Review

Mesenchymal stem cells: Stem cell therapy perspectives for type 1 diabetes

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Abstract

Mesenchymal stem cells (MSCs) are multipotent non-haematopoietic progenitor cells that are being explored as a promising new treatment for tissue regeneration. Although their immunomodulatory properties are not yet completely understood, their low immunogenic potential together with their effects on immune response make them a promising therapeutic tool for severe refractory autoimmune diseases. Type 1 diabetes is characterized by T cell-mediated autoimmune destruction of pancreatic β cells. While insulin replacement represents the current therapy for type 1 diabetes, its metabolic control remains difficult, as exogenous insulin cannot exactly mimic the physiology of insulin secretion. Pancreatic or islet transplantation can provide exogenous insulin independence, but is limited by its intrinsic complications and the scarcity of organ donors. In this context, stem cell therapy, based on the generation of insulin-producing cells (IPCs) derived from MSCs, represents an attractive possibility. In this review, we provide a brief characterization of MSC immunomodulatory effects, and present the current experimental evidence for the potential therapeutic efficacy of MSC transplantation in diabetes.
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Keywords: Mesenchymal stem cells; Type 1 diabetes; Insulin-producing cells; Stem cell therapy; Immunosuppression; Review

Résumé

Les cellules souches mésenchymateuses comme nouvelle approche thérapeutique du diabète de type 1.

Les cellules souches mésenchymateuses (CSM) sont des cellules progénitrices non hématopoïétiques multipotentes qui constituent aujourd'hui un outil thérapeutique prometteur pour la régénération et la réparation tissulaire. Bien que les mécanismes exacts de l'action immunomodulatrice des CSM in vitro ne soient pas totalement éclaircis, leurs propriétés immunosuppressives ont conduit à leur utilisation en clinique humaine pour le traitement de certaines maladies auto-immunes. Le diabète de type 1 est caractérisé par une destruction auto-immune des cellules β du pancreas, médiée en partie par les lymphocytes T. L'insulinothérapie permet, en partie, de palier le déficit en insuline endogène et de contrôler la glycémie, mais ne représente pas un traitement curatif de la maladie. La greffe de pancréas et la transplantation d'îlots pancréatiques permettent d'obtenir une insulino-indépendance mais sont limitées par les complications liées à ces approches thérapeutiques et par les difficultés inhérentes au don d'organe. Dans ce contexte, les thérapies cellulaires fondées sur l'utilisation de cellules productrices d'insuline (IPCs) dérivées des CSM représentent des perspectives thérapeutiques séduisantes. Dans cette revue, nous présentons les principales caractéristiques fonctionnelles des CSM ainsi que les données expérimentales qui suggèrent l'efficacité potentielle de la greffe de CSM ou de cellules dérivées des CSM dans le traitement du diabète de type 1.
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Mots clés : Cellules souches mésenchymateuses ; Diabète de type 1 ; Cellules productrices d'insuline ; Thérapies cellulaires ; Immunosuppression ; Revue

1. Introduction

Diabetes mellitus is a complex metabolic disease with an estimated worldwide prevalence of 171 million cases among

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the adult population [1]. It is associated with severe long-term micro- and macrovascular complications, and carries high rates of morbidity and mortality. Although it represents only 10% of the diabetic population worldwide, the ongoing increasing incidence of type 1 diabetes (up to 41/100,000 population/year in Europe and up to 25/100,000 population/year in North America) [2] represents a major global health issue.

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

Standard treatment strategies for type 1 diabetes are based on different regimes of insulin injections with careful blood glucose monitoring. However, good metabolic control is difficult to obtain, and insulin therapy is frequently associated with severe hypoglycaemic episodes, as exogenous insulin cannot exactly mimic the physiology of insulin secretion.

Now, however, β cell replacement therapies using either pancreatic or islet transplantation represent a therapeutic alternative. Human pancreatic transplantation, with 6600 transplant recipients reported worldwide since 1985 [3], has demonstrated partially successful results in reversing long-term renal and neural complications in selected type 1 diabetic patients [4]. Human islet transplantation (including islet cells from donor pancreata, and their administration into the liver via portal vein embolization) also offers glycaemic control and sufficient prevention of hypoglycaemia without the need for exogenous insulin administration [5].

Indeed, Shapiro et al. reported insulin independence in 21 out of 36 (58%) islet-transplant recipients after 1 year of follow-up, of whom five maintained insulin independence for 2 years [6]. In a 5-year follow-up study of 65 islet-transplant recipients, Ryan et al. reported a median duration of insulin independence of 15 months [7]. In fact, approximately 10% of all patients maintain long-term insulin independence. In its 2006 annual report, the Collaborative Islet Transplant Registry presented data from 225 islet-transplant recipients from 23 programmes carried out between 1999 and 2005. Nearly two thirds of the recipients achieved insulin independence (defined as no need for insulin administration for at least 14 days) during the year following transplantation, which dropped by one third over the second year [8]. However, the respective benefits from pancreatic or islet transplants are limited by the intrinsic (haemorrhagic or thrombotic) complications associated with the transplant procedure —specifically, the need for life-long immunosuppressant therapy, with its adverse side-effects, and the difficulty of obtaining transplant material and organ donations [5].

In late 2003, the group led by Voltarelli et al. started a phase I/II study of high-dose immunosuppression followed by autologous haematopoietic stem cell (HSC) transplantation for patients with new-onset type 1 diabetes. The aim of the treatment was to stop the autoimmune destruction of β cells with immunosuppressant drugs, and to "reset" the damaged immune system as a normal reconstituted one using autologous HSCs. The researchers obtained encouraging results in 15 patients after 7- to 36-month follow-ups. This study highlighted the stem cell immunomodulatory properties in human type 1 diabetes [9].

The major goal of future diabetes therapy is to promote β cell regeneration, which could be accomplished by β cell self-replication or differentiation from progenitor cells with the use of stem cell therapy to overcome autoimmunity and to improve endogenous insulin secretion. In this setting, mesenchymal stem cells (MSCs) represent an interesting therapeutic option due to their immunomodulatory properties and their potential for *in vitro* differentiation into insulin-secreting cells, therefore achieving the major therapeutic goals for type 1 diabetes.

2. MSCs: definition, identification and immunomodulatory properties

2.1. Definition, identification and sources of MSCs

Originally identified by Friedenstein et al. in 1976 [10] as a fibroblast-like cell population capable of generating osteogenic precursors, the mesenchymal stromal cells derived from the bone marrow (BM) are a rare, heterogeneous, stromal population of multipotent non-haematopoietic progenitor cells with the capacity to differentiate into multiple mesenchymal lineages, including bone, fat and cartilage. Due to this characteristic, Caplan [11] dubbed them "mesenchymal stem cells" (MSCs), which has been recently changed —by a consensus statement recommendation— to "multipotent mesenchymal stromal cells" [12]. Other studies have identified pluripotent cells capable of differentiation along endodermal and neurectodermal lineages, including neurons, hepatocytes and endothelial cells [13–15]. Such stem cells, isolated from BM, have been referred to as "multipotent adult progenitor cells" (MAPCs) [16], "marrow-isolated adult multilineage inducible cells" (MIAMIs) [17] and "very small embryonic-like stem cells" (VSELs) [18]. However, even if the transdifferentiation capacities of these primitive cell types is of major interest, obtaining them requires highly specific culture conditions and, so far, it has not been possible to isolate these cells from fresh BM. Whether or not they represent a culture phenomenon remains an unanswered question.

Compared with other stem cell sources, such as HSCs or stem cell populations from the liver, spleen or pancreas, MSCs appear to be a promising agent for overcoming autoimmunity in type 1 diabetes, given their ability to differentiate along various lineages in addition to their immunosuppressant capacities [19]. Although no specific membrane marker has yet been definitively identified on MSCs, several phenotypical characteristics have allowed these cells to be identified and enriched. MSCs express several cell surface antigens, such as CD73, CD90, CD105, CD146 and even the recently described CD200 [20], as well as various integrins and adhesion molecules. As MSCs are a non-haematopoietic cell line, they do not express haematopoietic markers such as CD34, CD14 and CD45 [21]. Adult human MSCs 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. In addition, MSCs differentiate into adipose, bone and cartilage cell lines [22], and can also synthesize trophic
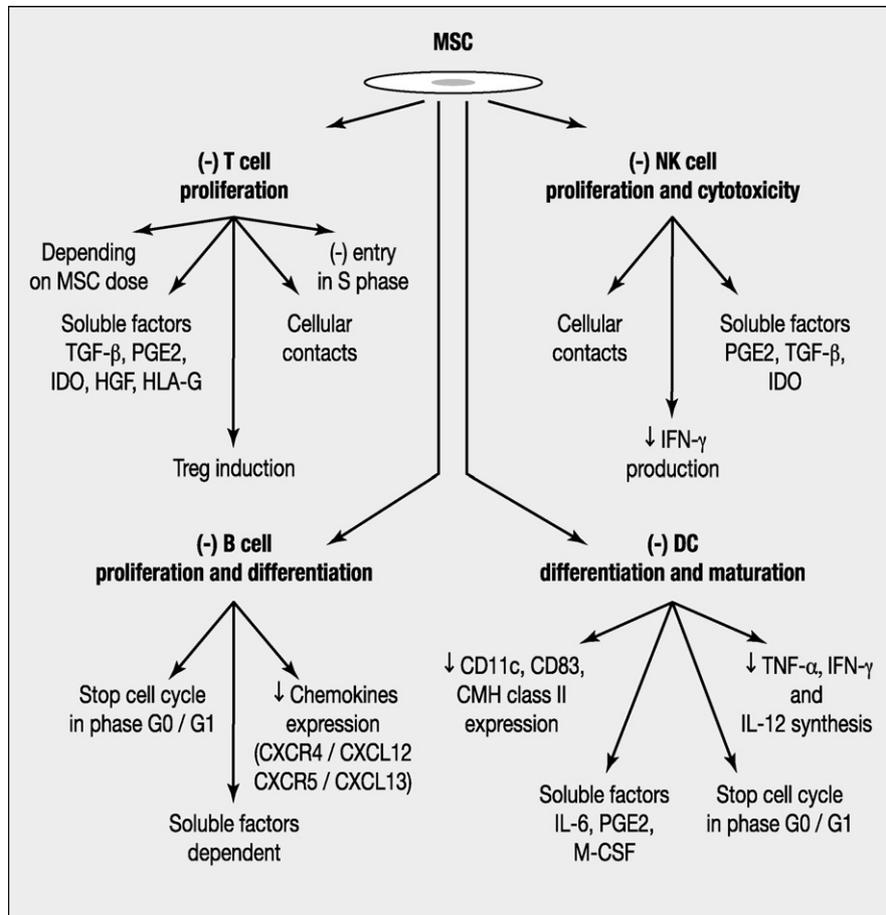
Fig. 1. Immunomodulatory properties of mesenchymal stem cells (MSCs). TGFβ: transforming growth factor beta; PGE2: prostaglandin E2; IDO: indoleamine 2,3-deoxygenase; HGF: hepatocyte growth factor; IFNγ: interferon gamma; TNFα: tumour necrosis factor alpha; M-CSF: macrophage colony-stimulating factor; IL-6: interleukin-6; IL-12: interleukin-12.

mediators such as growth factors and cytokines (M-CSF, IL-6, IL-11, IL-15, SCF, VEGF), involved in haematopoiesis regulation, cell signaling and modulation of immune response [23]. Because of the rarity of MSCs and the lack of a definitive marker to specifically isolate them, they are commonly obtained from BM rather than other sources such as adipose tissue, synovium, umbilical cord blood, fetal liver, amniotic fluid, umbilical cord or the perivascular cells derived from Wharton's jelly, or deciduous teeth [24–27].

2.2. MSC immunomodulatory properties: in vitro evidence of immunomodulation

MSCs can modulate the immunological activity of different cell populations, as shown by *in vitro* experiments, the most important being their inhibitory effect on T cell proliferation and dendritic cell (DC) differentiation, considered the principal key factors for activating autoimmune disorders (Fig. 1).

MSCs are also effective for inhibiting proliferation of CD4 and CD8 T cells, as well as memory and naïve T cells [28]. This mechanism may necessitate an initial cell-contact phase as well as several specific mediators produced by MSCs, such as transforming growth factor β (TGFβ), prostaglandin E2 (PGE2)

and indoleamine 2,3-deoxygenase (IDO); induced by interferon-gamma (IFNγ), this catalyzes the conversion of tryptophan to kynurenine and inhibits T cell responses by tryptophan depletion) [29].

The ability —reported in humans, rodents and primates— to suppress T cell responses to mitogenic and antigenic triggering is explained by a complex mechanism that can induce "division arrest anergy", responsible for maintaining T lymphocytes in a quiescent state. Thus, MSCs determine the inhibition of cyclin D2 expression, arresting cells in the G0–G1 phase of the cell cycle [30–32].

MSCs can also modulate immune response by stimulating the production of CD8+ Treg (regulatory T cells), which inhibit lymphocyte proliferation in allogeneic transplants [33]. However, the induction of regulatory T cells may be mediated by different factors in alloreactive and mitogen-stimulated lymphocyte cultures, as there are differences between the two systems.

Bone marrow MSCs (BM-MSCs) can indirectly reduce T cell activation by inhibiting DC differentiation (mainly DC type 1) from monocytes [34–37]. In addition, MSCs can inhibit B cell proliferation and activation in a dose-dependent manner, and modulate their differentiation, antibody production and chemotactic abilities [38].

2.3. MSC immunomodulatory properties in vivo: clinical evidence of immunomodulation and tissue regeneration

Due to their supportive function for HSC in BM, their selective activity on the cell cycle and their immunomodulatory effects, MSCs have already been used in clinical trials as a treatment for acute graft-versus-host disease (GVHD) following allogeneic HSC transplantation [39,40] and for autoimmune diseases such as systemic sclerosis [41].

The important capacity for differentiation of MSCs has made them a useful therapeutic means in orthopaedics for increasing new dense bone formation and total bone mineral content in osteogenesis imperfecta [42], and for providing early bone regeneration in osteonecrosis of the femoral head [43] and the repair of large bone defects [44].

The MSC capacity to synthesize certain growth factors and enzymes such as aryl-sulphatase A and α-L-iduronidase —which are deficient in metachromatic leukodystrophy and Hurler's disease— allow them, after *in vitro* expansion and intravenous administration, to enhance enzyme production and improve the symptomatology of this inborn error of metabolism [45].

MSCs have also been administered as autologous transcoronary transplants in human infarcted myocardium, alone or in association with endothelial progenitor cells (EPC), with satisfactory results in terms of improving myocardial contractility [46].

3. MSC therapeutic potential in type 1 diabetes: *in vitro* and *in vivo* evidence

3.1. In vitro differentiation of adult MSCs into insulin-producing cells (IPCs)

MSCs can be differentiated into IPCs by using a specific culture medium enriched with insulin-promoting factors (mainly glucose and nicotinamide). IPC identification is then based on the ability to express genes related to pancreatic development and function, such as insulin I and II, GLUT2, glucose kinase, islet amyloid polypeptide, nestin, and pancreatic duodenal homoeobox 1 (PDX1) and Pax6, and to synthesize C peptide and insulin [47].

3.1.1. BM-MSCs as a source of IPCs

IPCs can be obtained from rat BM-MSCs, using a high-glucose culture medium [48] or nicotinamide-enriched medium [49,50] to promote cell differentiation; the differentiated islet-like cells express insulin at both mRNA and protein levels, and are able to control glucose levels in non-obese diabetic (NOD) rats [50].

It has also been possible to induce *in vitro* IPC differentiation of BM-MSCs isolated from type 1 ($n = 5$) and type 2 ($n = 5$) uncomplicated diabetic patients through a specific 18-day, three-stage protocol [51]. The protocol includes using a combination of nicotinamide, activin A and β-cellulin in a high-glucose concentration (25 mmol/L) medium to effectively promote BM-MSC differentiation; at the end of the culture period, the differentiated cells show a similar morphology to that of pancreatic islet-like cells, high expression levels of PDX1, insulin and glucagon genes, and a positive response in terms of glucose dose-dependent insulin production [51].

To obtain β cell differentiation from 14 human BM-MSC donors, Karnieli et al. [52] used a PDX1 gene-transfer approach. PDX1 is a major transcription factor for pancreatic development and the β cell gene expression profile. The researchers showed that 40–60% of the PDX1-expressing MSCs produced insulin in response to increasing glucose concentrations.

The transfected MSCs, transplanted under the renal capsule of streptozotocin (STZ)-diabetic severe combined immune deficiency (SCID) mice, were able to reduce glucose levels from above 300 to 200 mg/dL after 5 weeks. However, an abnormal response to the glucose tolerance test was noted 6–8 weeks after transplantation [52].

3.1.2. Adipose-derived MSCs as a source of IPCs

Adipose tissue isolated from human lipoaspirates is an abundant and easily accessible source of stromal progenitor cells (adipose-derived stromal cells [ADSCs]), which resemble adult BM-MSCs and have the same differentiation capacities [53].

Timper et al. [54] and Eberhardt et al. [55] expanded human adipose tissue-derived MSCs from four donors in a fibroblast growth factor-containing medium. These cells had stem cell markers such as Scf and Thy1, but also Isl1 mRNA, which is essential for the generation of pancreatic endocrine cells. In addition, upregulation of transcription factors Ipf1, Isl1 and Ngn3, and islet gene insulin, glucagon and somatostatin, as well as expression of C peptide in differentiated cells were observed. As harvesting and producing adipose-derived MSCs appear to be more practical and less invasive in humans compared with BM-harvesting, further research is warranted in this field to confirm these promising results.

3.1.3. Umbilical cord blood MSCs (UCB-hMSCs) as a source of IPCs

The presence of mesenchymal stem or progenitor cells in human umbilical cord blood (hUCB) was revealed by the identification of mononuclear adherent cells, isolated from UCB, displaying a BM-MSC-like immunophenotype and differentiation capacities [56–58].

Pessina et al. showed that UCB cells, after culture in a medium supplemented with fetal calf serum (with no specific cytokines or growth factors), exhibited a panel of markers with the characteristics of epithelial cells, displaying genes considered essential for differentiation into pancreatic endocrine tissue (Isl1, PDX1, Pax4 and Ngn3) [58].

Chao et al. [59] obtained islet-like clusters differentiated from hUCB at the end of a four-stage differentiation protocol. The cell clusters showed insulin and other pancreatic β cell-related genes (PDX1, Hlxb9, Nkx2.2, Nkx6.1 and GLUT2), and released insulin and C peptide in response to physiological glucose concentrations *in vitro*. Since the first successful transplantation of UCB in 1988 [60], cord blood has become an important source of HSCs for the treatment of blood and genetic

disorders. With the increasing focus on cord blood as a source of tissue for regenerative medicine, UCB stem cells have attracted significant attention. Therefore, the use of hUCB as a source for IPCs should be considered an important potential therapeutic option.

3.1.4. Pancreatic MSCs

There is evidence to suggest that pancreatic stem or progenitor cells reside within pancreatic duct cells, where they differentiate and migrate to form new islets during both organogenesis and regeneration. Ramiya et al. first described the generation of new islets from pancreatic duct epithelial cells *in vitro*, isolated from prediabetic adult NOD mice. These *in vitro*-grown islets contained alpha and delta cells, responded to *in vitro* glucose challenge and, once implanted into NOD mice, reversed insulin-dependent diabetes [61].

Lu et al. [62] demonstrated that transcription factor PDX1 plays an important role in the differentiation of pancreatic stem cells into pseudo-islet 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 insulin. Therefore, pancreatic duct cells can be a source of pancreatic progenitor cells.

However, it is worthwhile asking whether or not these progenitor cells are MSCs. Seeberger et al. [63] showed that pancreatic stem cells could differentiate into osteogenic, chondrogenic and adipogenic lineages, and also express the transcription factors PDX1, Pax4 and Ngn3, suggesting that these progenitor cells are MSCs that can transform into β cells.

Initially, Gershengorn et al. [64] considered that pancreatic MSCs could represent an abundant source of islet progenitors and produce sufficient numbers of IPCs for transplants, as fibroblast-like cells residing within the pancreas are multipotent and capable of reversible endoderm-to-mesoderm transitions, just like MSCs. More recently, the same researchers [65], as well as Atouf et al. [66], concluded that mouse pancreatic β cells do not generate endocrine precursor cells by epithelial–mesenchymal transition (EMT) *in vitro*.

Using recombinant-based genetic cell tracing to determine the origin of proliferating fibroblast-like cells in mouse islet cultures *in vitro*, Chase et al. found that fibroblast-like cells in mouse islet cultures do not undergo EMT, but that these fibroblast-like cells represent MSCs like those isolated from BM [67].

Experimental approaches, using various phenotypic cell markers (such as nestin, Ngn3 and c-Met), may improve the isolation of pancreatic progenitor cells. Timper et al. [54] and Eberhardt et al. [55] found that cells expressing nestin can be isolated from human and rodent pancreatic islets and extensively expanded *in vitro*. Nestin-positive islet cells display endocrine differentiating capacity, so this intracytoplasmic filament protein could correspond to a pancreatic stem or progenitor cell marker [54,55,68]. Indeed, insulin, glucagon, PDX1/Ipf1 expression and low-level insulin secretion were detected in cultures of nestin-positive islet-derived stem or progenitor cells, suggesting that such cells can participate in the neogenesis of islet endocrine cells [54,55].

More recently, stem or progenitor cells were independently isolated from pancreatic ducts and islets in both developing and adult mice that, under specific differentiation conditions, were able to release insulin in a glucose-dependent manner [69]. After differentiation, these cells expressed specific developmental pancreatic endocrine genes (such as Ngn3, Pax4, Pax6 and PDX1). Some of these differentiated cells were nestin-positive and some were negative; yet, all cells had Ngn3, a specific marker for pancreatic endocrine growth and development. The important role of the Ngn3 gene in islet-cell progenitor activation and in expansion of the β cell mass has recently been confirmed in a mouse model of injured pancreas [70]. In that study, the authors showed that activation of Ngn3 gene expression increased β cell hyperplasia, whereas knockdown of Ngn3 impaired injury-induced β cell generation. Moreover, the report shed light on a new population of Ngn3-positive progenitor cells purified from adult mouse pancreas —specifically located in the duct lining. These cells were shown to differentiate into functional β cells *in vitro* and after culture in embryonic pancreas explants. In parallel, Suzuki et al. [71] isolated pancreatic progenitor cells from neonatal and adult mice, using flow cytometry and clonal analysis with the HGF receptor c-Met as a phenotypic marker. They determined that the interaction of c-Met and HGF is essential for growth and differentiation of pancreatic stem or progenitor cells during development, and that it contributes to regeneration and homoeostasis of the pancreas in adults.

3.2. MSC-based therapy in autoimmune animal models of diabetes

Immunocompromised animals such as the NOD and NOD/SCID mouse strains, with or without STZ-induced diabetes, represent the most commonly used experimental models for diabetic autoimmunity. The NOD strain (H-2KdDb), a model of spontaneous onset of diabetes, is characterized by anti-islet-cell antibodies, severe insulitis and evidence of autoimmune destruction of β cells similar to human autoimmune insulin-dependent diabetes [72]. NOD/SCID mice, which are homozygous for the SCID mutation, and severely deficient in T and B lymphocytes, represent an invaluable diabetic model for experimental research.

STZ-induced diabetic mice or rats were developed 30 years ago, based on the diabetogenic properties of the broad-spectrum antibiotic STZ [73–75]. Administered as multiple low-dose injections (30–50 mg/kg), STZ determines autoimmune T cell-mediated insulitis in mice or rats in a complex manner, while a single injection triggers toxic and non-immunological mechanisms to modulate the inflammatory process in pancreatic islets [74,75]. Experimental data for the therapeutic effects of BM-MSCs and UCB-MSCs in the above-mentioned animal models of type 1 diabetes (Table 1) are important tools for analyzing results before considering further human clinical applications.

3.2.1. Use of BM-derived MSCs to treat experimental diabetes

Allogeneic or syngeneic BM-MSC transplantation, alone or in association with HSCs, was performed on both STZ-induced

Table 1
Mesenchymal stem cell (MSC) treatment protocols for animal models of diabetes.

MSC source	Animal model	Transplant procedure	Therapeutic effects	Reference
Pancreatic duct cells	NOD mice ($n = 8$)	Implantation in subcapsular region of kidney (syngeneic)	Improved and normalized blood glucose levels; induction of angiogenesis	[61]
GFP-mouse c-kit+ BM-derived cells	MD-STZ-treated NOD/SCID mice (5 days, 35 mg/kg)	Intravenous injection (allogeneic)	Reduced glycaemic levels; proliferation of pancreatic cells	[79]
Human BM-MSCs	MD-STZ-treated NOD/SCID mice (4 days, 35 mg/kg)	Intracardial infusion (allogeneic)	Improved hyperglycaemia; repair of pancreatic and renal structures	[76]
Mouse syngeneic BM cells and syngeneic/allogeneic MSCs	STZ-treated C57Bl/6 mice	Intravenous injection	Improved blood glucose and insulin levels; pancreatic tissue regeneration	[77]
IPCs differentiated from rat MSCs	STZ-treated NOD/SCID mice	Transplantation into renal subcapsular space	Improved hyperglycaemia	[50]
IPCs from BM-MSCs	STZ-treated rats (60 mg/kg)	Portal vein injection (allogeneic)	Improved hyperglycaemia	[51]
hUCB-MSCs	B6.Y Lep(ob) mice	Injection into orbital plexus	Normalized glucose levels and renal morphology	[81]
hUCB-MSCs	NOD mice ($n = 25$)	Intravenous injection	Improved glucose levels; reduced pancreatic insulitis	[82]
ICC derived from hUC-MSCs	MD-STZ-treated mice (2 days, 50 mg/kg)	Intrahepatic injection	Improved glucose tolerance	[59]

MSC: mesenchymal stem cell; GFP: green fluorescent protein; MD-STZ: multidose streptozotocin; BM: bone marrow; IPC: insulin-producing cell; hUC-MSC: human umbilical cord mesenchymal stem cell; ICC: islet-like cell clusters.

diabetic mice and NOD models, with encouraging results in terms of improving glycaemia and renal lesions [50,76, 77].

In 2003, Ianus et al. [78] reported, in a controversial and unconfirmed study, that BM-GFP (green fluorescent protein)-labelled murine cells, transplanted into lethally irradiated C57BL/6 mice, could be identified as representing 1.7–3% of the recipient mice islet cells, and were able to express insulin, GLUT2 and transcription factors typically found in β cells. That same year, Hess et al. [79] showed that transplantation of either GFP-labelled whole marrow or GFP-labelled c-kit+ BM murine cells in STZ-induced diabetic NOD/SCID mice enhanced islet regeneration, lowered blood sugar and increased blood insulin levels.

These results were confirmed by Lee et al. [76] in STZ-induced diabetic NOD/SCID mice that were repeatedly transplanted with human MSCs via intracardial infusion. An increased production of endogenous β cells and higher levels of mouse circulating insulin were obtained, with improvement of hyperglycaemia and decreased inflammatory macrophage infiltrates in glomerular structures compared with non-transplanted diabetic mice. More recently, in a model of murine STZ-induced diabetes, concomitant administration, via a single injection, of BM cells with syngeneic or semi-allogeneic MSCs normalized blood glucose and serum insulin levels, and allowed regeneration of recipient-derived pancreatic insulin-secreting cells due to the immunosuppressive effect of MSCs on the β cell-specific T-lymphocyte response [77]. In addition, similar results were reported by Ezquer et al. [80] in the same model of STZ-induced type 1 diabetes. Reversion of hyperglycaemia and glycosuria was observed after injection of 0.5×10^6 MSCs, with increased morphologically normal β-pancreatic islets.

Allogeneic islet-like cells previously differentiated from BM-MSCs have been transplanted in STZ-induced diabetic rats via the portal vein. Differentiated MSCs were identified in the recip-

ient animals' liver, and were able to produce islet hormones (insulin, C peptide, glucagon, somatostatin and islet amyloid polypeptide) and alleviate hyperglycaemia [50].

Taken altogether, these *in vitro* and *in vivo* experiments demonstrate that the beneficial effects of MSCs in type 1 diabetes may be related to both their immunosuppressant activity and subsequent protective effects on damaged tissue, and their capacity to differentiate into IPCs.

3.2.2. Use of UCB-MSCs to treat experimental diabetes

Human UCB-MSCs are able to differentiate into functional IPCs which, after administration in multiple low doses (50 mg/kg per 2 days) to the liver of STZ-induced diabetic mice, can normalize and stabilize glycaemic levels [59]. Ende et al. also reported success with hUCB-MSCs as a treatment for type 1 and type 2 diabetes in mouse models [81,82].

Human UCB mononuclear-cell injections into the orbital plexus of obese B6.Y Lep(ob) mice, with spontaneous development of type 2 diabetes, improved blood glucose levels and survival rates, and led to normalization of glomerular hypertrophy and tubular dilatation [81]. Furthermore, improved glycaemic profiles associated with histological improvement of insulitis were obtained after intravenous administration of hUCB-MSCs to 25 NOD type 1 diabetic mice with insulitis [82].

Indeed, UCB-MSCs may represent a potential source of diabetic cell replacement because of their availability, their low risk for immune rejection and increased capacity for expansion.

4. Controversial issues

IPCs derived from both stem cells and islet cells isolated from donor pancreata offer hope as a way to adequately control blood glucose. However, the question of whether or not

stem cells isolated from various tissues in humans can be used to treat type 1 diabetes through *ex vivo* differentiation in IPCs, their immunosuppressive effects or other pathways is still a matter of debate. For this reason, the majority of MSC research has been limited by the use of rodent cell cultures and the use of several non-reproducible environmental manipulations, as well as by insufficient data on the qualitative and quantitative aspects of insulin secretion. While the ability of MSCs to differentiate into IPCs remains controversial, more is known of the immunomodulatory properties of MSCs, and these capacities have already been exploited in the clinical setting. Thus, MSCs can modulate immune responses in diabetes either directly —by releasing costimulatory and regulatory cytokines such as TGF-β or interleukin-10 (IL-10) to control autoreactive T cells— or indirectly via a DC mechanism. MSCs also exert anti-inflammatory effects, which could be important in maintaining peripheral tolerance, as type 1 diabetes represents a disease wherein the majority of patients are children and younger adults, in whom the safety of any therapeutic interventions is of paramount importance.

The precise therapeutic profile or main mechanism through which MSCs should be able to successfully treat type 1 diabetes has yet to be determined. It has been hypothesized —and not without controversy— that neither the immunomodulatory nor differentiation abilities of MSCs are able to support pancreatic regeneration. However, a possible indirect effect of differentiation towards endothelial cells could be to provide environmental support for pancreatic progenitors [79].

Indeed, stem cells represent an important source of novel gene-therapy strategies to prevent type 1 diabetes [83,84]. Genetic and microenvironmental manipulations could lead to human MSC differentiation into β cells by inducing, under certain stress conditions, the activation of several transcription factors of the β cell developmental pathway [85]. However, despite the ongoing enthusiasm for MSC-based cell therapies, the present encouraging results need to be tempered, especially given the potential risks associated with human MSC production and injection. Recent *in vitro* studies have shown that MSC expansion may be associated with cytogenetic abnormalities and tumour development in treated animals [86–88]. In addition, it has been shown in mice models that MSCs could exert tumoural effects and stimulate malignant-cell proliferation [89,90]. Preventative measures against unwanted transdifferentiation into other mesenchymal cell lines (such as bone in cardiac tissue replacements) [91] are now being investigated, and their eventual systemic administration or specific delivery into lymphoid tissues, where they can exert their immunomodulatory actions, will also need to be carefully evaluated.

Furthermore, human MSC culture is usually carried out on a medium containing fetal calf serum that could potentially lead to immune reactions with repeated administration. However, this adverse side-effect can be circumvented by replacing fetal calf serum with human serum [92] or platelet lysates [93] when manufacturing the MSCs.

Finally, the most difficult —and as yet unresolved— issue is how to generate functional IPCs from MSCs on a large scale.

In conclusion, the past 10 years have provided new and fascinating *in vitro* and *in vivo* data on the immunosuppressant properties of MSCs and their potential to differentiate into IPCs to support their theoretical use in clinical practice as a new therapeutic option to treat insulin-dependent diabetes. Yet, the majority of studies of β cell regeneration or IPC immunomodulation have involved animals (adult mice or rats), although the next step towards clinical applications in humans is close. Nevertheless, the recent cloud on the horizon arising from the oncogenic potential observed in animals has to be borne in mind when following-up ongoing clinical trials with careful monitoring of clinical and manufacturing procedures.

Conflicts of interest

No potential conflict of interest relevant to this article is reported by the authors.

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