Treating type-1 diabetes mellitus with insulin-secreting mesenchymal stem cells and haematopoietic stem cells followed by T-regulatory cells

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Introduction

Type-1 diabetes mellitus (T1DM), also known as juvenile-onset diabetes mellitus (DM), is characterized by beta-cell destruction, typically by an autoimmune T cell-mediated mechanism, which usually leads to an absolute insulin deficiency in the body required for glucose metabolism, confirmed with positive antibodies against glutamic acid decarboxylase (GAD). Symptoms of the disease appear when insulin making *β*-cell mass gets reduced by approximately 90% leading to severe insulin deficiency and hyperglycaemia[1]. At present, the only therapeutic option for management is life-long exogenous insulin. Stem cell therapy (SCT) has promising results in regenerative medicine. Stem cells are self-renewing, unspecialized cells that give rise to multiple specialized cell types through a process of differentiation. The development of strategies to avoid beta-cell mass reduction or to enhance beta-cell mass expansion, both *in-vivo and in-vitro* could provide a promising option for cell-based therapy as insulin-secreting cells (ISC) for the treatment of type-1 and type-2 DM[2]. We report a 13-year old male with 4-years of T1DM, treated with his own adipose-tissue derived insulin-secreting mesenchymal stem-cells (IS-AD-MSC) and his bone-marrow (BM) derived haematopoietic stem-cells (HSC) followed by $CD4^{+}CD25^{high}CD127^{low}$ forkheadBoxP3 ($Foxp3^{+}$) T-Regulatory Cells (T-regs).

Case report

A 13 year old adolescent male with T1DM since 2010, presented with weakness, fatigue and uncontrolled raised blood sugar for 12 months. He had a body-weight (BW) of 44kg and a height of 155cm and was admitted to G.R. Doshi and K.M. Mehta Institute of Kidney Diseases & Research Centre - Dr. H.L. Trivedi Institute of Transplantation Sciences for SCT in December 2013. His fasting blood sugar (FBS) and post-prandial blood sugar (PPBS) were 210mg/dl and 454mg/dl respectively with <0.01ng/ml serum C-peptide, 11.2% glycosylated haemoglobin and 98 IU daily biphasic-isophane insulin requirement. His urine sugar was +4 and there was absent serum acetone. He had 138 IU/ml (normal range: <10 IU/ml) GAD antibody by ELISA (Euroimmun, UK), 5% and 4% insulin porcine and bovine antibody (normal range: <6%, by chemiluminescence) respectively with 32 IU/ml of anti-islet cell antibody by ELISA (normal range: <40 IU/ml). He had unremarkable general, vital and systemic examinations. He was subjected to SCT after informed consent and approval by the Institutional Review Board (Figure 1).

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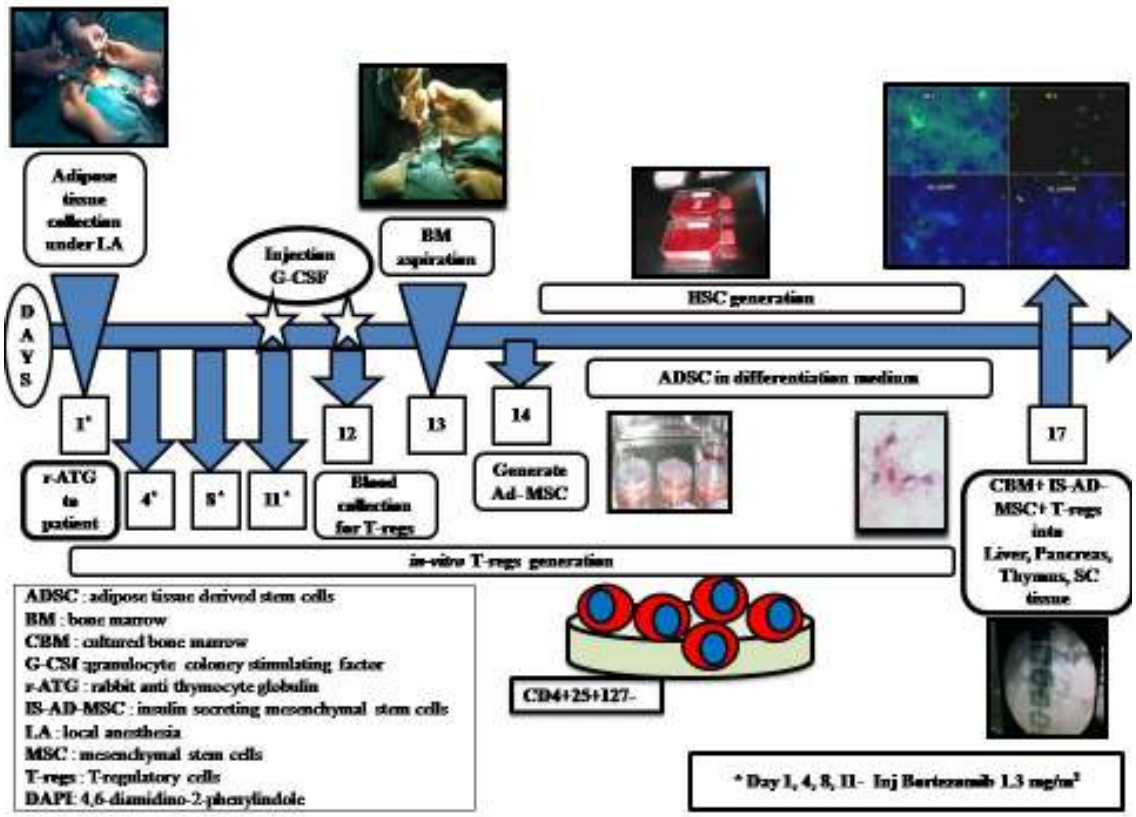

Figure 1: ***Protocol of stem cell therapy for Type-1 Diabetes Mellitus***

IS-ADMSC were generated as per our previously described technique[1] from 10g of adipose tissue from his anterior abdominal wall on Day-1, under local anaesthesia (LA) and subjected to generate *in-vitro* MSC on Day-14, which further differentiated into ISC on Day-17, quantified and tested for sterility, viability and insulin-secreting markers (pax-6, ipf-1 and isl-1) by immunofluorescence. C-peptide and insulin secretion were tested by chemiluminescence assay (Lumax, USA). On Day-17, *in-vitro* T-regs were generated using AD-MSC and peripheral blood mononuclear cells derived from 40 ml peripheral blood collection, confirmed with $CD4^+CD25^+$ $CD127^-$ by flow-cytometry. 100 ml BM was aspirated from posterior superior iliac-crest under LA from him on Day-13, followed by administration of 300µg granulocyte colony stimulating factor subcutaneously on Day-11 and 12 for *in-vitro* generation of HSC. On Day-17, 4ml IS-AD-MSC of 12.8×10^4/kg BW and 97.5 mL HSC of 142.8×10^6/kg BW were infused into subcutaneous tissue, 10ml (IS-AD-MSC,1ml+BM-HSC,9ml) and pancreatic artery to pancreas, 49ml (IS-AD-MSC,1ml+BM-HSC, 48ml) superior mesenteric artery to portal route, 35ml (IS-AD-MSC,1ml+BM-HSC,34ml)+ brachiocephalic artery to thymic, 3.5ml (IS-AD-MSC,1ml+BM-HSC, 2.5ml) circulation via femoral artery catheterization under LA with 2ml T-regs [$CD127^{low}CD25^{high}CD4^+$], 135.6×10^4/kg BW uneventfully.

Patient's blood sugar levels were monitored, 4 hourly for first 2 days after stem cell infusion and he was discharged at the end of 3rd day with advice to take insulin according to sliding scale of required-insulin according to blood sugar and monitor the FBS and PPBS daily for 5-days and then weekly for the first month, fortnightly for the next 2-months and monthly thereafter till the end of 1-year. Subsequently he was advised to check HbA1c every 3-months.

Over a follow-up of 11 months (Figure 2), patient is maintaining FBS and PPBS at 134 and 172 mg/dl respectively, serum C-peptide increased to 0.05 ng/ml from 0.01ng/ml, glycosylated haemoglobin reduced to 7.6% from 11.2% and daily biphasic-isophane insulin requirement reduced to 54 IU from 98 IU. There were no untoward side effects recorded. This was a safe, effective, reproducible and viable therapeutic option.

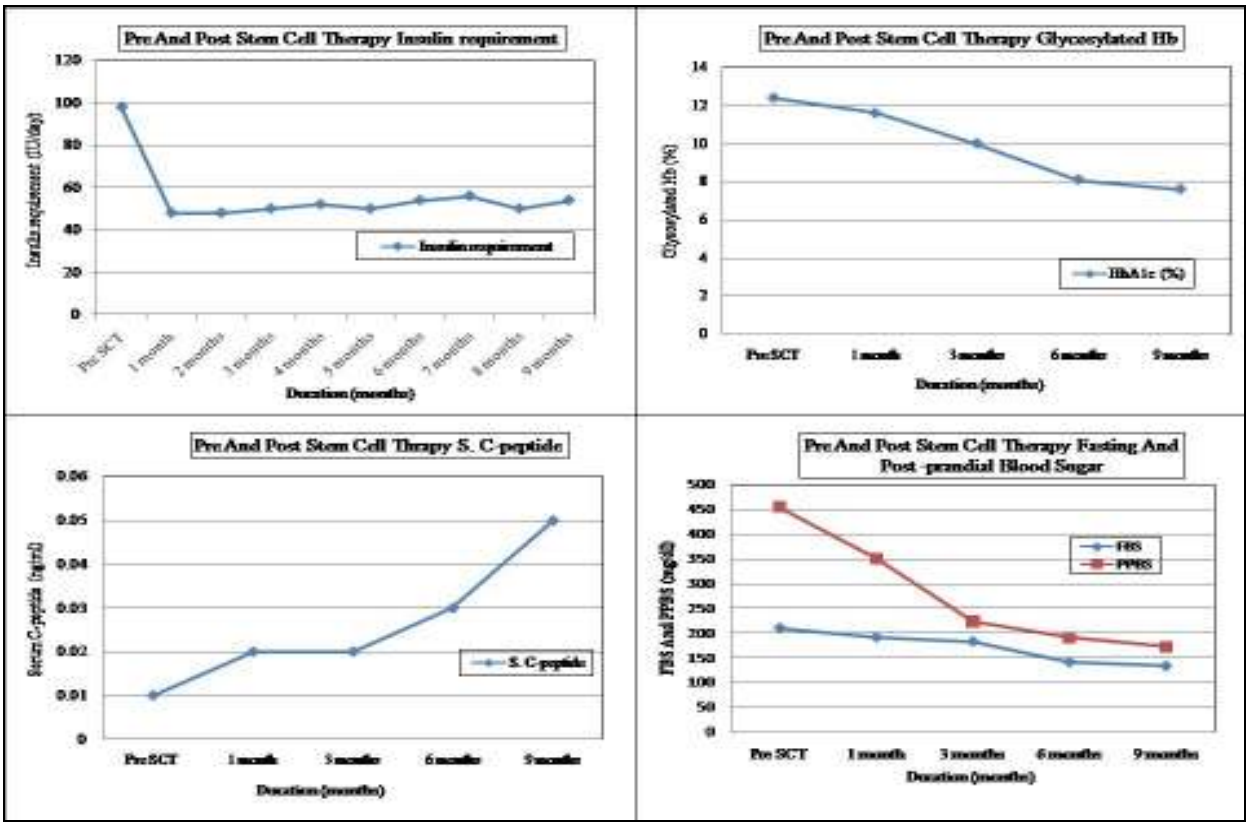

Figure 2: ***Stem cell therapy response to glycosylated haemoglobin, serum C-peptide, daily insulin requirement and fasting - post prandial blood sugar status***

Discussion

Potential therapy for T1DM needs to address insulin-replacement and immune dys-regulation arising in these patients. Islet cell transplantation is a well-known therapeutic option yet not feasible due to the shortage of available organs[3,4]. Optional cell therapy includes HSC and MSC, especially since MSC have the plasticity to adapt to pancreatic endocrine phenotype and migrate to the sites of tissue injury. They are also potent immunomodulators[5-9]. In animal models of T1DM, MSC have shown beneficial effects in glycaemic control, either isolated or combined with HSC[10].

We have generated MSC *in-vitro* from human adipose tissue which qualify the definition standardized by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy[1]. We further differentiated them to ISCs under defined culture conditions phenotypically identical to pancreatic *β*-cells. These cells expressed transcription factors ipf-1, pax-6, and isl-1. All three are central controlling genes capable of reprogramming non-pancreatic cells to surrogate *β*-cell functions[1]. Generated MSC were further differentiated to *in-vitro* T-regs, using peripheral blood mononuclear cells derived from 40ml collected peripheral blood, which were $CD4^{+}CD25^{+}CD127^{-}$ on flow cytometry. Sakaguchi et al reported that *in-vivo* and *in-vitro* T-regs carry out their suppressive action on cells causing autoimmunity by multiple mechanisms[11]. Thymic infusion was carried out in our patients to achieve central tolerance[12] and portal circulation was done to take the advantage of tolerogenicity of liver[13]. Subcutaneous tissue being an immunologically privileged site, we decided to inject part of the cells in abdominal subcutaneous tissue, so that it will serve as a "back-up reservoir" for insulin supply[14]. The HSCs were used along with IS-ADMSC to create active and passive tolerance by clonal deletion/T-cell suppression[15].

To our knowledge, this is the first case report of successfully treating T1DM patient with IS-AD-MSC +BM-HSC+Tregs [$CD127^{low/-}CD25^{high}CD4^{+}$] which is safe, effective and performed by relatively simple technique and will open-up the avenues for millions of diabetic children all across the world. However, the questions that remain unanswered are: clinical dilemma involving the issue of autoimmunity; will

the immune response to infused cells destroy the infused insulin-producing cells with longer time span? How much is the dose of cells required to achieve complete cure of T1DM? This is the first report of treating T1DM with autologous *in-vitro* generated IS-AD-MSC with BM-HSC along with T-regs [$CD127^{low}$ $CD25^{high}$ $CD4^{+}$], effectively with relatively simple techniques.

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