Mesenchymal stem cells derived in vitro transdifferentiated insulin producing cells: New approach to treat type 1 diabetes mellitus.

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- Type 1 diabetes mellitus
- Mesenchymal stem cells
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**ABSTRACT**

The pathophysiology of Type 1 diabetes mellitus (T1DM) is largely related to an innate defect in the immune system culminating in a loss of self tolerance and destruction of the insulin producing beta cells. Currently, there is no definitive cure for T1DM. Insulin injection does not mimic the precise regulation of beta cells on glucose homeostasis, leading long term to the development of complications. Stem cell therapy is a promising approach and specifically mesenchymal stem cells (MSC) offer a promising possibility that deserves to be explored further. MSC are multipotent, non-haematopoietic progenitors which have been explored as treatment option in tissue regeneration as well as potential of in vitro transdifferentiation into insulin secreting cells, achieving in this way the major therapeutic goals for T1DM. The regenerative capabilities of MSC have been a driving force to initiate studies testing their therapeutic effectiveness; their immunomodulatory properties have been equally exciting; which would appear capable of disabling immune dysregulation that leads to beta cell destruction in T1DM. Furthermore, MSC can be cultured in specially defined conditions and their transdifferentiation can be directed toward the beta cell phenotype and the formation of insulin producing cells (IPCs) can be targeted. To date, the role of MSC derived IPC in T1DM – a unique approach with some positive findings have been unexplored, but it is still in its very early phase. Herein has been summarized new approach that have been proposed and tested for MSC derived IPC’s potential therapeutic benefit for T1DM in experimental animal models as well as in humans.

1. Introduction

Type 1 diabetes mellitus (T1DM) is a T-cell mediated, organ-specific autoimmune disorder leading to beta cell destruction and reduced insulin production and has no definitive cure currently. Standard treatment strategies for T1DM are based on different insulin replacements. However, exogenous insulin cannot mimic exactly the physiology of insulin secretion, good metabolic control is difficult to reach and frequently associated with severe hypoglycaemic episodes. At present pancreas transplantation or islet transplantation as a treatment of T1DM is here to stay until something better comes along. The first efforts focused on whole pancreas transplants, which have been performed now for over 50 years. Although they have been shown to lead to insulin independence for several years, pancreas transplants to treat T1DM are not widespread for a number of reasons. Being a major surgery, the accompanying risk of mortality is one to three per cent and the complications that ensue include cardiac death and systemic infections. In addition, to prevent the body from rejecting the transplanted pancreas, recipients must take powerful immunosuppressions for the rest of their lives, leaving them susceptible to infections and a range of other diseases. Many feel that the immunosuppressions therapy could be a greater health threat than the diabetes itself.

In an effort to more tightly control blood glucose levels, researchers began to explore the possibilities of using cell-based therapies that would replace lost beta cells. Within recent years, stem cell research has become a very important part of the
scientific understanding of T1DM. Research has demonstrated that stem cells can be grown in the lab and could lead to a better availability of beta cells for future research purposes to treat T1DM. Many types of stem cells are candidates for the treatment of T1DM. The ideal stem cells would be one, with strong immunomodulatory and regenerative properties which would have to deal with active process of autoimmunity that probably target the newly formed insulin producing cells (IPC). Mesenchymal stem cells (MSC) have been a driving force to initiate studies testing their therapeutic effectiveness with their regenerative capabilities and their immunomodulatory properties. Generation of IPCs from MSCs represents an attractive alternative. This review was carried out because the sources of stem cells used in the generation of IPC have been previously reviewed however literature describing MSC alone as a source for in vitro transdifferentiation of IPC, further clinical use of IPC as a therapeutic agent in experimental animal models and in humans and its outcome are scarce.

Review

MSC therapeutic potential: in vitro and in vivo evidence

Friedenstein et al. in a ground-breaking study, isolated, clonogenic fibroblast-like cells, from whole bone marrow (BM) and showed that they were capable of forming bone- and cartilage-like colonies. Since then BM derived stem cells, are still the most frequently investigated cell type and often designated as the gold standard. Till date isolation of multipotent MSC from different sources has been reported. There is no universally agreed upon set or specific singular marker to identify these cells. As a result, a battery of negative and positive markers is generally used to phenotypically characterize these cells. As MSC are a non-haematopoietic cell lineage, they generally lack specific cell surface markers of HSC and do not express haematopoietic markers such as CD34, CD14 and CD45, CD11a/LFA-1, erythrocytes (glycophorin A), and platelet and endothelial cell adhesion molecules (CD31) but they express several other cell surface antigens, such as CD73 (SH3/4), CD90, CD105 (SH2), CD146 and CD200. They also express variable levels of CD44, stromal antigen 1, and a group of other adhesion molecules and receptors including CD166 (vascular cell adhesion molecule), CD54/CD102 (intracellular adhesion molecule), and CD49.[2] BM aspirate is considered to be the most accessible and enriched source of MSCs. Bone marrow derived MSC (BM-MSC) represent a rare population of cells that make up only 0.001 to 0.01% of total nucleated cells and are 10-fold less abundant than hematopoietic stem cells (HSC), but they can be readily grown and expanded in culture. [3] However MSC derived from adipose tissue (AD-MSC) [4,5], peripheral blood [6,7] umbilical cord blood (UCB) [8-10], have also shown promising potential for proliferation and differentiation into different cell types including IPC. Moreover, protein transduction technology also offers a novel approach for generating IPCs from stem cells including MSC.[11,12]

Why MSC?

The frequency of T1DM has been steadily increasing worldwide and T1DM prevention studies using immunosuppressants, self-antigens, and dietary interventions have far demonstrated largely disappointing results. [13] In T1DM selective and irreversible destruction of the insulin-secreting beta-cells in the pancreatic islets of Langerhans occurs by an autoimmune attack. While insulin replacement remains the cornerstone treatment for T1DM, the transplantation of pancreatic islets of Langerhans provides a cure for this disorder and yet, islet transplantation is limited by the lack of donor pancreas. The challenge involves the development of safe and effective means affording the prevention or reversal of T1DM. MSC have the capacity to differentiate into multiple mesodermal and non-mesodermal cell lineages including insulin producing cells in vitro. [14] MSC that have been pre-committed to one mesenchyme cell lineage can differentiate into other cell types in response to inducible extracellular cues by process known as transdifferentiation. These pre-committed cells proliferate and are able to dedifferentiate into a primitive stem cell stage through genome reprogramming. [15] On the one hand MSC have the potential to transdifferentiate into IPCs by genetic modification and/or defined culture conditions in vitro. On the other hand, MSC are able to serve as a cellular vehicle for the expression of human insulin gene and has promising role as therapeutic agents in the treatment, the complications of DM like cardiac function and in treatment of diabetic cardiomyopathy, nephropathy, diabetic polyneuropathy, and wounds in diabetic patients. MSC have generated marked interest and attention for their capacity to elicit tissue regeneration [23-26] and one of the most remarkable and least understood findings is the ability of MSC to migrate to sites of tissue injury. [27-30] Besides, MSC also possess immunosuppressive effects by modulating the immune function of the major cell populations involved in alloantigen recognition and elimination [31-33] and they have also shown promising results in the treatment of other autoimmune disorders (e.g., experimental autoimmune encephalomyelitis and rheumatoid arthritis). [34, 35] Adult human MSC express intermediate levels of major histocompatibility complex (MHC) class I molecules on their cell surface, but not MHC class II, properties that allow their transplantation across MHC barriers. Because of the lack in expression of MHC class II and most of the classical costimulatory molecules on MSCs, these cells have historically been regarded as hypo-immunogenic cells. The immunomodulatory properties of MSC were initially reported in T-cell proliferation assays using one of a variety of stimuli including mitogens, CD3/CD28, and alloantigens settings where the ability of MSC to suppress T-cell proliferation was readily determined. Such suppression occurs irrespective of donor source, including settings in which one uses “third party” MSC and suppress proliferation of both CD4+ and CD8+ lymphocytes; and are able to abrogate the response of ]
memory T-cells and naïve T-cells to their antigen. [36] MSC could regulate diabetes through a direct effect by presenting differential levels of negative costimulatory molecules and secreting regulatory cytokines such as transforming growth factor-β and IL-10 that control regulatory T-cells/auto-reactive T-cells. It is also possible that MSC could correct the dysregulation observed at the level of β-cells and natural killer cells as well. [37,40] MSC also exert anti-inflammatory effects that could be important in maintaining peripheral tolerance. [41-43] It has been shown that AD-MSC are capable of producing anti-inflammatory cytokines and angiogenic factors, which could potentially improve the diabetes-associated inflammatory and ischemic conditions. [44] According to one hypothesis MSC transplantation into diabetic animals can prevent apoptosis of injured pancreatic beta cells and enhance regeneration of endogenous progenitor cells through paracrine actions such as angiogenic, cytoprotective, anti-inflammatory, mitogenic and anti-apoptotic effects. [45] MSC have been also shown to provide cytokine and growth factor support for expansion of HSC and human-embryonic stem cells (ESC), which in turn during co-transplantation of IPC and HSC promote IPC engraftment and survival. Here HSC act as “feeder” cells for the IPC, supporting its protection, tissue revascularisation, and immune acceptance. [46-49]

**In vitro Directed Transdifferentiation of MSC into IPC**

In 2001, Assaday et al. reported that IPC can be generated from spontaneous differentiation using ESC. Although the number of IPC and the insulin content in these cells was low, it was the first proof-of-principle experiment showing that stem cells—ESC were a potential source for generating beta-like cells. [50]

MSC have the advantage over ESC in that they usually do not form teratomas and are free from the ethical issues of ESC. MSC can be easily obtained and are easily expanded and cultured in the laboratory. Different types of stem cells require different culture and induction media for transdifferentiation of IPCs to take place. The capacity of MSC to undergo functional transdifferentiation has been questioned over the years. Nonetheless, recent studies support that gene-therapy or factor-based transdifferentiation of MSC are two distinctive pathways to be considered as means of obtaining functional beta cells. Gene-therapy refers to the in vivo or in vitro transfer of a foreign gene into MSC, allowing it to produce insulin. The foreign gene in turn activates or represses on demand the insulin gene. The factor-based approach involves exposing MSC to a cocktail of insulin-promoting factors and cytokines over an extended period of in vitro culture, followed by transplantation of the transdifferentiated IPC into the receiving diabetic patient.

MSC can be differentiated into IPC by using a specific culture medium enriched with extrinsic insulin-promoting factors. IPC transdifferentiation period varies greatly with use of different protocols, it may last from several days to several months. Addition and withdrawal of a combination of extrinsic insulin-promoting factors in a stage-wise manner is required. Many extrinsic insulin-promoting factors which are biologically active compounds that have been used in endocrine pancreas differentiation have been shown to promote beta cell proliferation and differentiation and increase insulin content of IPC. A number of these factors have been commonly observed in protocols for IPC transdifferentiation. Commonly known insulin-promoting factors include epidermal growth factor, activin A, betacellulin, nicotinamide, exendin-4, hepatocyte growth factor, fibroblast growth factors and gastrin. Careful use of serum and glucose in the induction media has also been indicated for successful transdifferentiation of IPC. Signaling by these factors in MSC allows the induction of the transcription factors those are prerequisite for pancreas development.

IPC identification is then based on the ability to express genes related to pancreatic development and function, such as Isl1 and II, Glut2, glucose kinase, islet amyloid polypeptide, nestin, and Pdx 1 and Pax 6, and to synthesize C-peptide and insulin which have been shown to play a role in the development of the pancreas and/or the differentiation of insulin-producing beta cells. Although pancreas development has been partly deciphered by identification and characterization of many transcription factors little is known about their function and molecular mechanism of action. Most of these transcription factors are sequentially and transiently expressed during pancreas development. However for successful transdifferentiation of MSC into IPC in vitro culture procedure should follow at least the major part of transcriptional program.

There are evidences to suggest that pancreatic stem or progenitor cells derived MSC, [51-57] UCB derived MSC, [58-60] BM-MSC, [61-67] and AD-MSC [68-71] can be isolated from rodents and/or human and extensively expanded in vitro, where they differentiate to form new IPC and exhibited with the characteristics genes and a panel of markers considered essential for differentiation into pancreatic endocrine tissue (Isl1, Pdx1, Pax4 and Ngn3, Pax6). (Table 1)

Majority of researchers have described a multistep protocol for generation of insulin-producing islet-like clusters from MSC derived from different sources. The glucose challenge tests revealed the production of insulin, and such production was regulated via physiological signaling pathways i.e. in glucose responsible manner and they believed that insulin-producing cells derived from MSC could be potentially used for cell therapy of T1DM in human models followed by trials in experimental animal models. (Figure 1)

**Success Story: MSC derived IPC-based therapy in autoimmune animal models of T1DM**

Experimental data for the therapeutic effects of MSC derived IPC in animal models of T1DM are important tools for analyzing results before considering further human clinical applications. NOD/SCID mice, which are severely deficient in T and B
Table 1: *In vitro* approaches to generate IPC from different MSC sources

<table>
<thead>
<tr>
<th>MSC source</th>
<th>Characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic MSC</td>
<td>Expression of Pdx1, Hlxb9, Nkx2.2, Nkx6.1 and GLUT2. Released insulin and C-peptide in response to physiological glucose concentrations <em>in vitro</em>.</td>
<td>51-57</td>
</tr>
<tr>
<td>Human UCB -MSC</td>
<td>Expression of Isl1, Pdx1, Pax4 and Ngn3</td>
<td>58-60</td>
</tr>
<tr>
<td>BM - MSC</td>
<td>Generation of islet-like cells, Expression of PDX1</td>
<td>61-67</td>
</tr>
<tr>
<td>ADSC- MSC</td>
<td>Up-regulation of transcription factors Ipf1, Isl1 and Ngn3 and islet gene insulin, glucagon and somatostatin, as well as expression of C-peptide</td>
<td>68-71</td>
</tr>
</tbody>
</table>

Figure 1: Schematic representation showing *in vitro* transdifferentiation of insulin producing cells from mesenchymal stem cells; IPC *in vivo* infusion in an experimental animal model, T1DM human model and therapeutic effect.
lymphocytes, represent an invaluable diabetic model for experimental research. Use of IPC derived from UCB-MSCs, [72-74] BM-MSC alone or in association with HSC, [75-79] and from AD-MSC [80-83] represent a potential source of diabetic cell replacement because of their availability, low risk for immune rejection and increased capacity for expansion with encouraging results in terms of improving glycaemia, enhanced islet regeneration, lowered blood sugar and increased circulating blood insulin levels, increased production of endogenous beta cells, reversion of glycosuria, with increased morphologically normal beta-pancreatic islets, aquired functional beta cell phenotype, partially restored pancreatic function in compared with non-transplanted diabetic mice. Histological examination of IPC transplanted organ showed the presence of transplanted cells with formation of tissue-like structure, which stained positive for insulin. (Table 2)

Table 2: Approaches to treat experimental diabetic model using IPC generated from different MSC sources

<table>
<thead>
<tr>
<th>MSC source</th>
<th>Animal model</th>
<th>Infusion procedure</th>
<th>Therapeutic effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCB-MSCs</td>
<td>NOD/SCID mice</td>
<td>Intravenous/Implantation in subcapsular region of kidney</td>
<td>Improved blood glucose levels and survival rates, led to normalization of glomerular hypertrophy and tubular dilatation</td>
<td>72-74</td>
</tr>
<tr>
<td>BM-MSC</td>
<td>NOD/SCID mice</td>
<td>Intraportal/Intravenous injection</td>
<td>Reversion of hyperglycaemia and glycosuria, improved renal lesions, increased circulating insulin and decreased inflammatory macrophage infiltrates in glomerular structures</td>
<td>75-79</td>
</tr>
<tr>
<td>ADSC-MSC</td>
<td>NOD/SCID mice</td>
<td>Intravenous / intraperitoneal injection</td>
<td>C-peptide decreased, Reversion of hyperglycaemia and glycosuria</td>
<td>80-83</td>
</tr>
</tbody>
</table>
Table 3: Insulin replacement therapy using MSC derived IPC in humans

<table>
<thead>
<tr>
<th>MSC source; year</th>
<th>No. of patients</th>
<th>Infusion procedure</th>
<th>Therapeutic effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-MSC; 2008</td>
<td>N=2</td>
<td>intravenous</td>
<td>Data not published</td>
<td>84</td>
</tr>
<tr>
<td>ADSC-MSC; 2008</td>
<td>N=5</td>
<td>intraportal</td>
<td>Mean follow-up 2.9 months. Decreased exogenous insulin requirement, Hb1Ac, raised serum c-peptide levels, no immunosuppression</td>
<td>85</td>
</tr>
<tr>
<td>ADSC-MSC; 2010</td>
<td>N=11</td>
<td>intraportal</td>
<td>Mean follow-up: of 23 months. Decreased exogenous insulin requirement, Hb1Ac, raised serum c-peptide levels, and no diabetic ketoacidosis events, weight gain, no any immunosuppression</td>
<td>86</td>
</tr>
</tbody>
</table>

Figure 2: IPC transdifferentiated from MSC: Learning points

- MSCs can be isolated from many sources
- IPC can be differentiated in vitro using pancreatic as well as non-pancreatic origin of MSC
- IPC provides potential treatment option for TIDM patients
- IPCs generated in vitro could correct hyperglycemia in animal models
- IPC therapy could remove in the near future diabetes from the list of incurable, chronic diseases
MSC or MSC derived IPC-based therapy in T1DM humans

The most effective protocols till date have produced cells that express insulin and have molecular characteristics that closely resemble bonafide IPC; however, these cells are often unresponsive to glucose, which is also a most vital characteristic concern which needs to be solved before finding a definite clinical application. There are very few reports noted till now using MSC derived IPC as a treatment of T1DM in humans. In 2008, researchers studied role of MSC in humans with T1DM. The protocol includes bone marrow biopsy under general anesthesia in first-degree relatives for the collection of MSC. These cells were sent to a laboratory to be stimulated to proliferate for a month and were later infused into the patient through a gelatinous solution of approximately 100 ml without chemotherapy. After 1 month, the patients were given another infusion. So far, they are not sure about how many infusions will be necessary. Two patients had been included in this protocol but still results are not published. [84]

Same year in 2008 our group published results using AD-MSC transdifferentiated IPC in combination with HSC to treat T1DM in 5 patients having disease duration of 0.6-10 years. Intraportal administration of in vitro generated IPC and HSC were carried out. We decided to infuse the cells in portal circulation since liver is the most tolerogenic organ. [85] Results showed 30% to 50% decreased insulin requirements with 4- to 26-fold increased serum c-peptide levels, with a mean follow-up of 2.9 months without any untoward effects and without use of any immunosuppression. [86]

In 2010 again our group published results of insulin replacement therapy using AD-MSC derived IPC in combination with HSC to treat T1DM in another 11 patients having disease duration of 1-24 years. Cells were administered intraportally. In vitro generated IPC showed presence of pancreatic transcriptional factors Pax-6, Isl-1, and Pdx-1(by immunofluorescence assay). Chemiluminescence assay detected secretion of glucose and C-peptide in response to glucose concentration in vitro. Over mean follow-up of 23 months, treated patients showed a decreased mean exogenous insulin requirement, Hb1Ac, raised serum c-peptide levels, and became free of diabetic ketoacidosis events with weight gain on normal diet and physical activities without any untoward effects, without use of any immunosuppression. [87] (Table 3)

Conclusions and Future Perspectives

Taken altogether, these in vitro and in vivo experiments demonstrate that the beneficial effects of MSC in T1DM may be related to both their immunosuppressant activity and subsequent protective effects on damaged tissue, and their capacity to transdifferentiate into IPC. (Figure 2)

With sporadic reports of use of MSC derived IPC and their further use in clinical trials to treat T1DM it can be concluded that i) the present study is only a first step toward using MSC derived IPC as a cell-based treatment appear to be suitable for treatment of for T1DM, ii) MSC derives IPC are an ideal population of personal stem cells for cell replacement therapy and also MSC could be induced to differentiate into physiologically competent functional islet like cell aggregates, which may provide as a source of alternative islets for cell replacement therapy in T1DM. Generation of MSC into IPC is feasible and promising making the transplantation of IPC a promising approach for the treatment of T1DM. However, there is no standard method for IPC generation and the wide variations in induction techniques used may be a challenge to researchers. As most of these stem cells are being tested in pre-clinical T1DM with rare clinical use in human further exploration is necessary for the in vitro generation of sufficient IPC that can produce sufficient insulin for wide clinical use. The biggest challenge for the future trials using this approach is to prevent or treat relapses and maintain complete or very good partial responses for very long time.

Abbreviations

AD-MSC: adipose tissue derived mesenchymal stem cells
BM: bone marrow
BM-MSC: bone marrow derived mesenchymal stem cells
ESC: embryonic stem cells
HSC: hematopoietic stem cells
IPC: insulin producing cells
MHC: major histocompatibility complex
MSC: mesenchymal stem cells
T1DM: type 1 diabetes mellitus
UCB: umbilical cord blood
UCB-MSC: umbilical cord blood derived mesenchymal stem cells

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