Using Mesenchymal Stromal Cells in Islet Transplantation

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SUMMARY

Islet transplantation has the potential to cure type 1 diabetes, but current clinical transplantation protocols are inefficient because of the extensive loss of functional islets during the immediate post-transplantation period. Studies in rodent models have demonstrated that co-transplanting mesenchymal stromal cells (MSCs) with islets improves graft functional survival and transplantation outcomes, and some of the beneficial effects of MSCs are attributable to bioactive molecules secreted by MSCs. Clinical islet transplantation is almost exclusively via the hepatic portal vein, which does not facilitate co-engraftment of islets and MSCs, so attention is currently focused on using cell-free cocktails of MSC-derived products to treat islets prior to transplantation. This approach has the potential to overcome many of the technical and regulatory hurdles associated with using MSCs as an adjuvant therapy for human islet transplantation. STEM CELLS TRANSLATIONAL MEDICINE 2018;7:559–563

SIGNIFICANCE STATEMENT

This concise review considers some of the current issues with islet transplantation as a therapy for type 1 diabetes and how using mesenchymal stromal cells as an adjuvant therapy may improve transplantation outcomes. The review focuses on the possibility of using MSC-derived secretory products as a cell-free approach to improving islet graft survival and function.

INTRODUCTION

Islet Transplantation and Type 1 Diabetes

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterized by hyperglycemia caused by insulin deficiency due to the selective destruction of β-cells within pancreatic islets of Langerhans. People with T1DM are dependent on the administration of exogenous insulin to survive, either by (multiple) daily injection or infusion. However, sporadic administration of exogenous insulin often fails to maintain tight glycemic control, such that hyperglycemic and hypoglycemic excursions are common, both of which are associated with devastating side effects [1, 2]. β-cell replacement offers the potential for providing physiological glycemic control, so avoiding hyperglycemic and hypoglycemic episodes. Whole pancreas transplantation is surgically invasive and associated with significant comorbidity [3] whereas transplantation of the endocrine islets, which comprise only 2%–3% of the total pancreas, is a safe procedure with little or no comorbidity [4]. Transplantation of isolated islets as a therapy for T1DM is therefore an attractive therapeutic option.

The UK Islet Transplant Consortium (UKITC) was established in 2000 and the UK National Health Service established the first government-funded islet transplant service focused on people with T1DM with severe hypoglycemia unawareness. Worldwide, over 1,500 people with T1DM have now received intraportal islet transplantation in 40 centers (https://citregistry.org/content/citr-9th-annual-report). Islet transplantation outcomes have improved year-on-year [5, 6], with recent reports that approximately 50% of graft recipients remain insulin independent at 5 years and more, which places the success rate of islet transplantation on par with that of whole pancreas transplantation [7].

Current Issues with Islet Transplantation

The availability of islet transplantation as a therapeutic option to a wider population of people with T1DM is severely limited by a shortage of tissue donors. Current isolation techniques recover only around half of the islets from a pancreas, so a single transplantation requires multiple donors. The scarcity of donor islets is exacerbated by the loss of functional islets

during the islet isolation and preimplantation culture period due to ischemia, physical and oxidative stresses, and the deleterious effects of inflammatory cytokines. Culturing human islets for 24–72 hours prior to transplantation has been adopted by most islet transplant centers. This allows for the initiation of time-dependent immunosuppressive regimens in the graft recipient and enables quality control testing of the donor islets and their shipment to distant transplantation centers. However, maintaining islet viability after isolation remains challenging. It is well documented that islets deteriorate rapidly during culture [8, 9], with reported average losses of 20% of the islet cell mass after 20 hours, and occasional losses of >50% of the islet cell mass resulting in the cancellation of the planned transplantation procedure [10]. There is also convincing evidence that a substantial proportion of the functional islet graft (up to 80%) is lost in the immediate post-transplantation period (24–48 hours), because of deleterious responses of transplanted islet cells to a hypoxic, inflammatory, immunogenic host environment [11]. Strategies that improve the functional survival of islets both before and after transplantation will improve the outcome of individual grafts and also enable the limited pool of donor islets to treat many more people with T1DM by avoiding the current clinical practice of administering multiple grafts to achieve normoglycemia [5]. Genetic manipulation strategies have been reported to improve the survival and/or function of transplanted islets [12], but this is unlikely to be acceptable in clinical-grade human transplant material, whereas autologous, cell-based treatments are clinically attractive. One emerging strategy is the use of mesenchymal stromal cells (MSCs) to take advantage of their anti-inflammatory, immunoregulatory, angiogenic, and regenerative properties. This Perspective will focus on the potential of MSC-derived soluble mediators to improve transplantation outcomes primarily by influencing graft function rather than affecting the host environment.

MESENCHYMAL STROMAL CELLS

The International Society for Cellular Therapy has designated the term "multipotent MSCs" to refer to cells often referred to previously as mesenchymal stem cells, which can be isolated from bone marrow and many other tissues including adipose, placenta, cord blood, and the ocular limbus [13]. To define cells as MSCs, they must meet three main criteria [14]. First, cells must be plastic-adherent when maintained in standard culture conditions. Second, ≥95% of cells must express CD105, CD73, and CD90, while they must lack hematopoietic antigen expression that includes CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA class II. Finally, MSCs must be capable of trilineage differentiation potential into osteoblasts, adipocytes, and chondroblasts under the standard differentiation conditions in vitro.

MSCs AND ISLET TRANSPLANTATION

MSCs are currently being investigated as adjuvants to improve the outcomes of islet transplantation. It is well established from studies in other tissues that MSCs play a major role in tissue repair by migrating to the site of injury and depositing extracellular matrix (ECM), which acts as a repair scaffold and as a reservoir for a wide range of MSC-derived biologically active molecules with anti-inflammatory, immunomodulatory,
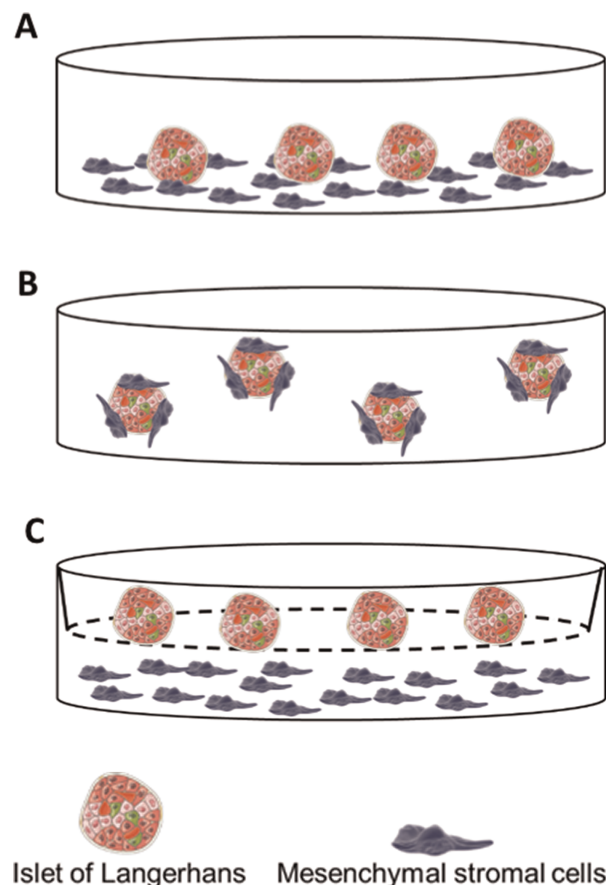
Figure 1. Different coculture systems for islets and mesenchymal stromal cells (MSCs). **(A):** Direct contact coculture system [18], where MSCs are seeded in adherent monolayers in a treated tissue culture dish and islets are placed in direct contact on the layer of MSCs. **(B):** Direct contact coculture system [26], where MSCs are seeded in a nontreated culture and maintained in suspension culture with islets. **(C):** Indirect coculture system [27]; in this system, MSCs are seeded as adherent monolayers into the bottom of a transwell treated culture dish and islets are placed into the insert in the upper compartment of the well.

and angiogenic properties [11–13], all of which might be predicted to enhance the functional survival of islet grafts.

Studies in rodent models of diabetes have demonstrated that cotransplanting MSCs with islets improves the outcome of islet transplantation in terms of glycemic control [15–20]. These beneficial effects have been attributed to the ability of MSCs to enhance revascularization [18, 21] or to suppress the host immune responses to the graft [22–24]. However, MSCs also improve transplantation outcomes of syngenic grafts [17], which do not provoke an immune response, and in microencapsulated islet grafts [18], which do not revascularize, suggesting that MSCs improve graft outomes via multiple mechanisms.

Many of these in vivo islet transplantation studies use the renal subcapsular graft site because it is surgically accessible, it facilitates the anatomical colocalization of islets and MSCs, and it enables surgical retrieval of the graft material (and thus reversion to hyperglycemia) by unilateral nephrectomy. However, clinical islet transplantation is almost exclusively via the hepatic portal vein, which does not facilitate coengraftment of islets and MSCs. Thus, after intraportal delivery the islets

Table 1. Proposed mechanisms of action for MSCs to improve islet function

Author/year	MSCs	Proposed underlying mechanisms modulating islet functional viability
Arzouni et al. [36]	Human adipose-derived MSCs	Annexin A1 MSC-derived extracellular matrix
Lavoie et al. [32]	Human bone marrow -derived MSCs	EMILIN-1 ILK-1
Rackham et al. [37]	Mouse adipose-derived MSC	Annexin A1
Yamada et al. [31]	Human adipose-derived MSCs	VEGF
Bell et al. [33]	Human umbilical cord blood/ Bone Marrow-derived MSCs	Matrix metalloproteases TGF-beta Epidermal growth factor receptor-activating ligands
Park et al. [18, 38]	Human umbilical cord blood-derived MSCs	IL-6 Hepatocyte growth factor TGF-beta VEGF-A
Karaoz et al. [39]	Rat bone marrow-derived MSCs	IL-6 TGF-beta1 Fibronectin Phosphoprotein1 (SSP1)

Abbreviations: IL-6, interleukin-6; ILK-1, integrin-linked protein kinase; MSCs, mesenchymal stromal cells; TGF-beta, transforming growth factor-beta; VEGF, vascular endothelial growth factor.

(100–200 μm diameter) lodge in the hepatic microcirculation where they revascularize, while the much smaller MSCs (15–30 μm) pass through the liver and most likely end up in the lung microcirculation [25]. Attempts to colocalize islets and MSCs by generating islet:MSC composites have shown limited efficacy in vitro with no significant improvement in the in vivo transplantation outcomes in diabetic mice [26].

An alternative strategy to cotransplanting islets and MSCs is a pretransplantation coculture period in vitro to enhance islet functional survival before their subsequent engraftment, and several different configurations have been used to investigate the functional effects of coculture, as shown in Figure 1. Direct contact coculture of islets with MSCs is consistently reported to improve glucose-stimulated insulin secretion (GSIS), whether configured as monolayer MSCs, or in suspension culture as composites [27–30]. However, discrepancies in the effect of MSCs on islet function have been observed in studies using indirect, noncontact coculture systems (Fig. 1), with some reporting MSC-dependent improvements in GSIS [18, 31], and others reporting no effects [32–34]. The efficacy of direct contact coculture strategies may be due, at least in part, to the ability of MSCs to secrete abundant amounts of ECM [35] which interacts with localized islet cells. Thus, elastin microfibril interface 1 (EMILIN-1) and integrin-linked protein kinase (ILK-1) were reported to be highly expressed in MSCs populations with islet regenerative capabilities [32], implicating ECM-integrin interactions in mediating the beneficial effects of MSCs on islet function. Consistent with this, MSCs which support islet function secrete protein matrix metalloproteases (MMP1 and MMP13) that are involved in ECM remodeling [33], and coculture of mouse and human islets with decellularized MSC-derived ECM is alone sufficient to improve GSIS [24].

The ECM secreted by MSCs acts not only as a physical scaffold but also as a reservoir for numerous biologically active molecules to influence the function and regeneration of the surrounding tissues [34]. Mass spectrometry analysis of human bone marrow MSC-derived ECM [34] has identified a wide range of secretory products, including growth factors and anti-inflammatory molecules, which reinforces the notion of MSC-derived ECM acting as a "mobile drug dispensary" for tissue regeneration. Indeed, many of the factors implicated in the beneficial effects on islets are associated with MSC-derived ECM [34] (Table 1). This, in turn, raises the possibility of using MSC-derived molecules (Table 1) to improve islet graft function without the requirement for delivering MSCs as part of the graft material. We have recently used a nonbiased quantitative reverse transcription polymerase chain reaction (qRT-PCR) strategy to screen MSC populations for the expression of peptide and protein ligands of G-protein-coupled receptors known to be expressed by islet β-cells [40]. The anti-inflammatory molecule annexin A1 (ANXA1) was identified as a highly expressed secretory product in both mouse [37] and human [36] MSC populations, and has been localized to MSC-derived ECM [36]. Subsequent functional studies demonstrated that preculturing islets with exogenous ANXA1 improved GSIS in vitro [24, 27], protected islet cells against cytokine-induced apoptosis [37], and improved their ability to regulate glycemia in diabetic mice [37]. However, ANXA1 is not the sole mechanism through which MSCs influence islet function since genetic deletion or siRNA-induced knockdown of ANXA1 in MSCs impaired, but did not completely abolish, their capacity to enhance islet function [37]. Two other molecules which are highly expressed by MSCs—stromal cell-derived factor1/C-X-C motif chemokine 12 (SDF-1/CXCL12) and collagen type III alpha 1 (Col III A1)—are also reported to have beneficial effects on islet function [41], and CXCL12 has been reported to enhance islet graft survival and function in a mouse model of diabetes [42]. This raises the possibility of defining a "cocktail" of MSC-derived molecules which mimics the beneficial effects of MSC coculture on islet function, and which therefore offers the prospect of cell-free treatment of islet populations during the isolation and pretransplantation culture periods.

TRANSLATION TO CLINICAL HUMAN ISLET TRANSPLANTATION PROTOCOLS

The use of a precisely-defined cocktail of MSC-derived products could overcome many of the technical hurdles of cellular therapy, such as upscaling MSC production while maintaining a stable phenotype, quality control between different MSC populations, the establishment of reliable safety and efficacy profiles, and the challenges of in vitro coculture of MSCs and human islets on the scale required for clinical transplantation. MSC-derived products can be manufactured in GMP conditions, and evaluated for safety, dosage, and potency in a manner similar to that applied to current pharmaceuticals. Indeed, several of these MSC biotherapeutic molecules and/or their receptors are currently under investigation for their therapeutic potential in other clinical situations [43–45].

A cell-free "cocktail" treatment of islets prior to transplantation may also offer regulatory advantages over cell-based adjuvant therapy. In the U.K., human islets for clinical transplantation are covered by the Tissues and Cells Directive (2004/23/EC) and are considered as graft material rather than medicinal products by the Human Tissue Authority, so are exempt from the stringent regulations covering new medicinal products. If, as seems likely, pretreating islets with MSC biotherapeutic molecules is maintaining their functional viability rather than inducing any fundamental modification, they will continue to be classified as graft material rather than a medicinal product. Thus, for example, the biotherapeutic cocktail could be used as a supplement to the islet culture medium in an analagous manner to the inclusion of human serum albumin which is beneficial for islet survival since it neutralizes endogenous pancreatic enzymes [46]. Translation of current experimental studies into modifications to clinical human islet transplantation protocols may therefore be relatively straightforward in regulatory terms.

CONCLUSION

There is a convincing body of experimental evidence demonstrating that MSCs can exert beneficial effects on the outcomes of islet transplantation as a therapy for T1DM, most likely through a number of different mechanisms involving MSC-derived molecules having direct effects on the islet graft to improve its functional survival and on the host environment to suppress inflammatory and immune responses to the graft. The MSC-dependent effects on the graft material may be mimicked, to some extent, by defined modifications to pretransplantation culture protocols, offering a simple and effective means of improving the cinical outcomes of islet transplantation.

AUTHOR CONTRIBUTIONS

A.A.A., A.V.-S. and P.M.J. wrote the manuscript. N.N. and A.J.F.K., edited the manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

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