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Preserved β -Cell Function in Type 1 Diabetes by Mesenchymal Stromal Cells

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The retention of endogenous insulin secretion in type 1 diabetes is an attractive clinical goal, which opens possibilities for long-term restoration of glucose metabolism. Mesenchymal stromal cells (MSCs) constitute, based on animal studies, a promising interventional strategy for the disease. This prospective clinical study describes the translation of this cellular intervention strategy to patients with recent-onset type 1 diabetes. Twenty adult patients with newly diagnosed type 1 diabetes were enrolled and randomized to MSC treatment or to the control group. Residual β -cell function was analyzed as C-peptide concentrations in blood in response to a mixed-meal tolerance test (MMTT) at 1-year follow-up. In contrast to the patients in the control arm, who showed loss in both C-peptide peak values and C-peptide when calculated as area under the curve during the 1st year, these responses were preserved or even increased in the MSC-treated patients. Importantly, no side effects of MSC treatment were observed. We conclude that autologous MSC treatment in new-onset type 1 diabetes constitutes a safe and promising strategy to intervene in disease progression and preserve β -cell function.

Despite intensive research, tested treatments have thus far at best only temporarily arrested the progressive loss of β -cells in type 1 diabetes (1). Retention of endogenous insulin secretion is an attractive goal, since it causes better glycemic control (2) and decreases the risk of microvascular complications and severe hypo- or hyperglycemia episodes (3,4).

Mesenchymal stromal cells (MSCs) are rare non-hematopoietic cells in the bone marrow that produce matrix proteins and contribute to tissue regeneration and repair (5). MSCs also have unique immunomodulatory capacities (6). They can suppress T-cell proliferation in response to nominal antigens or alloantigens (7) and upregulate the number of regulatory T cells (8,9). MSC-mediated immunosuppression is also associated with a reduction in inflammatory cytokine production (10). Several factors have been implied to mediate immunomodulation, including transforming growth factor- β , hepatocyte growth factor, prostaglandin-E₂, interleukin-10, indoleamine-2,3-dioxygenase (11), nitric oxide (12), heme-oxygenase-1 (13), and matrix metalloproteinases 2 and 9 (14).

MSCs can be expanded *ex vivo* and have in a number of studies in animal models of type 1 diabetes been forwarded as an attractive therapy to ameliorate or reverse manifest diabetes (15–17). Besides having systemic immunomodulatory properties, the MSCs have been observed to specifically home to the damaged islets and to local pancreatic lymph nodes. MSCs show distinct efficacy in graft-versus-host disease (GvHD) patients (18,19) with no, or only minor, side effects. Importantly, no increased risk of tumor development in patients is known, and no ectopic tissue formation has been observed (20,21).

The current study aimed to evaluate the safety and efficacy of autologous MSCs in treatment of patients recently diagnosed with type 1 diabetes.

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RESEARCH DESIGN AND METHODS

Patient Selection

Patients newly diagnosed with type 1 diabetes at Uppsala University Hospital or neighboring admitting hospitals were enrolled according to the preset criteria: 18–40 years of age with type 1 diabetes diagnosed <3 weeks before enrollment and with a stimulated C-peptide level >0.1 nmol/L. None of the patients were allowed to be pregnant; have BMI >30; have tested positive for HIV, viral hepatitis B or C, or *Treponema pallidum*; be immune suppressed; or have known or previous malignancy. All participants were given oral and written information about the study and signed a written consent prior to inclusion to the study. The study was approved by the Uppsala ethics board, and the reported investigations were performed in accordance with the principles of the Declaration of Helsinki as revised in 2000.

Study Design

The study was an open, single-center, randomized pilot study at Uppsala University Hospital to evaluate the safety using autologous MSCs in treatment of recently diagnosed type 1 diabetes. Patients meeting the inclusion criteria were randomized in equal numbers (10 in each group for either MSC treatment or only insulin treatment). As secondary end points, treatment efficacy was evaluated and assessments included maintained fasting C-peptide and evoked C-peptide response to a mixed-meal tolerance test (MMTT), blood glucose control by HbA_{1c}, changes in insulin doses per kilogram, and changes in levels of autoantibodies to β -cells (GAD65 and IA2 antibodies). Study end points were evaluated at 10 weeks after diagnosis and at the 1-year follow-up.

Randomization and Masking

Randomization without any blocking restriction was performed by the selection of 1 of 20 identical envelopes containing a unique identification number and group assignment by third party member. No masking was performed. All patients received intensive insulin therapy (i.e., four or more injections per day) with insulin aspart and insulin detemir on the basis of patient-measured blood glucose levels, as well as by continuous glucose monitoring performed on two occasions during the year. Target levels of plasma glucose were between 5 and 8 mmol/L, and HbA_{1c} had a target level of <7.0% (53 mmol/mol). The database was maintained at Uppsala University Hospital, and measurements of C-peptide, HbA_{1c}, GAD65, and IA2 antibodies were performed at Uppsala University Hospital central laboratory.

MSC Treatment

Within 3 weeks of randomization, bone marrow was aspirated from the iliac crest of the participants in the MSC group. Clinical-grade MSCs were then generated under good manufacturing practice conditions as accredited by the Swedish National Board of Health and Welfare in growth media supplemented with lysed human platelets

(final concentration 10^8 /mL) (19). MSCs expressed (>95%) CD73, CD90, CD105, and HLA-ABC and were negative (<5%) for CD14, CD31, CD34, CD45, and HLA-DR (Supplementary Fig. 1). All cells were harvested in passage 1–2 and cryopreserved before infusion. MSC release criteria for clinical use included the following: spindle shape morphology, absence of contamination by pathogens, and viability >95%. Three to four weeks after bone marrow harvest, 2.1 – 3.6×10^6 autologous cells/kg (median 2.75×10^6 cells/kg) were given as one single intravenous 20-min infusion without premedication.

Safety Tests

At each outpatient visit, patients in both study groups underwent history taking to monitor for adverse events. Specific emphasis was put on increased tendency for infections, development of malignancy or other disease, as well as hypo- or hyperglycemic events. During hospitalization, additional physical examinations and blood analyses were performed.

Efficacy Tests

Residual β -cell function was analyzed as C-peptide concentrations in blood in response to an MMTT (6 mL/kg, maximal dose of 360 mL; Resource Protein; Novartis) at an early time point after diagnosis (10 weeks) and at the 1-year follow-up. No concomitant insulin was given at time of testing. In order to avoid potential influence also of their insulin detemir, this was avoided the night before testing in all patients. Instead, the patients were admitted to the diabetes ward and received a combined intravenous glucose rapid-acting insulin infusion, keeping plasma glucose levels between 3.9 and 6.3 mmol/L until the performance of MMTT in the morning. Plasma samples for C-peptide measurements were obtained at 0, 15, 30, 60, 90, and 120 min after meal intake and analyzed with the use of a Roche Modular E and cobas e 601 at Uppsala University Hospital. In addition to the preset criteria of analysis of peak C-peptide value, the repeated measurements allowed calculations of area under the curve (AUC). Also, daily insulin doses per kilogram and HbA_{1c} levels were recorded.

Immunological Monitoring

GAD65 and IA2 antibodies were analyzed at diagnosis, 10 weeks after diagnosis, and at the 1-year follow-up.

Statistics

An unpaired, two-tailed Student *t* test was used to compare differences between two groups of parametric data, whereas Wilcoxon rank sum test was used for nonparametric data. All values are given as means \pm SEM. *P* values <0.05 were considered statistically significant.

RESULTS

Characteristics of the Patients

Twenty-six patients newly diagnosed with type 1 diabetes between April 2010 and May 2012 provided interest for the study and fulfilled screening criteria (Supplementary

Fig. 2). Four of these declined to participate before randomization, whereas the remaining 22 were randomized. Two of these declined after randomization due to assignment to the control group, and these two participants were therefore substituted in the randomization. Two of the total 20 patients in the study (one in the MSC group and one in the control group) withdrew during the 1-year study period. The patients who withdrew stated lack of time (the subject in the MSC group moved) and motivation. Most patients interested in participating in the study were males. The participants did not differ in age, weight, BMI, or clinical or laboratory parameters (Table 1).

Adverse Events

All patients ($n = 10$) randomized to autologous treatment with MSCs tolerated the procedure well. No side effects were observed. During the 1st year, no tumors or chronic infections have been diagnosed in any of the study patients. Neither did any of the patients report infections requiring treatment with antibiotics. Several of the patients have had episodes of viral upper respiratory tract infections, but with similar frequency in both study groups. One of the patients in the control group has been diagnosed with microscopic colitis and Horton headache. None of the study patients have had any episodes of either hyperglycemic ketoacidosis or assisted hypoglycemia.

Efficacy Evaluation

In order to avoid effects of glucose toxicity and β -cell exhaustion, the first MMTT was performed 10 weeks after the start of insulin treatment. At this time point, there were no differences in HbA_{1c}, insulin doses per kilogram, fasting C-peptide, or MMTT-evoked C-peptide response (Table 2). Similarly, at the 1-year follow-up, the study groups did not differ in these parameters. Changes during the 1st year in C-peptide response to an MMTT were evaluated as primary efficacy end point. In response to the MMTT, patients in the control arm had a mean decrease in both C-peptide peak values and C-peptide when calculated as AUC during the 1st year. In contrast, these responses were preserved in MSC-treated patients (Fig. 1). For individual subjects in the control group, 8 out of 9 patients decreased in peak C-peptide, and 7 out of 9 decreased in AUC response to the MMTT during this 1st year (Fig. 2). Notably, only 3 of the 9 MSC-treated patients decreased their peak C-peptide or AUC response to the MMTT during the same period.

Table 1—Characteristics of the patients at diagnosis

Characteristic	Control ($n = 9$)	MSC treated ($n = 9$)
Sex (male/female)	5/4	8/1
Age (years)	27 \pm 2	24 \pm 2
Body weight (kg)	68 \pm 4	78 \pm 3
BMI (kg/m ²)	22.5 \pm 0.9	23.3 \pm 1.1
GAD65 antibodies (no. of all)	8/9	6/9
IA2 antibodies (no. of all)	4/9	6/9
Both GAD65 and IA2 antibodies (no. of all)	4/9	3/9
Diabetes-associated HLA alleles		
DR4 (no. of all)	8/9	7/9
DR3 (no. of all)	0/9	0/9
Neither DR3 nor DR4 (no. of all)	1/9	2/9
DQ8 (no. of all)	9/9	7/9
DQ2 (no. of all)	4/9	4/9
DQ2/8 (no. of all)	3/9	2/9
Neither DQ2 nor DQ8 (no. of all)	0/9	0/9
DR4-DQ8 (no. of all)	8/9	7/9
Diabetic ketoacidosis (no. of all)	1/9	1/9
Polyuria and weight loss (no. of all)	8/9	9/9

Plus-minus values are means \pm SEM. There were no statistically significant differences between the two groups. Concentrations of GAD65 and IA2 antibodies were determined by ELISA technique, where values of GAD IgG \geq 5 units/mL and IA2 IgG \geq 8 kU/L indicated their presence. HLA class II alleles were measured with PCR amplification and sequence-specific hybridization.

There were no changes in frequency of GAD65 or IA2 antibodies between control and MSC-treated patients. During the year, two patients in the control arm and one in the MSC group lost GAD65 antibodies. No seroconversion for presence of IA2 antibodies occurred at all.

DISCUSSION

This is the first study to report on the possibility to intervene in the course of type 1 diabetes by systemic MSC treatment. During the 1st year, we observed preserved or even increased C-peptide response to an MMTT in MSC-treated patients.

Similar to what has been reported in interventional studies with MSCs for other diagnoses, e.g., GvHD (19), Crohn disease, and multiple sclerosis, we observed no adverse events.

Composite type 1 diabetes TrialNet data indicate a mean 15% decrease in the AUC response to an MMTT

Table 2—Comparison in functional parameters between the two groups at 10 weeks after diagnosis and at 1-year follow-up

Functional parameter	10 weeks control	10 weeks MSC-treated	1-year control	1-year MSC-treated
HbA _{1c} [% (mmol/mol)]	6.9 \pm 0.4 (52 \pm 4)	6.5 \pm 0.4 (48 \pm 4)	6.6 \pm 0.2 (49 \pm 2)	6.3 \pm 0.2 (46 \pm 2)
Insulin dose (IU/kg/day)	0.39 \pm 0.13	0.43 \pm 0.05	0.37 \pm 0.07	0.39 \pm 0.05
Fasting C-peptide (nmol/L)	0.28 \pm 0.02	0.29 \pm 0.05	0.29 \pm 0.04	0.32 \pm 0.05

All values are given as means \pm SEM for nine patients in each group. There were no statistically significant differences between the groups.

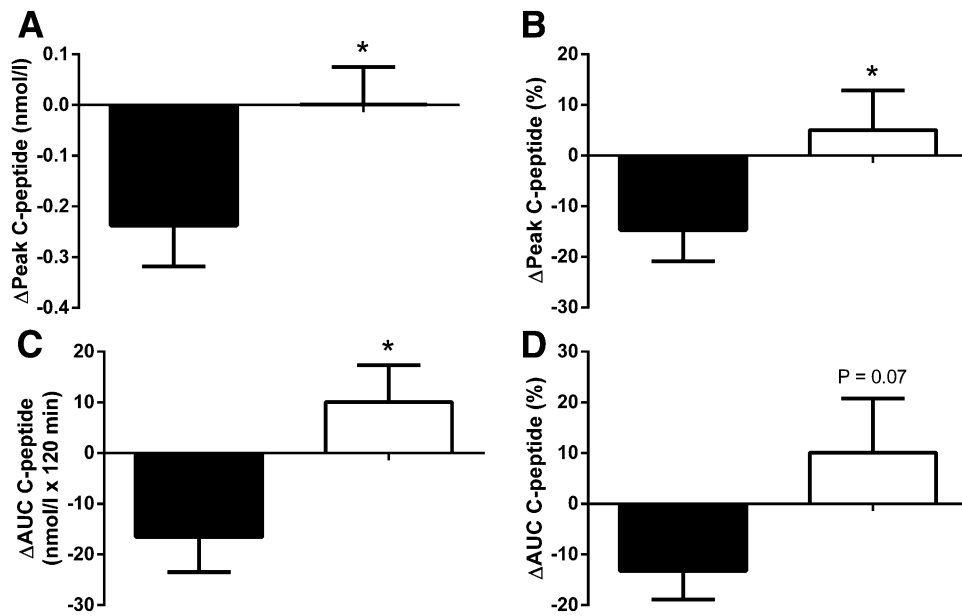


Figure 1—Changes in C-peptide response to an MMTT between 10 weeks after diagnosis and the 1-year follow-up for control (closed bars) and MSC-treated type 1 diabetic patients (open bars). Absolute (A) and percent (B) changes in peak C-peptide concentrations between the loads. Absolute (C) and percent (D) changes in the AUC response of C-peptide concentrations between the loads. All values are expressed as means \pm SEM for nine individuals in each group. **P* < 0.05 when compared with control group. Comparisons were made using Student unpaired *t* test.

during the 1st year in 21–46-year-old patients, and 24% in 15–21-year-old patients (22). In our study, a mean 13% decrease in the AUC response to an MMTT was observed during the 1st year in patients randomized to the control group. In contrast, most patients randomized to MSC

treatment increased their capacity for C-peptide response to the MMTT during the study period, with increased delta values for both peak C-peptide response and AUC C-peptide response to the MMTT, when compared with the control group. There were more females in the control

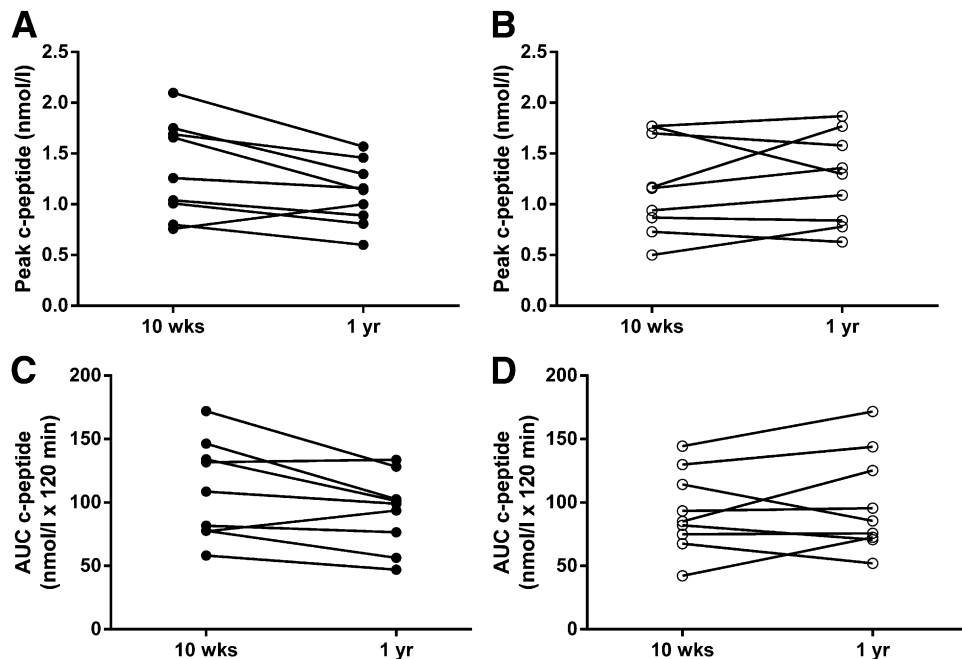


Figure 2—C-peptide response to an MMTT at 10 weeks after diagnosis and at the 1-year follow-up for control (closed circles) and MSC-treated type 1 diabetic patients (open circles). Peak C-peptide concentrations for all individuals in control (A) and MSC-treated group (B). AUC response of C-peptide concentrations for the same individuals in control (C) and MSC-treated group (D).

group, which partially reflected the open design of study, where two male patients randomized to control group declined after assignment and subsequently were substituted by chance by two female patients. However, no influence of sex on loss of C-peptide has been reported in larger studies (22,23). Moreover, although the male predominance in the MSC group caused a tendency for these patients to weigh more, there were no differences in BMI or insulin doses per kilogram between the groups.

MSCs cannot only be harvested from bone marrow, as in this study, but also from several other organs. Moreover, culture and expansion protocols vary, as well as the use of allogeneic or autologous cells. All these factors may affect the cell characteristics. We chose autologous MSC therapy to avoid any potential risk of HLA immunization or transfer of donor-derived infections or other diseases. In our previous studies of GvHD after hematopoietic stem cell transplantation, the 1-year survival rate of patients was substantially higher in patients receiving MSCs harvested in early passages (passage 1–2) compared with later passages (passage 3–4) (20). Short-term expanded MSCs, as in the autologous setting, also elicit less innate immune attack when infused systemically, which supports their survival and function in vivo (24). There are reports of diabetes-induced changes in MSCs, including their regenerative potential, but these seem to mainly reflect effects of long-term hyperglycemia by, e.g., advanced glycation end products (25). In this study, we observed no defects in the expansion potential of MSCs after autologous harvest in subjects with recent-onset type 1 diabetes.

Results presented show that autologous MSC treatment of new-onset type 1 diabetes may be a safe and feasible strategy to intervene in the disease process and preserve β -cell function. These encouraging results call for larger, randomized, and double-blinded studies, with a longer duration of follow-up, to validate the findings obtained.

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approved the final version of the manuscript. P.-O.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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