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Multipotent Mesenchymal Stromal Cells Interact and Support Islet of Langerhans Viability and Function

Naomi Koehler[1], *Leo Buhler*[2], *Bernhard Egger*[1,2] *and Carmen Gonelle-Gispert*[1]*

[1] Surgical Research Unit, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland, [2] Department of Surgery, Cantonal Hospital Fribourg, Fribourg, Switzerland

Type 1 diabetes (T1D) is a widespread disease, affecting approximately 41.5 million people worldwide. It is generally treated with exogenous insulin, maintaining physiological blood glucose levels but also leading to long-term therapeutic complications. Pancreatic islet cell transplantation offers a potential alternative treatment to insulin injections. Shortage of human organ donors has raised the interest for porcine islet xenotransplantation. Neonatal porcine islets are highly available, can proliferate and mature *in vitro* as well as after transplantation *in vivo*. Despite promising preclinical results, delayed insulin secretion caused by immaturity and immunogenicity of the neonatal porcine islets remains a challenge for their clinical application. Multipotent mesenchymal stromal cells (MSCs) are known to have pro-angiogenic, anti-inflammatory and immunomodulatory effects. The current state of research emphasizes the great potential of co-culture and co-transplantation of islet cells with MSCs. Studies have shown enhanced islet proliferation and maturation, insulin secretion and graft survival, resulting in an improved graft outcome. This review summarizes the immunomodulatory and anti-inflammatory properties of MSC in the context of islet transplantation.

Keywords: MSC, neonatal porcine islets, diabetes, cell encapsulation, xenotransplantion

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****Correspondence:***
Carmen Gonelle-Gispert
carmen.gonelle@unifr.ch

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INTRODUCTION

Human pancreatic islet transplantation through portal vein infusion, is a current clinical beta-cell replacement therapy to treat patients with advanced Type I Diabetes (T1D). However, live-long immunosuppression, difficulties to achieve long-term islet graft function and insulin independence as well as the shortage of suitable pancreata from heart-beating brain-dead donors for islet isolation, are still important limitations for ongoing allo-transplantation programs.

Pig islet xenotransplantation is a promising alternative to overcome the bottleneck of islet availability for the treatment of T1D. However, clinical application of pig to human islet transplantation will depend on genetic engineering of pigs to overcome immune barriers and to reduce risks of pathogen infection of porcine viruses (1). Recently, significant progress has been achieved with the transplantation of pig organs presenting several genomic modifications to prevent hyperacute rejection (2, 3) and cellular immune responses (4, 5). In immunosuppressed nonhuman

primates, long-term control of diabetes by the transplantation of adult porcine islets had been successfully achieved (6, 7). Other strategies to protect porcine islets from the host immune system include islet encapsulation in semi-permeable hydrogel, such as alginate (8) functionalized by bioactive ligands or by poly(ethylene glycol) (PEG) derivatives (9, 10). Mesenchymal stem cells (MSCs) are multipotent cells and play an important role in tissue repair, angiogenesis and their immunomodulatory action on immune cells have been widely studied (11, 12). In the field of islet transplantation MSC are investigated for the improvement of islet function and graft survival after transplantation. Numerous studies of co-culture and co-transplantation with MSCs indicate a functional support. However, due to variable transplantation settings and origins of MSCs the immunomodulatory role, as well as their ability to reduce inflammatory processes *in vivo* remains controversial. This review summarizes the immunomodulatory and anti-inflammatory properties of MSC in the context of islet transplantation and evokes some of the current challenges of islet xenotransplantation.

MULTIPOTENT MESENCHYMAL STROMAL CELLS (MSCs), ALSO CALLED MESENCHYMAL STEM CELLS

Multipotent mesenchymal stromal cells (MSCs) are self-renewing multipotential progenitor cells, differentiating along the osteogenic, chondrogenic and adipogenic lineages (13). MSCs have first been isolated from the bone marrow over 50 years ago and bone marrow-derived MSCs (BM-MSCs) still represent the most conventional source. A variety of other tissues also contain MSCs, including adipose tissue, umbilical cord blood, Wharton's jelly, amniotic fluid, endometrium, skin and skeletal muscle (14–22). It remains unknown which source is most suitable for the clinical use in the context of islet cell transplantation and further research is needed concerning this matter.

To facilitate isolation and expansion as well as to standardize characterization, the International Society for Cellular Therapy (ISCT) proposed three minimal criteria for defining mesenchymal stem cells. First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules. Third, MSCs must differentiate to osteoblasts, adipocytes and chondroblasts *in vitro* (23). MSCs have been shown to perform various beneficial functions, making them highly interesting for application in cell-based therapy, especially also for islet transplantation.

MSCs SUSTAIN ANGIOGENESIS

One major limitation of islet graft survival is a delayed revascularization after transplantation. After isolation, islet cells are cut off from their oxygenation *via* micro-vascularization and are temporarily dependent after transplantation on diffusion of nutrients and oxygen in order to ensure survival. Neovascularization is finalized after approximately two weeks, however with a lower capillary density and a significantly reduced perfusion compared to islets prior to transplantation (24). Further remodeling takes up to another three months (16).

Several studies have shown a pro-angiogenic potential of MSCs. MSCs promote angiogenesis through expression and release of different pro-angiogenic cytokines, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor beta (TGF-β), as well as annexin 1 (ANXA1), matrix metalloproteinase (MMP) and Angiopoietin-1 (Ang-1) (16). The impact of VEGF is contentious, showing not only beneficial proangiogenic but also damaging proinflammatory effects (16). MSCs seem to keep a balance through its valuable anti-inflammatory property, discussed later. Kinnaird et al. demonstrated further that co-culturing islets with MSCs enhanced neovascularization of islets through promotion of proliferation and migration of endothelial and smooth muscle cells (25).

Also, other studies showed that co-transplantation of islets with MSCs improves graft survival and function by increased neovascularization, shortening the post-transplantation ischemia period (26–28).

IMMUNOMODULATORY PROPERTIES OF MSCs

MSCs do not express co-stimulatory molecules that activate the immune system, such as CD40, CD80 or CD86 (29). Originally, it was thought that MSCs express only low or no human leukocyte antigen (HLA) class I and II molecules. It has since then been demonstrated that MSCs, like all somatic tissues, express MHC class I molecules constitutively and have the ability to express MHC class II when exposed to inflammatory cues such as interferon-γ (30). *In vitro* studies showed that, attracted by a number of complement proteins, growth factors, proinflammatory cytokines and chemokines, MSCs migrate towards sites of inflammation supporting the hypothesis that MSCs possess anti-inflammatory properties (17, 31, 32). MSCs can express potent inhibitory molecules of both, innate and adaptive immune effectors (33), however, after transplantation, this may not allow to circumvent acquired alloimmunization, as observed in human trials (30). Nevertheless, immediate events such as acute toxicity associated with the administration of MSCs have not been described (34, 35).

MSCs have also shown to exert an immunomodulatory effect through phenotype-alteration of different immune cells, including dendritic cells (DC), T- and B-cells, as well as natural killer cells (NK cells) (36). Several authors have described an inhibitory effect of MSCs on immune cell proliferation, generating an immunosuppressive local milieu (16, 37–40). Research has further shown that MSCs induce modifications of the adaptive immune system, notably T-cells,

entailing T-cell anergy. MSCs act on T-cells through physically hindering contact with antigen-presenting cells (APCs) (41) or by an indirect suppression of T-cell activation *via* MSCs by hindering the maturation of DCs through cell-to-cell contact. These semi-mature DCs possess a tolerogenic phenotype, thus restraining T-cell activation (42). Also, MSCs are able to inhibit T-cell reactivity through the downregulation of proinflammatory cytokines (37, 43) and to escape cytotoxic T-cell-mediated apoptosis (44, 45). Importantly, MSCs inhibit T-lymphocyte proliferation through soluble factors, such as TGF-β1 and HGF (41, 46) or nitric oxide (47). TGF-β1 plays a well-documented role in MSCs immunomodulation, including a role in regulatory T cell (Treg) induction and/or expansion (48–50). MSCs promote the expression of regulatory T-cells (Treg) (43, 51). Early studies showed that stable islet allograft function in cynomolgus monkey was associated with increased numbers of regulatory T-cells in peripheral blood (43). Further, when co-transplanted with allogeneic islets in diabetic cynomolgus monkeys, MSCs derived from islet recipient were more efficient to prolong islet survival, when compared with 3rd party MSCs or islet derived MSCs from the donor. Using recipient-derived MSCs, they observed decreased number of memory T cells, reduced anti-donor T cell proliferation and higher Treg:T cell ratios (52).

CO-TRANSPLANTATION OF ISLETS WITH MSCs FROM DIFFERENT SOURCES

Various possible sources of MSCs have been tested for co-transplantation with islet cells so far.

In murine models, several studies showed improved and prolonged graft survival, function, morphology and revascularization, as well as induction of beta cell proliferation following transplantation of murine islets with autologous (53), syngeneic (27, 54, 55), allogeneic (27, 56–59) or xenogeneic MSCs (60). In mice, co-transplantation of autologous MSCs delayed islet allograft rejection and generated a local immune-privileged site in mice (53). Rackham et al. studied the effects of co-transplantation of syngeneic murine MSCs and islet cells, and observed an improved graft outcome (54). In a subsequent study they examined the underlying factors, suggesting Annexin A1 to be a key contributor to the improved graft function through direct and indirect mechanisms (61). The exact mechanisms remain unclear.

Co-transplantation of islets with MSCs in syngeneic rodent models showed better outcomes of islet survival and function than islets transplanted alone (26, 27, 62). Karaoz et al. described an improved islet function after co-culturing allogenic rat MSCs and islet cells, suggesting paracrine actions through IL-6, TGF-β1, osteopontin and fibronectin (59). Further, allogeneic MSCs resulted in improved islet xenograft survival and function in immune-competent diabetic mice (63). In cynomolgus monkeys, intraportal co-infusion of allogenic MSCs and islets, increased islet engraftment and function, shown by a reduced number of islets necessary to reach normoglycemia (43).

Co-encapsulation studies using islets versus islets and MSC also showed beneficial effects on islet function (64–66). Intraperitoneally syngeneic transplantation of co-encapsulated islets and MSCs showed significantly lower glycaemia compared to islets encapsulated alone. By week 6, 71% of mice transplanted with islets and MSCs were cured, whereas only 16% of the islets-alone group was cured at that point. Interestingly, islet area in recovered capsules was significantly higher when co-encapsulated with MSCs suggesting that MSCs promote survival of islet cells independently from its effects on revascularization. In this study co-encapsulation of islets with MSC did not inhibit pericapsular fibrotic overgrowth (PFO), suggesting that MSCs have no influence on the inflammatory process that causes fibrotic overgrowth (64). PFO is an inflammatory host reaction, induced through the leakage of antigens from semi-permeable microcapsules, that severely impairs islet viability and graft function. However, in a mouse model of islet allotransplantation, co-encapsulation of MSCs (stimulated or not with a cocktail of pro-inflammatory cytokines) with islets in alginate microcapsules, prevented pericapsular fibrotic overgrowth (PFO) compared to islets encapsulated alone (66). Further mice receiving islets co-encapsulated with stimulated and unstimulated MSC achieved higher percentages of normoglycemic mice (100% versus 71.4%, respectively) compared to mice transplanted with islets encapsulated alone (9.1%). Similarly, *in vitro* rat MSCs and rat islet cells when co-encapsulated in a ligand-functionalized polyethylene glycol (PEG) hydrogel (67) led to a doubling of the stimulation index compared to islets encapsulated alone. Co-encapsulation of islet cells and MSCs in addition with cell adhesion peptides led to a significant sevenfold increase of the stimulation index compared to islets encapsulated alone (67).

Human islets co-cultured in direct cell contact with human MSCs compared to islets co-cultured with MSCs but without cell-to-cell contact, displayed significantly enhanced insulin secretion in the presence of cell-to-cell contact. This effect was identified to be dependent on N-cadherin interaction, since impeding N-cadherin interaction with antibodies led to a reversal of the enhanced insulin secretion. Additionally, mice transplanted intraperitoneally with human islets co-encapsulated with MSCs in hydrogel microspheres, composed of calcium alginate and covalently crosslinked to polyethylene glycol showed significantly lower blood glucose levels and prolonged islet graft survival (57). Others have shown that improved graft function correlates with enhanced revascularization of islets transplanted under the kidney capsule (68–70). Accordingly, research findings revealed significantly higher apoptosis rates in islet cells cultured without MSCs (16).

Taken altogether, these findings support the hypothesis that co-transplantation of MSCs and islet cells is beneficial and that MSC**s** are useful for future therapeutic applications.

IMPROVED NEONATAL PORCINE ISLET FUNCTION, SURVIVAL AND GRAFT OUTCOME

The main disadvantage of neonatal or juvenile porcine islets, also called porcine pancreatic islet cell clusters (ICCs), is their lack of integrity and maturity. ICCs are obtained by *in vitro* digestion of neonatal or juvenile pig pancreas with subsequent short time culture in a specific maturation media to increase the beta cell mass for transplantation (**Figure 1**) (71). Also, porcine pancreatic ICC co-transplanted with human MSC into immune deficient diabetic mice reached normoglycemia significantly earlier than mice transplanted with ICC alone (60).

He et al. demonstrated an improved and accelerated development of ICCs in diabetic rhesus monkeys after co-transplantation with allogeneic simian MSCs into diabetic rhesus monkeys (72). Additionally, the group described an enhanced expression of genes implicated in the development of endocrine cells and insulin and further demonstrated enhanced expression and activation of PDGFR-α in neonatal islets through MSCs confirming earlier studies demonstrating the capability of PDGFR-α to stimulate beta-cell proliferation (73). Further, He et al. suggest an inhibition of the Notch1 signaling provoked by PDGFR-α, leading to an improved islet development and maturation. It is known that Notch1 downregulates the expression of several genes and transcription factors implicated in the development of endocrine cells and insulin (72, 74). Juvenile porcine exocrine pancreas-derived MSCs (pMSCs) co-cultured with direct cell to cell contact of juvenile porcine ICCs significantly enhanced beta-cell function, suggesting that cell signaling *via* adhesion molecules are important (57, 65). However, co-encapsulation of such ICCs with pMSCs do not effectively prevent PFO and graft survival was rapidly impaired after transplantation of capsules in immunocompetent mice. Therefore, further research is required to enable efficient long-term survival of encapsulated juvenile porcine islets. Possible approaches being evaluation of modified alginate chemical composition (75) or the use of different anti-fibrotic polymers (65).

IMMUNOMODULATION STRATEGIES TO INCREASE XENOGRAFT SURVIVAL

To overcome the immunological barrier between pig and humans, genetic modifications have been performed in pig strains to reduce immunogenicity of organs and tissue. The first genetically modified pig, i.e. with a single human transgene for a complement regulating gene (hDAF), allowed survival of pig organs in immunosuppressed non-human primates for several months. Since then, genetic engineering, using CRISPR-CAS9, allowed cloning of animals with additional genetic modifications. Today, pigs with over 10 genetic modifications, both, deletions of pig antigens and inclusions of human transgenes are under investigation for transplantation purposes (76). Immunosuppressive regimens are still necessary but recently heart transplantation from genetically modified pigs [α1,3-galactosyltransferase-knockout and knock in human CD46 (77, 78) and thrombomodulin (79)] to a non-human primate (baboon) reached long term survival of 195 days (80).

Furthermore, immunoregulatory therapies (tolerance induction) using Treg-based therapeutic approaches are under investigation. Regulatory T cells (Tregs) are immune-suppressive T cells that are critical for the maintenance of tolerance *in vivo* (81). Chimeric antigen receptors (CARs) are synthetic fusion proteins that have been developed to genetically modify T cells in order to create a specificity toward designated antigens. The application of the CAR technology to Tregs, may allow to reduce immune responses for solid organ and cell transplantation. CAR Treg therapies are currently developed using genetic modifications for xenogenic pig antigens with the aim to improve graft acceptance of xenotransplanted tissue i.e. porcine islets. This might be achieved through infusion of *ex vivo* expansion of donor-specific Tregs (55, 82).

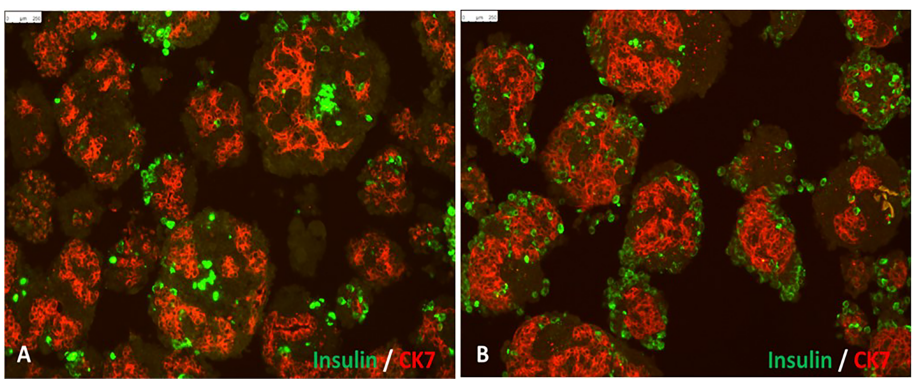

FIGURE 1 | *In vitro* differentiation of isolated porcine pancreatic islet cell clusters: Panel **(A)** shows cell clusters containing insulin-positive beta cells (green) and CK7-positive pancreatic exocrine tissue (red) at 3 days after isolation. Panel **(B)** shows pancreatic islet cell clusters 7 days after culture in neonatal pig islet differentiation media (scale bar represents 250 μm).

CAR-Tregs technology started with a study of MacDonald and colleagues which successfully transduced human Tregs with a CAR targeting the human leukocyte antigen (HLA) class I-A2 (A2-CAR) (83). In a human skin xenograft transplant model, HLA class I-A2 specific CAR-Tregs alleviated rejection of skin transplants (84). Since co-transplantation of autologous MSC delayed islet allograft rejection, it is possible that genetically modified, MSC, could be exploited as a target cell in porcine ICC xenografts to foster islet function and to increase trafficking and activation of adoptive transferred CAR-Treg cells to increase tolerance toward pig ICC xenografts.

An additional challenge for islet transplantation is the precise quantification of beta cell mass (BCM) or endocrine cell mass (ECM) *in vivo*. Imaging the progressive loss of beta cells following islet transplantation should allow the development of individualized therapies for the management of patients post-transplant (85). Recently, a suitable biomarker for beta cell quantification, the dipeptidyl aminopeptidase-like protein 6 (DPP6) has been identified as a promising target for human BCM imaging in healthy individuals as well as diabetic patients (86, 87). First imaging and biodistribution studies using SPECT/CT and radiolabeled high-affinity camelid single-domain antibody (nanobody) directed specifically against human DPP6, allowed to visualize transplanted DPP6-expressing Kelly neuroblastoma cells or insulin-producing human EndoC-βH1 cells in immunodeficient mice. Importantly, neonatal pig islets expressing near-infrared fluorescent protein (iRFP) were non-invasively monitored through multispectral optoacoustic tomography (MSOT). MSOT signals, obtained after islet transplantation under the kidney capsule in mice, and obtained after subcutaneous and intramuscular islet transplantation in pigs, allowed to distinguish graft mass changes (88). Such reporter gene-expressing islets are also promising tools to evaluate the efficacy of newly developed biomaterials for encapsulation and transplantation of porcine islets.

CONCLUSION

Diabetes is a worldwide disease, affecting over 40 million people and putting an important burden on the healthcare system. Exogenous insulin represents the predominant treatment modality for type 1 diabetes, but is associated with long-term complications. Islet cell transplantation is a highly promising approach for treating type 1 diabetes aiming at reestablishing a physiological insulin secretion through replacement of the endocrine tissue. Despite improving preclinical and clinical results over the past decades, the need for immunosuppression and donor shortage limits the clinical application of this procedure. The implementation of porcine pancreatic ICCs with porcine MSC might represent a promising alternative to help to overcome the problem of donor shortage; especially neonatal or juvenile pigs providing high islet yields. Encapsulation techniques could resolve the need for immunosuppression, shielding the islets from immune attacks while still enabling the exchange of oxygen, insulin and nutrients. Yet, delayed and impaired graft outcome due to immature islet cells and the formation of pericapsular fibrosis continue to severely limit the clinical application of encapsulated islet transplantation.

AUTHOR CONTRIBUTIONS

NK: designed and wrote the manuscript; LB contributed to manuscript writing; LB and BE: critically revised the manuscript; CGG: designed, contributed to manuscript writing; supervised the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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