MESENCHYMAL STEM CELLS – STEM CELL THERAPY PERSPECTIVES FOR TYPE 1 DIABETES

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Mesenchymal stem cells (MSC) are multipotent non-haematopoietic progenitors, which have been explored as a promising treatment in tissue regeneration. Although their immunomodulatory properties are not yet completely understood, their low immunogenic potential together with their complex and profound effects on immune responses can be used as a therapeutic tool not only for regenerative medicine, but also for the treatment of variate autoimmune pathologies, such as graft-versus-host (GVH) disease after transplantation. Several severe refractory autoimmune diseases as well as the prevention of organ rejection Phase I-II clinical trials are testing the therapeutic efficiency of MSCs as such or in several autoimmune pathologies such as GVH disease, and systemic sclerosis.
Type 1 Diabetes is characterised by T-cell mediated autoimmune destruction of pancreatic β cells. While insulin replacement represent the current therapy of this disease, the metabolic control is still difficult and limited by the fact that exogenous insulin cannot mimic exactly the physiology of insulin secretion. Pancreatic or pancreatic islet transplantation can provide exogenous insulin independence, but the scarce availability of transplantation material and the thrombotic complications associated, limitate significantly the benefits and the success of the procedure. In this context, stem cell therapy based on generation of insulin producing cells (IPCs) from MSC, might represent an attractive perspective.
In this review we provide a brief characterisation of the immunomodulatory effects promoted by MSCs, and present the state of experimental evidence (*in vitro* as *in vivo*) related to the effects of the transplantation of mesenchymal stem cells in diabetes.

Key words: Mesenchymal stem cells; Type 1 diabetes; Insulin producing cells; Stem cell therapies.

INTRODUCTION

Diabetes mellitus is a complex metabolic disease, with an estimated prevalence worldwide among adult population of 171 million cases[1]; it is associated with severe long-term, microvascular and macrovascular complications, therefore with a high level of morbidity and mortality.

Diabetes mellitus is commonly classified in Type 1 Diabetes, which has been divided by expert ADA comitees in Type 1A (immune-mediated) and type 1B (other forms of diabetes with severe insulin deficiency) and Type 2 Diabetes.

Although representing only 10% of the diabetic population worldwide, the ongoing increasing incidence of Type 1 Diabetes (of up to 41 per 100.000/year, in Europe and up to 25 per 100.000/year, in North America[2]) represents an important global health issue.

Type 1A Diabetes mellitus is a T cell mediated, organ-specific autoimmune disorder leading to beta cell destruction and reduced insulin production, characterized by the presence of anti-islet cell antibodies (which is the best current criterion for diagnosis of type 1A diabetes), severe insulitis, and evidence for autoimmune destruction of the beta cells.

Standard treatment strategies in type 1 Diabetes are based on different schemes of insulin replacement administered through an injectable form combined with careful blood glucose monitoring. However, good metabolic control is difficult to reach and frequently associated with severe hypoglycaemic episodes, as exogenous insulin cannot mimic exactly the physiology of insulin secretion.

Therefore a major goal of future diabetes therapy would be to promote proficient strategies of overcoming autoimmunity, and improving endogenous insulin secretion, based on beta cell regeneration (which could be accomplished by beta cell self replication or differentiation from progenitor cells).

Current beta-cell replacement therapies are represented by pancreatic or pancreatic islet transplantation, which have been recently followed by stem cell transpalntation.

Human pancreatic transplantation has been achieved in selected patients suffering from type 1 diabetes mellitus, starting from 1985, with currently reported 6576 transplanted patients worldwide[3] with partially successful results in reversing the long-term renal and neural complications of diabetes[4].

A therapeutic alternative is represented by the transplantation of islet cells isolated from donor pancreata and administered into the liver through portal vein embolisation. Human pancreatic islet transplantation realised following the Edmonton protocol (using meticulous islet isolation techniques, transplantation of islets from two pancreata, and improved immunosuppressive regimens)[5], allows glycaemic control without exogenous insulin administration, and a proficient prevention of hypoglycaemia .

Shapiro *et al.* present one year follow-up results after islet transplantation, with 58% insulin independent patients, of which only 31% maintained insulin independence for two years[6]. In a five year follow-up study after clinical islet transplantation, only 10% of patients maintained insulin independence for a median time of 15 months[7]. In its 2006 annual report, the Collaborative Islet Transplant Registry, funded by the National Institute of Diabetes and Digestive and Kidney Diseases, presented data from 23 islet transplant programs on 225 patients who received islet transplants between 1999 and 2005. According to the report, nearly two-thirds of recipients achieved "insulin independence" (defined as unnecessity of insulin administration for at least 14 days) during the year following transplantation, which dropped by one third over de second year of follow-up (http://www.diabetes.niddk.nih.gov).[8]

These therapies not only do not manage to provide long term insulin independence, but are also associated with risks related to the transplantation procedure (haemorrhagic or thrombotic complications), life-long immuno-suppression complications and difficulties in obtaining the transplantation material[5].

In late 2003, the research group conducted by Voltarelli et al started a phase I/II study of high dose immunosuppression followed by autologous hematopoietic stem cell transplantation for patients with new-onset type 1 Diabetes. The aim of the treatment was to stop the autoimmune destruction of β-cells with immunosuppressive drugs and to "reset" the deleterious immunologic system to a normally reconstituted one with autologous hematopoietic stem cells, the research team presenting encouraging results obtained with 15 patients after a 7-36 month follow-up.[9]

In this context, mesenchymal stem cells could provide more benefit, representing an interesting therapeutic option, due to their immunomodulatory properties (realised by inhibition of β- cell specific T cell response and production of trophic mediators with antifibrotic and proangiogenetic proprieties)[10], as well as potential of *in vitro* differentiation into insulin secreting cells, achieving in this way the major therapeutic goals for type I Diabetes.

SC–definition, identification, immunomodulatory properties

Definition, identification, sources of MSC

Originally identified by Friedenstein in 1976[19] as a fibroblast-like cellular population capable of generating osteogenic precursors, the bone marrow derived mesenchymal stromal cells are a rare, heterogenous, stromal population of multipotent nonhematopoietic progenitor cells, capable of differentiation towards mesodermal, as well as endodermal and ectodermal lineages. Due to this characteristic, Caplan[20] introduced the name of mesenchymal stem cells (MSC), which has been recently changed, on a consensus statement recommandation, into multipotent mesenchymal stromal cells, with the acronym MSC[21].

Compared to other stem cell sources, such as haematopoietic stem cells (HSC), or stem cell population from liver, spleen and pancreas, the Mesenchymal Stem Cells seem to be a promising source for overcoming autoimmunity in Type 1 Diabetes, because of their abilities of transdifferentiation towards various cell lines, including the endodermal lines, and their particular immunosuppressive capacities[22].

Their identification as MSC relies upon the ability of the cells to differentiate «in vitro» into multiple lineage. Although no specific membrane marker has ever been identified on MSC, several phenotypical characteristics have allowed their identification and enrichment. MSC express several surface antigens, such as CD73, CD 90, CD 105, CD 146 and even the recently described CD 200[23], integrins and adhesion molecules; as MSC are a non-haematopoietic cell line, they do not express haematopoetic markers such as CD 34, CD14 and CD45[24]. Due to their hypoimmunogenicity (as they faintly express HLA I molecules, and no HLA II), MSCs could represent an appropriate stem cell source for allogeneic transplantation, irrespective of HLA compatibility. They can also synthesize trophic mediators, such as growth factors and cytokines (M-CSF, IL-6, IL-11, IL-15, SCF, VEGF) involved in haematopoesis regulation, cell signalling and modulation of the immune response[10].

Due to the rarity of the cells, and the lack of a definitive marker that specifically isolates MSCs, the cells are commonly obtained from the bone marrow, rather than other sources, (adipose tissue, cord blood, foetal liver, or in skin, gut, brain or kidney[25–28] isolated by adherence to plastic, followed by *in vitro* expansion.

MSC–immunomodulatory properties – in vitro evidence for immunomodulation

MSC can modulate the immunologic activity of different cellular population, as shown by *in vitro* experiments, of most importance being the inhibitory effect on T cell proliferation and dendritic cell differentiation, considered to be principal steps for activating autoimmune disorders

Adult human mesenchymal stem cells express on their cell surface intermediate levels of MHC class I molecules, and there are no detectable levels of MHC class II; moreover MSCs differentiated into adipose, bone and cartilage cells express HLA class I but no HLA class II[29], properties that would allow transplantation across major histocompatibility complex barriers.

MSCs are effective in inhibiting proliferation of CD4 and CD8 T cell, as well as memory and naïve T cells[33]. This mechanism might necessitate initial cell contact phase, as well as several specific mediators, produced by MSCs, such as TGFβ, PGE2 and indoleamine 2,3 deoxygenase (IDO) (IDO is induced by IFN-γ, catalyses the conversion of tryptophan to kynurenine and inhibits T-cell responses by tryptophan depletion)[34].

The ability, reported in humans, rodents and primates to suppress the response of T cells to mitogenic and antigenic triggering, is explained by a complex mechanism of induction of « division arrest anergy », responsible for the maintaining of T lymphocytes in a quiescent state (MSC determine the inhibition of cyclin D2 expression arresting cells in the G0–G1 phase of the cell cycle).[30–32]

MSC s can also modulate the immune response by stimulating the production of CD8+Treg (regulatory T cells), which could inhibit lymphocyte proliferation in allogenic transplantation. However, the induction of regulatory T cells may be mediated by different factors in alloreactive and mitogen-stimulated lymphocyte cultures as differences exist between the systems. MSCs also produce bone morphogenic protein-2, which mediates immunosuppression via the generation of CD8+ regulatory T cells.[35]

They can also reduce T-cell activation indirectly by reducing the formation of dendritic cells (DC) from monocytes[36].

Moreover MScs can inhibit B cell proliferation and activation in a dose dependent manner, modulate their differenciation, antibody production and chemotaxis abilities, activities mediated via soluble factors, and induce in vitro, on very high concentrations, a reduction of the secretion of IFNγ by IL-2-stimulated NK cells[37].

MSC–immunomodulatory properties – clinical evidence for immunomodulation and tissue regeneration

MSCs, due to their supportive function for HSC in BM niche, their selective activity on the cell cycle, and immunomodulatory effects, have already been used in clinical trials as a treatment for acute graft *versus* host disease (GvHD) following allogeneic transplantation for leukemia[11], haematological malignancies[12] and autoimmune diseases such as systemic sclerosis[13].

The important differentiation capacity of MSCs has made MSC an useful therapeutic mean in

orthopaedics by increasing new dense bone formation and total bone mineral content in osteogenesis imperfecta[15], provide early bone regeneration in osteonecrosis of the femoral head [16] and provide repair of large bone defects[17].

The MSCs capacity of expressing certain growth factors and enzymes such as aryl-sulphatase A and α-L-iduronidase which are deficient in metachromatic leukodistrophy and Hurler's disease, made them capable, after in vitro expansion and intravenous administration to enhance enzyme production and improve symptomatology in this kind of inborn errors of metabolism[14].

Due to their propriety of homing to injured tissues, MSCs have also been administered as autologous transcoronary transplantation in infarcted human myocardium, alone or in association with endothelial progenitors (EPC), with satisfactory results regarding myocardial contractility improvement.[18]

MSCs – *in vitro* and *in vivo* (autoimmunity animal models)evidence of therapeutic potential in type 1 diabetes

In vitro–differentiation of adult mesenchymal stem cells into insulin producing cells

MSCs can promote spontaneous or induced differentiation into insulin-producing cells (IPCs) under special *in vitro* conditions, using specific culture medium enriched with insulin promoting factors (mainly glucose and nicotinamide). Identification of IPCs is based on the ability of expression of several genes related to pancreatic development and function, such as insulin I and II, Glut2, glucose kinase, islet amyloid polypeptide, nestin, and pancreatic duodenal homeobox-1 [PDX-1] and Pax6, and assessment of functionality, respectively synthesis of C peptide and insulin, assessed by qPCR analysis[38].

A. Bone marrow MSCs

Karnieli *et al.* used a PDX1 (pancreatic duodenal homeobox 1, a transcription factor with key roles in pancreas development and β-cell gene expression) gene transfer approach in order to obtain beta cell differentiation from 14 human donors BM-MSC. Insulin expression has been detected in 40-60% of the cells expressing PDX1 in 9/14 human donor cultures. The functional differentiation of the PDX1 transfected BM-MSCs was evaluated by glucose-induced secretion *in vitro* and the ability to replace beta-cell function *in vivo*. Insulin secretion occurred in response to glucose concentrations above the physiological range. Transplantation of the transfected MSCs under the renal capsule of STZ-diabetic SCID mice, led to a amelioration of hyperglycemia (from over 300 mg/dl to 200mg/dl after 5 weeks)[43].

Insulin producing cells (IPCs) have been differentiated *in vitro* from BM-MSC, isolated from five type 1 and five type II uncomplicated diabetic patients; the differentiation into IPCs has been realised through a specific 18 day-three stage protocol by Yu *et al.* (39). The protocol consisted in the use of a combination of nicotinamide, activin A and β-cellulin in high glucose concentrations (25 mmol/l) medium in order to effectively promote bone marrow derived MSCs differentiation; moreover, at the end of the third stage of the protocol the differentiated cells had a similar morphology to pancreatic islet-like cells, expressed high levels of PDX-1, insulin and glucagon genes and had a positive response regarding glucose dose dependent insulin production[39].

Insulin producing cells have also been isolated from rat bone marrow, using similar protocols of differentiation in high glucose culture medium[40], or nicotinamide enriched medium[41,42]; the differentiated islet-like cells expressed insulin, at both mRNA and protein levels and managed to control glucose levels in diabetic NOD rats.[42]

B. MSCs from adipose tissue

It has recently been shown that adipose tissue isolated from human lipoaspirates is an abundant and easily accessible source of stromal progenitor cells (ADSCs, adipose-derived stromal cells), resembling the mesenchymal stem cells (MSCs) obtained from adult bone marrow, and showing potential of differentiation *in vitro* into adipogenic, osteogenic, chondrogenic, myogenic and neurogenic lineages[44].

Zulewski, Timper *et al.*[45,46] expanded isolated human adipose – tissue-derived MSC from four donors in FGFcontaining medium. These cells were shown to express stem cell markers such as Scf, Thy-1, but also Isl-1 m RNA, Isl-1 being crucial for the generation of pancreatic endocrine cells, also involved in the devolpment of central nervous system. The research team revealed an up-regulation of the transcription factors Ipf-1, Isl-1 and Ngn-3 and the islet genes insulin, glucagons and somatostatin, as well as expression of c-peptide in differentiated cells.

In a recent study, Peroni *et al.*[47] found that BM-MSCs and ADSCs express an identical transcriptional profile for genes related to stem cells phenotype such as Pou5f1 (Oct4) or other genes found in embrionic stem cells, such as UTF1 and Nodal, suggesting a close relationship between BM-MSCs and embryonic stem cells.

C. Umbilical cord blood –MSC

Recent reports suggested the existence of mesenchymal stem/progenitor cells in human umbilical cord blood (UCB-hMSC). Mononucleated adherent cells, isolated from UCB, displayed an immunophenotype similar to BM-hMSC and under appropriate conditions, these cells differentiated into mesenchymal lineages such as osteoblasts, chondrocytes, adipocytes and skeletal myoblasts[48–50].

To date, Pessina *et al.* brought the evidence that umbilical cord blood cells, after culture in medium supplemented with foetal calf serum (in the absence of specific cytokines or growth factors), show a panel of markers consistent with the characters of epithelial cells expressing genes considered essential in the differentiation steps towards pancreatic endocrine tissue (Isl-1, PDX-1, Pax-4 and Ngn3)[50].

Recently Chao *et al.* obtained IPCs differentiated from hUCB. HUCB MSC have been harvested in neural conditioned medium, differentiated initially into nestin-positive cells, that changed into insulin-producing cells under medium containing high concentrations of glucose, insulin and nicotinamide and at the end of a four stage differentiation protocol, islet-like clusters have been obtained. The cell clusters expressed insulin and other pancreatic β-cell-related genes, such as Pdx1, Hlxb9, Nkx2.2, Nkx6.1, and Glut-2, and released insulin and C peptide in response to the physiological glucose concentrations *in vitro*.[51]

D. Pancreatic MSCs

The pancreas is known to have the capacity to regenerate under certain circumstances, by mature beta-cell replication, as analyzed in mouse models[52,53].

Georgia and Bhushan[54] demonstrated that cyclin D2 expression in the endocrine pancreas coincides with a massive increase in islet mass, with ß-cell replication being the main mechanism for the maintenance of β-cell mass. Using cyclin D2–/– mice, β-cell replication was reduced 4-fold and cyclin D2–/– mice were glucose intolerant. These results suggested that cyclin D2 plays a key role in regulating the transition of β-cells from a state of quiescence to replication. In addition, many other investigators support the hypothesis of self-replication as the main source of β-cell mass.

Identification of pancreatic cells with progenitor features might open an important and promising strategy for cell replacement/ regeneration therapy.

In vitro, there is strong evidence that new pancreatic islets can derive from progenitor cells present within the ducts and islets in a process called “neogenesis”. Furthermore, when these pseudo-islets were transplanted into non-obese diabetic (NOD) mice, diabetes reversal was observed[55].

There is some evidence to suggest that pancreatic stem/progenitor cells reside within pancreatic ductal cells, where they can differentiate and migrate to form new islets during both organogenesis and regeneration. Ramiya *et al.* first described the generation of new islets from pancreatic ductal epithelial cells *in vitro*. The authors grew pancreatic ductal epithelial cells isolated from prediabetic adult non-obese diabetic (NOD) mice in long-term cultures, where they were induced to produce functioning islets containing alpha and delta cells. These *in vitro*-generated islets showed temporal changes in mRNA transcripts for islet cell-associated differentiation markers, responded *in vitro* to glucose challenge, and reversed insulin-dependent diabetes after being implanted into diabetic NOD mice[56].

Lu *et al.* demonstrated that transcriptional factor-pancreas duodenum homeobox-1 (PDX-1) might play an important role in differentiation of pancreatic stem cells into pseudo-islets cells; moreover, with the use of hepatocyte growth factor (HGF), neonatal pig pancreatic duct-derived cell monolayers could be induced to form three-dimensional islet-like cells that synthesize and release proinsulin and subsequently insulin[55]. These data suggest that duct cells are a source of pancreatic progenitor cells.

Are these progenitor cells mesenchymal stem cells? Their potential of differentiation into osteogenic, chondrogenic and adipogenic lineages has been analysed by Seeberger *et al.*[57]; in addition, further differentiation in the presence of growth factors resulted in the expression of the transcription factors PDX1, Pax4 and ngn3, suggesting that these MSC could derive β cells.

The exciting observation that nestin-positive islet cells display endocrine differentiating capacity led to the hypothesis that this intracytoplasmatic filament protein might correspond to a pancreatic stem/progenitor cell marker. Nestin is believed to play an important role in the partitioning of cytoplasmic components during the division of stem cells so as to maintain one daughter cell as a stem cell and the other daughter cell as a differentiated cell[58].

Timber *et al.* and Zulewski *et al.*[45,46] have reported that cells expressing the intermediate filament protein nestin, a marker of neural stem cells, can be isolated from human and rodent islets and expanded extensively in vitro. Insulin, glucagon, PDX-1/IPF-1 expression, and low-level insulin secretion, were detected in cultures of nestin-positive islet-derived stem/progenitor cells after addition of differentiating cytokines and growth factors, suggesting that nestin-positive islet-derived progenitor cells may participate in the neogenesis of islet endocrine cells.

More recently, a population of cells in the developing and adult mouse pancreas was identified, which, under differentiation conditions, released insulin in a glucose-dependent manner[59]. After differentiation, these cells expressed specific developmental pancreatic endocrine genes (e.g. Ngn3, Pax-4, Pax-6 and PDX-1) and contamination with mature beta cells was ruled out. In the study by Seaberg and collaborators, using a single-cell colony approach, stem/progenitor cells were independently isolated from duct and islet subpopulations. Interestingly, some of these particular cells with differentiating capacity were nestin positive and some were negative. Yet, all cells expressed Ngn3–a specific marker for pancreatic endocrine faith. These results support the study of Suzuky *et al.*[60] who isolated cells by means of flow cytometry and clonal analysis; the marker used to identify these cells was c-met–the hepatocyte growth factor HGF receptor. The authors suggest that interaction between c-met and HGF is crucial for growth and differentiation of pancreatic stem/progenitor cells during development, and that it contributes to regeneration and homeostasis of the pancreas in adult life

However, if, as postulated recently by Gershengorn *et al.*[61] fibroblast-like cells residing within the pancreas are multipotent and capable of reversible endoderm to mesoderm transitions, perhaps pancreatic MSCs could represent an abundant source of islet progenitors and potentially derive sufficient numbers of insulin-producing cells required for transplantation.

Anyhow, to be clinically relevant, *in vitro* proliferation of progenitor cells from human pancreas must produce large amounts of cells, in order to allow cells isolated from one single donor to be sufficient to treat a given diabetic patient. It would be even better to have one single donor for several diabetics. For these reasons, acinar isolated stem/progenitor cells might be of interest, considering that exocrine tissue constitutes 90% of pancreatic tissue and is discarded during islet isolation

In vivo – experimental evidence in autoimmune animal models of diabetes

Experimental animal models of diabetes for stem cell therapy

Animal models of autoimmune diseases are commonly used in order to test the immunomodulatory abilies of the MSCs; mouse experimental autoimmune encephalomyelitis (EAE) can be successfully treated with *in vitro*-expanded murine allogeneic MSC, with amelioration of clincal signs, related to inhibition of T cell proliferation and induction of immunological tolerance[62], while in SLE mouse models (BXSB mice), MSC injections can inhibit T and B cell autoreactivity[63].

Regarding experimental models of diabetic autoimmunity, the most commonly used ones, in order to test the functionality and the proficiency of β cell regeneration, induced either by MSCs per se or by the differentiated IPCs, are the immunocompromised animals with or without streptozotocin-induced diabetes mellitus.

The non obese diabetic (NOD) mouse strain (H-2KdDb), model of spontaneous onset of diabetes, provides the most relevant similiarities with the human autoimmune (insulindependent) diabetes mellitus. The disease in these animals is characterized by anti-islet cell antibodies, severe insulitis, and evidence for autoimmune destruction of the β cells.[64] Moreover, as diabetes can be tranferred to a recipient from a NOD mouse donor by haematopoietic stem cell transplantation (HSCT), suggests that diabetes in NOD mouse models, is also the result of a genetic hematopoietic stem cell predisposition.

Ikehara *et al.* showed that allogeneic bone marrow transplantation prevented insulitis and

diabetes in NOD mice, confirming the autoimmune involvement in the NOD mice diabetic pathology, and its similarities with human type I diabetes[65].

Streptozotocin – induced diabetic mice or rats, represent a widely used model for experimental research for new therapies in diabetes, that has been developed thirty years ago, based on the oncogenic and diabetogenic properties of a broad spectrum-antibiotic – streptozotocin.[42,66]

Whether administered as a single injection, or at multiple low-dose injections (30–50 mg/kg), streptozotocin determines autoimmune T-cell mediated insulitis in mice or rats, in a complex and controversial manner, as there are also toxic and non-immunological mechanisms modulating the inflammatory process in the pancreatic islets[42,67,68].

The functionality of theMSC differentiated IPCs is tested in vivo, using the above mentioned diabetic models.

Therapeutic effect of MSCs in animal models of type I diabetes (Table 1)

Table 1

Mesenchymal stem cell (MSC) treatment protocols in animal models of diabetes

MSC source	**Animal model**	**Transplantation procedure**	**Therapeutic effects**	**Ref**
Pancreatic ductal cell MSC	NOD mice (n=8)	Implantation in the subcapsular region of the kidney (syngeneic)	Amelioration and normalisation of blood glucose levels, induction of angiogenesis	52
GFP-Mouse c-kit+ BM derived cells	MD-STZ treated NOD/scid mice (5d 35mg/kg)	Intravenous injection (allogeneic)	Reduction of glycaemic levels and proliferation of pancreatic cells	66
Human BM-MSCs	MD-STZ treated NOD/scid mice (4d 35mg/kg)	Intracardial infusion (allogeneic)	Improvement of hyperglycaemia and repair of pancreatric and renal structures	63
Mouse syngeneic BMCs and syno- or allogeneic MSCs	STZ treated C57Bl/6 mice	Intravenous injection	Improvement of blood glucose and insulin levels and pancreatic tissue regeneration	64
PDX1-transfected hBM-MSC	STZ-treated scid mice	Implantation under renal capsule	Mild glycaemic amelioration	
IPCs diff from rat MSCs	STZ treatedNOD /scid mice	transplantation into the renal subcapsular space	Amelioration of hyperglycaemia	42
IPCs from BM-MSCs	STZ treated rats (60mg/kg)	portal vein injection (allogeneic)	Amelioration of hyperglycaemia	43
hUCB-MSCs	B6.Y Lep ob mice	Injection into orbital plexus	Normalisation of glucose levels and renal morphology	67
hUCB-MSCs	NOD mice (n=25)	Intravenous injection	Amelioration of glucose levels, and reduction of pancreatic insulitis	68
ICC derived from hUCB-MSCs	MD-STZ treated mice (2d 50mg/kg)	Intrahepatically injection	Amelioration of glucose tolerance	51

MSC – mesenchymal stem cells, *GFP* – gren fluorescent protein, *MDSTZ mice* – multi-dose streptozotocin treated mice, *IPC* – insulin producing cells, *hUCB-MSC* – human umbilical cord blood mesenchymal stem cells, *ICC* – islet-like cell clusters.

a. Bone marrow MSC

Allogeneic or syngeneic bone marrow MSC transplantation, alone or in association with haematopietic stem cells (HSC) has been performed on streptozotocin induced diabetic mice, or in NOD models, and brought encouraging

results regarding amelioration of glycemia and of renal lesions.[42,71,72]

Using transplants of bone marrow cells from male mice that express, using a CRE-LoxP system, an enhanced green fluorescent protein (EGFP) if the insulin gene is actively transcribed into lethally irradiated recipient female mice, Ianus *et al.*[69], demonstrated in a controversial and further not re-confirmed study that 1,7–3% of the islet cells of the recipient mice were marrowderived and that GFP-labeled donor cells isolated from the islets expressed insulin, glucose transporter 2 and several transcription factors typically found in β cells.

Hess *et al.*[70] reported that in NOD/scid mice with streptozotocin induced diabetes, partial marrow ablation, followed by transplantation of either GFP-labeled whole marrow or GFP-labeled c-kit+ cells from murine marrow, enhanced regeneration of islets, lowered blood sugar, and increased blood insulin levels, despite no observed differentiation into insulin producing cells.

This was confirmed by Lee RH *et al.*[71] Repeated transplantation of human mesenchymal stem cells (MSCs) *via* intracardial infusion, in NOD/scid mice after streptozotocin-induced pancreatic damage, has not only enhanced the number of endogenous β cells, with consecutive increase of mouse circulating insulin, and improvement of hyperglycaemia, but has also been associated with a marked decrease of inflammatory macrophage infiltrates in glomerular structures, compared to nontransplanted diabetic mice.

Moreover, a recent study published by Urban *et al.*[72] reported that concomitant administration of bone marrow cells and syngeneic or semi-allogeneic mesenchymal stem cells as a single injection of a mixture of BMCs and MSCs in a model of murine streptozotocin induced diabetes, normalized blood glucose and serum insulin, due to regeneration of recipient derived pancreatic insulin secreting cells, associated with an immunosupressive effect of MSCs on β-cell specific T lymphocyte response.

Wu *et al.* administered allogeneic islet-like cells previously differentiated from bone marrow-MSCs in streptozotocin – induced diabetic rats (by intraperitoneal injection of 60 mg/kg STZ), by transplantation via the portal vein; after transplantation, the differentiated MSC have been identified in the recipient's liver expressing islet hormones and alleviating the hyperglycaemia of the rat models[42].

Interpretation of these results *in vitro* and *in vivo* in mouse diabetic models demonstrate that the beneficial effects of infused MSCs in type 1 diabetes is mostly associated with anti-inflammatory activity coupled with a protective effect on the damaged tissues, and is not due to MSCs transdifferentiation into insulin producing cells.

b. Umbilical cord blood MSC

Ende et al examined the effect of transplanted human UCB mononuclear cells on blood glucose levels, survival, and renal pathology in obese mice with spontaneous development of type 2 diabetes (the B6.Y Lep ob mouse model). The results show that injection of h UCB mononuclear cells into orbital plexus of mice caused improvement not only in blood glucose levels and survival rate but also normalization of glomerular hypertrophy and tubular dilatation[73].

Using 25 female non obese diabetic (NOD) mice with insulitis (as type I diabetic model) treated by intravenous administration of human umbilical cord MSC, the same research team, observed amelioration of glycemic profiles, together with histological improvement of insulitis at around 148 days after treatment[74].

Recent publications evidentiated the ability of h UCB MSC to differentiate into functional IPCs, which, transplanted into the liver of mouse with multiple low-dose (50 mg/kg/2days) stretpozotocin-induced diabetes mellitus, normalised and stabilised the glycaemic levels on the 12 week follow up period[51].

Due to their simple availability, without risk for the donors and associated with a low risk for immune-rejection of transplanted grafts, cord blood-derived MSC might represent a potential source for diabetes cell-replacement.

Benefits and risks related to MSC therapies

Both insulin-producing cells derived from stem cells and islet cells extracted from donor pancreata provide the hope of sufficiently tight control of blood glucose, being thus capable to prevent the diabetic late complications that current diabetes drug therapies have been almost impossible to avoid.

Whatsoever, stem-cell-based therapy is much different from traditional islet cell transplantation therapy. Stem cells provide a theoretically unlimited supply of insulin-producing cells for transplantation, as they could be easily isolated and

harvested within a few weeks, and may also be induced from the remained stem cells *in vivo*.

Human MSCs transplantation could be practised with minimal risks compared with HSC or BM (bone marrow) transpantation, due to their hypoimmunogenicity.

Furthermore, stem cells represent a fruitful subject for the development of new gene therapy strategies to prevent T1D[75,76].

An overview of the ongoing clinical trials related to mesencchymal stem cells therapies has been realised by Giordano A *et al.*[77] who concluded that despite the ongoing enthusiasm on the part of scientists and physicians regarding cellular and gene therapies and related treatments therapies , the results are overshadowed by many difficulties, especially with regard to the immunological risks, to ethical aspects, (in the case of cell therapies) as well as the efficacy and safety of delivery of exogenous genes to target cells and tissues by viral vectors in the case of the prospective gene therapies.

The efficacy of MSCs as well as their products of differentiation in still debatable, and results of the ongoing clinical trials are needed, before assessing new clinical perspectives.

Despite extensive research, there are still problems related to the specificity of the stem cells as a true marker of MSC "stemness" and multipotentiality has not yet been defined .The most difficult problem is related to the availability of physiologically functional insulin-producing cells from MSCs.

Besides, the majority of studies on beta cell regeneration or IPC immunomodulation have been performed in adult mouse or rat and not in humans.

In mice, MSCs might exert a tumoral effect, and stimulate malignant cell proliferation[78]; therefore there is a risk that administration of autologous hMSCs in human subjects will enhance to growth of an unsuspected tumor.

A recent study published by Lu *et al.*[79] evidentiated a number-dependent growth inhibitory effect of MSC on murine tumor cell lines in vitro and in vivo without host immunosuppression. MSCs inducing apoptotic cell death and G0/G1 phase arrest of cancer cells.

There is also a risk of embolisms if the cells are allowed to aggregate in suspension before i.v. infusion. In addition, culture of hMSCs usually is performed in medium containing FCS (fetal calf serum), that the cells internalize and that can produce immune reactions with repeated administrations of the cells. However, the calf proteins can be removed metabolically by short-term culture with human serum[80] or avoided by culture in medium containing platelet lysates in place of the FCS[81].

Future clinical perspectives for stem cell therapies

At present there is an ongoing debate related to the mechanisms by which beta-cell mass is maintained in the pancreas of an adult human[53,54]. However there is recent evidence for the presence of mesenchymal cells within the human adult pancreas that act as progenitors[54], which broadenes the research perspectives related to pancreatic cell regeneration.

Better understanding of the intimate nature of the intracellular pathways of signalisation transmitted by the stimuli that would promote ß cell replication or potential neogenesis, as well as further research on the tissular and intracellular mechanisms of potential immune reconstitution would be necessary in future, in order to be able to extent clinical use of MSC therapies in a safe manner, as a potential treatment of type 1 diabetes.

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