulin requirement.

Variable	Range	Mean	± SD
Age	18.000 - 22.000	19.900	1.449
Weight (Kg)	55.000 - 78.000	65.700	6.651
Height (cm)	153.000 - 190.000	167.400	10.002
BMI (Kg/m2)	21.000 - 25.700	23.510	1.730
Systolic blood pressure (mm/Hg)	120.000 - 135.000	128.500	5.798
Diastolic blood pressure (mm/Hg)	70.000 - 85.000	77.500	5.401
Total insulin requirement (pre injection) u/d.	42.000 - 80.000	61.000	11.136
Duration of type 1 DM (years)	3.000 - 5.000	3.700	0.423



Figure(1): Change in total insulin requirement after transplantation

Total insulin requirement after transplantation increased in 20% of cases, Decreased in 70% of subjects (statistical highly significant) and no change in 10% of cases

Table (2): Fasting blood sugar pre-transplantation and 3 and 6 months after transplantation

	Fasting blood sugar			Difference			Paired t-test	
	Range	Mean	± SD	Comp.	Mean	±SD	t	*P*-value
Pre transplantation	138.00 - 250.00	200.40	38.66					
After 3ms	123.00 - 230.00	167.60	34.35	Pre-6ms	48.30	34.64	4.41	0.00
After 6ms	115.00 - 216.00	152.10	31.86	3ms-6ms	15.50	6.55	7.48	0.00

The mean of fasting blood sugar pre transplantation showed highly significant decrease after the 3rd month & the 6th month of transplantation.

Table (3): Post prandial blood sugar pre-transplantation and 3 and 6 months after

	Post prandial sugar			Difference			Paired t-test	
	Range	Mean	± SD	Comp.	Mean	±SD	t	*P*-value
Pre transplantation	155.00 - 350.00	247.80	62.25					
After 3months	125.00 - 300.00	197.20	55.44	Pre-6ms	70.60	38.33	5.82	0.00
After 6months	120.00 - 280.00	177.20	51.17	3ms-6ms	20.00	8.25	7.67	0.00

The mean of post prandial blood sugar pre transplantation showed highly significant after the 3rd month & the 6th month of transplantation

Table (4):hemoglobin A1C level pre-transplantation and 3 and 6 months after transplantation.

	HbA1C			Difference		Paired t-test	
	Range	Mean	± SD	Mean	±SD	t	*P*-value
Pre transplantation	7.000 - 9.300	8.220	0.646				
After 3ms.	7.000 - 9.000	8.050	0.635	0.170	0.295	1.825	0.101
After 6ms.	7.200 - 9.000	7.840	0.643	0.380	0.539	2.229	0.048*

The mean of HbA1C pre transplantation dropped significantly after 6 months of transplantation, however insignificant decline was recorded after 3 months.

Table (5): SerumC-peptide level pre-transplantation and 3 and 6 months after.

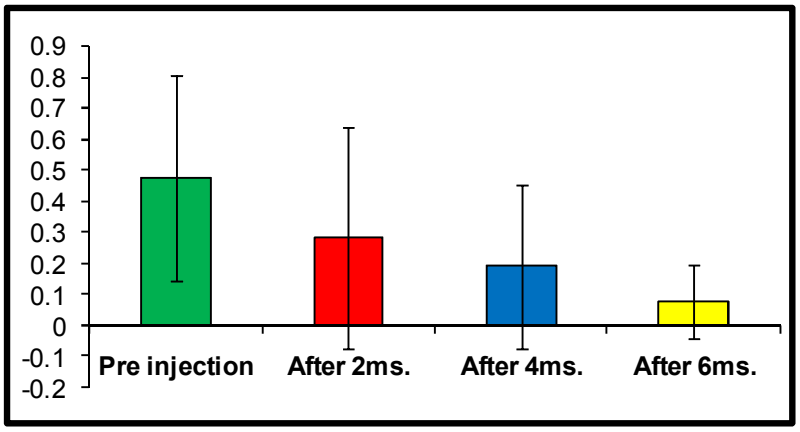
	C-peptide			Difference		Paired t-test	
	Range	Mean	± SD	Mean	±SD	t	*P*-value
Pre transplantation	0.500 - 3.600	1.330	0.907				
After 3ms.	1.500 - 6.300	4.190	1.718	-2.860	1.798	-5.029	0.001*
After 6ms.	2.000 - 9.000	6.120	2.649	-4.790	2.730	-5.549	0.000*

The means of serum C-peptide pre transplantation showed highly significant increase after 3 & 6 months of transplantation.

Table (6): Seruminterleukin 4level pre-transplantation and 2, 4 and 6 months after.

	Interleukin 4			Difference		Paired t-test	
	Range	Mean	± SD	Mean	±SD	t	*P*-value
Pre transplantation	30.000 - 90.000	64.100	18.387				
After 2ms.	40.000 - 120.000	84.800	20.666	-20.700	14.221	-4.603	0.001*
After 4ms.	88.000 - 122.000	103.900	13.000	-39.800	22.783	-5.524	0.000*
After 6ms.	95.000 - 130.000	111.200	11.053	-47.100	19.439	-7.662	0.000*

The mean of IL- 4 pre transplantation showed highly significantly increase after transplantation by 2 months with further highly significant rise after 4 & 6 months.



Figure(2): Gamma interferon pre and after transplantation 2, 4, 6 months.

The mean of Gamma interferon pre transplantation showed highly significant decline after transplantation by 2 months with further drop after the 4th and 6th months.

4. Discussion

Type 1 diabetes is an autoimmune disease and results from T cell-mediated destruction of insulin-producing pancreatic beta cells. Patients at onset of type 1 diabetes usually have limited beta cell mass and depend on exogenous insulin treatment. Although intensive insulin treatment can achieve adequate glycemic control, it does not completely prevent the development of diabetic complications. Hence, the development of new therapies to control T cell autoimmunity and to preserve the remaining beta cell function will be of great significance in managing patients with type 1 diabetes. ***(30)***

Autologous hematopoietic stem cell transplantation (AHSCT) has been tested for the treatment of patients with new onset of type 1 diabetes. This therapeutic strategy can result in exogenous insulin independence by destroying pathogenic memory T cells and preserving the remaining beta cell function. However, little is known about the efficacy of AHSCT in the dynamics of immunocompetent cell reconstitution and how the reconstituted immune system regulates beta cell-specific antibody response. ***(31)***

The cytokine profile of T-helper cells is crucial to the development of an effective immune response. Th-1 cells secrete IL-2, IFN-γ and tumor necrosis factor beta (TNF-β) which may cause diabetes in different ways. These mediators may stimulate immune inflammatory responses such as the activation of cytotoxic T-cells. This, in turn, may lead to destruction of pancreatic β-cells and activation of macrophages leading to the secretion of pro-inflammatory cytokines such as IL-1, TNF-α, IFN-γ and free oxygen or nitrogen radicals which are cytotoxic to beta cells ***(32).***

On the other hand, Th-2 cells secrete cytokines such as IL-4 and IL-10 which may be protective for beta cells. Along with its ability to drive T-helper cells to a Th-2 phenotype, IL-4 also possesses strong down regulatory properties with respect to Th-1 cells not excluding autoreactive Th-1 cells. The expression of IL-4 in the target organ significantly reduced the diabetogenic potential of islet-specific T-cells. These findings have led to the development of the hypothesis that prevention of type-1 diabetes can be achieved by inhibition of Th-1 reactions and stimulation of Th-2 reactions ***(33).***

The aim of our study was to evaluate the possible role of autologous bone marrow transplantation done for cases of type 1 DM in modulating the immune response from T helper 1 to T helper 2 cells.

This study was conducted on 10 patients with auto immune type 1 diabetes mellitus, selected according to inclusion and exclusion criteria and who agreed to sign a written consent.

In our study, the mean of fasting and post prandial blood sugar showed highly significant decrease after 3 and 6 months following transplantation and despite the insignificant decline recorded for HbA1C after 3 months post transplantation, a significant decline was observed after 6 months of transplantation. these results came close to study done by ***Dave et al. (34);*** who found improvement in mean Hb1Ac from 10.99 to 6.72% after treating IDDM patients with autologous adipose tissue-derived MSC mixed with hematopoietic stem cells (HSC). Our results also agreed with the study done by ***Couri et al. (35);*** who recorded a highly significant decline in the mean pre-transplant HbA_{1C} from 8% to 5.4%, 5.7%, 5.7%, 5.5% and 6.0% at 3, 12, 24, 36 and 48 months post AHSCT respectively, following AHSCT done for 23 type 1 diabetic cases. Furthermore our results agreed with the study done by ***Otonkoski et al. (36);***who recorded a significant drop in HbA1c concentration from 11.5 % pre transplantation to a level of 5.88% at 6 months and 5.76% at 12 months following AHSC transplantation in15 patients (age 19 - 32) with early diagnosis of type 1 diabetes.

In our work the means of C-peptide level before transplantation (1.330 $\pm$ 0.90) showed a significant increase at 3 and 6 after autologous bone marrow transplantation (4.19$\pm$1.71), (6.12$\pm$2.64) respectively this result agreed with the study done by ***Carlos et al. (37);***who dida prospective study on 23 patients with type 1 DM (aged 13-31 years) recently diagnosed and confirmed by measurement of serum levels of anti-glutamic acid decarboxylase antibodies showed that the C-peptide levels increased significantlyafter a mean follow-up of 29.8 months following autologous HSCT. Also our result agreed with ***Ablamunits et al. (38);*** who recorded a significant elevation in serum C-peptide level 6 months following AHSCT performed for 13 diabetic patients. In contrast to our result, ***Giannopoulou et al. (39);*** who found insignificant changes in serum C-peptide level at 12 months follow-up between seven children with newly diagnosed type 1 diabetes underwent a single autologous cord blood infusion and 10 children who were enrolled as natural controls children.

In our study, the daily total insulin requirement increased in 20% of studied subjects, but the total insulin dose decreased significantly in 70% of studied subjects, this result came close to the study done by ***Weiqiong et al. (40);***whofound a significant decrease in the average daily insulin dose requirements at 1 month after AHSC transplantation, however the lowest dose was reached after 3 months, and remained at a

stable level for at least 24 months in a study involved 28 type 1 diabetic patients. In contrast to our study where no subject recorded insulin free time, ***Couri et al. (35);***Who found after a mean follow-up period of 29.8 months post AHSCT, 20 patients out of 23 one to become insulin free; 12 patients maintained this status for a mean of 31 months (range 14–52 months), while 8 patients relapsed and resumed insulin use at a low dose (0.1–0.3 IU/kg). Also ***Snarski et al. (31);*** recorded that all of the enrolled eight naïve type 1 diabetic patients in his study to become insulin free after the transplantation but one patient resumed using low-dose insulin 7 months after the transplantation, and six patients were given acarbose for better glycemic control after transplantation but actually the procedure performed in that study involved performing 2 to 3 plasmaphereses, hematopoietic stem cell mobilization with colony stimulating factor, collecting of at least 3 × 10(6) per kg of CD34+ cells, and conditioning with anti-thymocyte globulin followed by stem cell infusion.

In the present study the mean of Interleukin 4 significantly increasedafter transplantation by 2, 4 and 6 months to (84.8±20.6), (103.9±13) and (111.2±11) respectively this came close to the study done by ***Voltarelli et al. (41);*** who found an increase in the numbers of regulatory CD4+ T cells and Th2 cytokines-producing cells, following AHSCT done for 21 newly diagnosed type 1 diabetic cases. Also our result came close to the study done by ***Mesples et al. (42);*** who showed negative value in ICA, GAD and anti-insulin antibody levels, with an increased levels of C- peptide and decreased levels of blood glucose and HbA1c in two naïve type 1 diabetic patients (≤ 8 years old) after 12 months of treatment with autologous bone marrow stem cell. However our result came in contrast to the study done by***Lirong et al. (43);*** who found insignificant difference between serum level of IL-4 before AHSCT and throughout the observation period (31–54 months) following the transplantation done for thirteen naïve type 1 diabetic patients.

In our study, the means ofserum Gamma interferon level significantly decreased ($p<.001$) after transplantation at 2, 4 and 6 months, thisresult came along with the study done by***Zhang et al. (44);***who found The acute responses in lymphocytes at six-month follow-up include declined CD3(+)CD4(+), CD3(+)CD8(+) T cell population, concluding that AHST may eliminate the islet specific autoreactive T cells thus improving the islet function in newly diagnosed type 1 diabetic patients. In contrast to our finding,***Lirong et al. (43);*** found insignificant difference between serum level of Gamma interferon before AHSCT and throughout the observation period (31–54 months) following the transplantation done for thirteen naïve type 1 diabetic patients. Also our result disagrees with ***Giannopoulou et al (39);*** who found insignificant changes in immune response (islet autoantibody titer and T-cell response) at 12 months follow-up for seven children with newly diagnosed type 1 diabetes underwent a single autologous cord blood infusion.

In conclusion, the present work documented that the Autologous hematopoietic stem cell transplantation (AHSCT) for autoimmune type 1 DM can modulate the immune response from T helper 1 to T helper 2, this conclusion based on measurement of INF γ (marker of T helper 1) which was significantly decreased and IL4 (marker of T helper 2) which was significantly increased after AHSCT.

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