Accessory cells for beta cell transplantation

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ABSTRACT

Despite recent advances, insulin therapy remains a treatment, not a cure, for diabetes mellitus with persistent risk of glycemic alterations and life-threatening complications. Restoration of the endogenous beta cell mass through regeneration or transplantation offers an attractive alternative. Unfortunately, signals that drive beta cell regeneration remain enigmatic and beta cell replacement therapy still faces major hurdles that prevent its widespread application. Co-transplantation of accessory non-islet cells with islet cells has been shown to improve the outcome of experimental islet transplantation. This review will highlight current travails in beta cell therapy and focuses on the potential benefits of accessory cells for islet transplantation in diabetes.

ABBREVIATIONS

ASCs: adipose-derived stem cells
BOECs: blood outgrowth endothelial cells
CCL: chemokine (C-C motif) ligand
CGM: continuous glucose monitoring
DCs: dendritic cells
EC: endothelial cell
E(P)Cs: endothelial (progenitor) cells
FasL: Fas ligand
FGF: fibroblast growth factor
GAD: glutamic acid decarboxylase
GM-CSF: granulocyte-macrophage colony stimulating factor
HepSCs: Hepatic stellate cells
HGF: hepatocyte growth factor
IBMIR: instant blood-mediated inflammatory reaction
IL: interleukin
iNOS: inducible nitric oxide synthase
KDR: kinase insert domain receptor
MDSCs: myeloid-derived suppressor cells
MHC: major histocompatibility complex
MMP: matrix metalloproteinase
MSCs: mesenchymal stem cells
NCSCs: neural crest stem cells
NOD: non-obese diabetic
RA: retinoic acid
T1D: type 1 diabetes
T2D: type 2 diabetes
TGF: transforming growth factor
tol-DCs: tolerogenic dendritic cells
(i/n)Tregs: (inducible/natural) regulatory T cells
Tsp: thrombospondin
VEGF: vascular endothelial growth factor

I. INTRODUCTION

Although crucial for diabetes management, multiple daily insulin injections and systematic blood glucose monitoring impose a major burden on the quality of life of patients with diabetes mellitus. Despite therapeutic advances such as the use of insulin analogues, continuous glucose monitoring (CGM) systems and, more recently, closed loop devices or “artificial pancreases”, insulin therapy often fails to adequately prevent secondary complications while carrying an inherent risk of life-threatening hypoglycemia. Strategies aimed at restoring the beta cell mass via transplantation or at protection of residual and/or formation of new beta cells (regeneration) should thus be further investigated.

Since several decades, beta cell replacement therapy has been applied to brittle type 1 diabetes (T1D) patients, resulting in an improvement of glycemic control and of micro- and macrovascular complications (1, 2). Advances in islet isolation procedures and in immune suppressive regimens have substantially improved the outcome of clinical islet transplantation to approximate that of whole organ pancreas transplantation (reviewed in (3)). Despite these advances, major hurdles still need to be overcome for islet transplantation to become a universal treatment for T1D and, possibly, T2D. Several pre- and post-transplantation approaches such as alternative islet cell sources and transplantation sites as well as novel immunosuppressives and graft encapsulation, have already been preclinically and clinically explored to improve the outcome of islet transplantation (summarized in **Figure 1** and comprehensively reviewed in (4-6)). Here, we elaborate on the benefits of co-engrafting accessory nonislet cells with islet cells to prevent post-transplant islet graft loss and dysfunction. Thanks to their pleiotropic effects, accessory cells might prove to be superior compared to genetic or protein-based approaches that often target only a single component of islet graft failure.

II. POST-TRANSPLANT ISLET GRAFT LOSS AND DYSFUNCTION

The first few days after islet transplantation are characterized by dynamic changes, with substantial islet cell death and dysfunction preceding tissue remodeling and stable engraftment (7). In mouse models, approximately 60% of syngeneic transplanted islet tissue is lost by beta cell apoptosis and necrosis during the first 3 days after transplantation (8). In man, quantification of early beta cell loss after transplantation remains difficult due to a lack of appropriate markers. Although further standardization is needed, a beta cell loss varying from 5 to 47% after intra-portal transplantation was recently estimated based on plasma glutamic acid decarboxylase 65 (GAD65) levels (9). However, this number might still underestimate the actual loss since only a fraction of GAD65 from damaged or dead beta cells is likely to end up in the circulation (9). To compensate for this loss, an excessively high number of human donor islets needs to be transplanted (10), further depleting the already scarce pool of donor tissue. Factors that have been implicated in graft loss after intraportal transplantation include: (i) insufficient graft revascularization (11) and reinnervation (12), (ii) an inflammatory reaction following intra-vascular islet infusion (a.k.a. “instant blood-mediated inflammatory response” (IBMIR)) (13), (iii) alloimmune rejection and recurrence/persistence of autoimmunity (14), (iv) toxicity of the immunosuppressive regimens (15), (v) gluco- and lipotoxicity (16, 17), (vi) amyloid formation (18) and (vii) liver ischemia with subsequent cytotoxicity (19). Angiogenic, graft-supporting and immunomodulatory properties of accessory cells including stem cells and an armamentarium of differentiated nonislet cell types, have been exploited to tackle some of these obstacles and improve islet transplantation outcome. An overview of the results obtained so far (summarized in **Table 1**) as well as the putative mechanism of action of each accessory cell type (summarized in **Table 2**) will be discussed in the following section.

III. ACCESSORY CELLS TO PREVENT ISLET GRAFT FAILURE

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent, self-renewing cells capable of differentiation into cells of mesodermal origin. They are found in the perivascular space and can be isolated from virtually every adult organ and tissue. In the bone marrow, MSCs regulate maintenance and proliferation of hematopoietic stem cells. MSCs are the most extensively studied accessory cell type for islet transplantation. Due to their adhesive capacity, MSCs are able to "coat" islets in culture, prior to transplantation (20). Thanks to their pro-angiogenic (21) and anti-apoptotic effects (22-24), MSCs improve islet function and survival in rodents and primates (25-33) (see Table 1). Pro-angiogenic effects of MSCs are augmented by hypoxia and result from their release of angiogenic factors and proteases that degrade the extracellular matrix, thereby promoting endothelial cell (EC) migration and growth factor bioavailability (22, 23, 34), resulting in improved graft revascularization. Furthermore, MSCs contribute to vessel stabilization through their differentiation into perivascular smooth muscle cells (“pericytes”) (35). In addition, MSCs are immunomodulatory (36), given their ability to decrease the activation and proliferation of various immune-competent cells such as natural killer cells, dendritic cells (DCs), cytotoxic T cells and B cells (37-52), to modulate neutrophil and B cell function, differentiation and chemotaxis (53, 54) and to generate regulatory T cells (Tregs) (22, 23, 47, 50, 51, 55). Finally, MSCs protect islets from destruction mediated by pro-inflammatory cytokines via their secretion of a.o. hepatocyte growth factor (HGF) (56). Although the ability of MSCs to survive, engraft and suppress immune responses in an allogeneic transplantation setting has been questioned (57-59), a recent prospective clinical trial indicates that autologous MSCs infusion arrests disease progression and preserves beta cell function in new-onset T1D patients (60). Although larger studies with extensive follow-up are required, these data support the future use of MSC in new-onset T1D and as accessory cells in islet transplantation.

Adipose-derived stem cells

MSCs derived from adipose tissue (a.k.a. adipose-derived stem cells (ASCs)) are obtained from the adipose stromal vascular fraction, a population of cells obtained after enzymatic dissociation of adipose depots followed by density separation from adipocytes (61). While ASCs are functionally similar to bone marrow MSCs, they are more easily accessible with minimal risk to the patient. This accessibility is especially attractive with regard to autologous transplantation (62). Similar to MSCs, ASCs exposed to hypoxic conditions increase their secretion of angiogenic growth factors (63-65). A number of reports indicate that co-transplantation of islets with ASCs improves islet function and survival. Subcutaneous implantation of mouse ASCs combined with minced adipose tissue gives rise to a vascular-rich bed that supports subsequent syngeneic islet transplantation and long-term reversal of diabetes in immune competent mice (66). Combined transplantation of syngeneic mouse ASCs with a marginal allogeneic islet mass results in prolonged graft survival and glucose tolerance in immune competent diabetic mice (67). Hybrid grafts had a well-preserved islet structure compared to grafts consisting of native islets alone and demonstrated increased revascularization. In addition, a decreased accumulation of $CD4^+$ and $CD8^+$ T cells and macrophages was observed, reflecting the anti-inflammatory effect of co-transplanted ASCs (67, 68). Co-transplantation of rat islets and human ASCs within a fibrin gel improves glycometabolic outcome of subcutaneous xenogeneic islet transplantation in diabetic immune deficient mice, which was further enhanced by prior inclusion of fibroblast growth factor (FGF)-2 in the fibrin gel [50]. ASCs improve islet viability by secreting growth factors that protect islets from hypoxic damage and promote neovascularization via overexpression of VEGF-A (69). Preconditioning of human ASCs with a mixture of acids (hyaluronic, butyric and retinoic acid) to increase expression/secretion of vascular endothelial growth factor (VEGF)-A, kinase insert domain receptor (KDR, a.k.a. VEGF-R2) and HGF, followed by co-culture with rat islets and intrahepatic transplantation of the mixed graft diabetic immune competent rats further improves graft function (70).

Endothelial progenitor and blood-outgrowth endothelial cells

Endothelial Progenitor Cells (EPCs) are a population of rare circulating cells with the ability to adhere to endothelium at sites of hypoxia with subsequent differentiation into endothelial cells (71). EPCs can be isolated from bone marrow, cord blood, vessel walls or peripheral blood. These beneficial effects on vasculogenesis have generated great clinical interest in EPCs to promote neovascularization at sites of hypoxia or injury as demonstrated in ischemic injury models such as myocardial infarction (72) and limb ischemia (73). Similar to MSCs/islet co-transplantation, numerous reports have shown beneficial effects on islet transplantation outcome when islets are co-transplanted with EPCs in rodent models of diabetes (74-78). Alternatively, peripheral mobilization of recipient bone marrow-derived EPCs by Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) enhances islet revascularization and engraftment in a model of syngeneic islet transplantation (79). EPC-derived effects are various and are mediated via direct differentiation into new vessels and pericytes, through secretion of paracrine factors (angiogenic and beta cell mitogenic (75)), via thrombospondin (Tsp)-1-mediated activation of transforming growth factor (TGF) beta-1, resulting in enhanced insulin secretion (80, 81) and through modulation of the expression of the beta cell gap junction protein connexin 36, a key element in coordinated beta cell function (78). In addition, after incorporation into islet endothelium, microvesicles, released from EPCs and carrying proangiogenic microRNAs, enhance syngeneic islet graft function, survival and vascularization in diabetic mice (82). Importantly, endothelial cells readily tolerate contact with blood, thereby avoiding IBMIR as shown in composite pig islet- human endothelial cell grafts in vitro (83) and after transplantation in immune deficient diabetic mice (76). Similar to MSCs, incorporation into or coating of islets by EPCs prior to transplantation may represent an improved cell therapy approach to enhance function, survival and revascularization (76).

Blood Outgrowth Endothelial Cells (BOECs) represent a late-outgrowing subtype of EPCs (84), derived from *in vitro* clonal expansion of peripheral blood mononuclear cells. Their endothelial commitment is evident from their cobblestone morphology and the presence of specific markers such as von Willebrand Factor and CD31,

combined with their capacity for *in vitro* tube formation (85). In an experimental murine model of wound healing, BOECs have been shown to improve wound revascularization and subsequent healing, both by integrating into functional vessels and providing trophic support for angiogenesis (85). Combined with their minimally invasive procurement from peripheral blood (85, 86), favoring their use in autologous transplantation protocols, these observations inspired us to employ BOECs to improve post-transplantation islet graft function. Co-transplantation of rat islets with human BOECs in diabetic immune-deficient mice resulted in a significant improvement in metabolic outcome. Moreover, BOECs recipients displayed reduced beta cell death and increases in beta cell proliferation and graft-vessel and beta cell volume. Comparable metabolic benefits were observed when using BOECs derived from a T1D patient, thereby underscoring the clinical potential of BOECs (87).

Neural crest stem cells

Neural crest stem cells (NCSCs) are self-renewing multipotent cells located in the pre-migratory neural crest that give rise to neurons (88). NCSCs play a role in beta cell differentiation by regulating beta cell mass during development (89). NCSCs produce several angiogenic and neurotrophic growth factors, including VEGF-A, glial cell line-derived neurotrophic factor and ciliary neurotrophic factor, all exerting beneficial effects on islets (90-92). In addition, NCSCs secrete matrix metalloproteinases (MMP)-2 and -9 to promote extracellular matrix-reorganization, endothelial cell migration and vascularization (93). *In vitro,* NCSCs stimulate beta cell proliferation by direct cell-cell interaction (94) and protect beta cells from cytokine-induced cell death in rodents (92). When co-transplanted with mouse pancreatic islets in diabetic immune competent mice, mouse NCSCs enhance insulin release and beta cell proliferation of the islet graft (95). Recently, mouse NCSCs were shown to stimulate neural and vascular engraftment as well as proliferation of human beta cells when co-transplanted under the kidney capsule of immune-deficient nondiabetic mice (96) Accordingly, surface coating of mouse pancreatic islets with mouse NCSCs improves islet revascularization, reinnervation and function after intraportal transplantation in immune competent diabetic mice (93). Notably, NCSCs

harbor the potential for autologous use since they can be retrieved from adult tissues such as skin (97).

Regulatory T cells

Regulatory T cells constitute a subpopulation of $CD4^+$ T cells that modulate the immune system by maintaining tolerance to self-antigens thereby preventing auto-immune disease. While naturally occurring regulatory forkhead box P3 $(FoxP3)^+CD4^+CD25^+$ T cells (nTregs) arise in the thymus from birth onwards, inducible regulatory T cells (iTregs) are generated from peripheral T cells upon antigen encounter under tolerogenic conditions such as exposure to TGF-beta (reviewed in (98)). Many different subsets of inducible Tregs have been described, including T regulatory type 1 (Treg1) cells that constitutively produce high levels of interleukin (IL)-10 (99). Absence of or defects in Tregs are associated with several auto-immune diseases, including T1D (100). As Tregs modulate self-tolerance and autoimmunity, strategies have been developed to induce local tolerance through the accumulation of immune-suppressive Tregs at the islet transplantation site. Major histocompatibility complex (MHC)-mismatched mice that are intramuscularly engrafted with islets and a plasmid encoding chemokine (C-C motif) ligand (CCL)22, a Treg-attracting chemokine, display delayed graft rejection (101). Adoptive transfer of expanded mouse Treg1 cells obtained by *in vitro* exposure to rapamycin and IL-10, prevents allograft rejection in immune competent diabetic mice (99, 102). Co-aggregates of mouse Tregs and allogeneic mouse islet cells support long-term survival of intraportal allogeneic islet cell grafts without the use of immune-suppressive drugs (103, 104). In line with these findings, adoptive transfer of *ex vivo* expanded human Tregs delays human islet rejection in recipient humanized immune competent diabetic mice (105). Mechanistically, Tregs inhibit islet infiltration of innate immune cells and of $CD4^+$ T cells by downregulating islet graft-derived CCL2 (105).

Other tolerogenic cells

Besides Tregs, several other cell types have been evaluated for their tolerogenic potential in the context of islet transplantation.

Myeloid-derived suppressor cells (MDSCs) consist of a heterogeneous population of myeloid-derived cells including myeloid progenitors, immature macrophages, granulocytes and dendritic cells. While, during steady state, MDSCs reside in the bone marrow, pathological conditions such as cancer, infection and autoimmune disease result in MDSC expansion and mobilization from the bone marrow. Direct cell-cell contact is crucial for the immune-suppressive effects of MDSCs, the latter which involves both inducible nitric oxide synthase (iNOS) and arginase activity, respectively suppressing T cell function and proliferation through NO production and L-arginine depletion (106). In addition, MDSCs promote development of Tregs through the costimulatory molecule B7-H1 (107). Adoptive transfer of mouse MDSCs prevents onset of T1D in mouse models through Treg expansion and effector T cell inhibition (108). In addition, co-transplantation of mouse islets with mouse MDSCs prolongs islet allograft survival though iNOS-mediated T cell inhibition in immune competent diabetic mice (109).

Hepatic stellate cells (HepSCs) are liver-derived pericytes that contribute to liver fibrosis after transdifferentiation into collagen-producing myofibroblasts. HepSCs store retinoic acid (RA), secrete cyto- and chemokines such as IL-17 and CCL2, and may act as antigen-presenting cells. HepSCs display immune-suppressive properties by promoting Treg cell induction through the release of RA and TGF-beta1 (110, 111). Furthermore, HepSCs induce T cell apoptosis and inhibit cytotoxic $CD8^+$ T cells ((112, 113)). In addition, mouse islet co-transplantation with mouse HepSCs induces immune tolerance towards islet allografts in mouse via the recruitment of MDSCs (107, 114, 115). Notwithstanding these apparent beneficial effects, additional research on the effects of HepSCs on beta cell function, replication and survival after co-transplantation is mandatory since in vitro co-culture of pancreatic stellate cells with mouse islet cells was recently shown to result in decreased beta cell proliferation and viability and a reduction of islet insulin content (116).

Dendritic cells (DCs) are unique antigen-presenting cells that play a crucial role in innate and adaptive immunity. Tolerogenic DCs (tol-DCs) constitute a subset of DCs that mediate central and peripheral tolerance. Tol-DCs are immature, alternatively activated DCs that express several chemokine-receptors including CCR5, CCR6 and CCR7 while showing decreased expression of MHC II and co-stimulatory molecules. Tol-DCs promote tolerance through deletion of T cells, the induction of regulatory and anergic T cells, the expression of immune-modulatory and immune-suppressive molecules and, discovered only recently, the induction of regulatory B cells (reviewed in (117-119)). Cell therapy with *ex vivo* directed tol-DCs has been applied to T1D patients. These tol-DCs were well tolerated and up-regulated the frequency of $B220^{+}CD11c$-B cells (120). Others have loaded autologous DCs, isolated from T1D patients, with insulin- and glutamic acid decarboxylase 65 antigen to induce hyporesponsiveness of antigen-specific T-lymphocytes (121). Currently, a beneficial effect of tol-DCs in clinical islet transplantation remains unexplored. Nonetheless, experiments in rodents have provided evidence for a potential benefit of tol-DCs in islet transplantation since combination of autologous tol-DCs with short-term anti-CD3 treatment results in permanent acceptance of pancreatic islet allografts in diabetic rodents (122, 123) while *ex vivo* exposure of murine DCs to TGF-beta induces tol-DCs that, upon co-engraftment, lead to long-term syngeneic islet graft survival in diabetic non-obese diabetic (NOD) mice(124).

Sertoli cells are testicular cells that nourish developing sperm cells in the seminiferous tubules via growth factor secretion. In addition, Sertoli cells create an immune-privileged testicular microenvironment through secretion of immune-protective factors such as Fas ligand (FasL) and TGF-beta1 (125). Their nourishing and immune-suppressive potential focused attention on Sertoli cells as accessory cells for islet transplantation. Prolonged survival of allogeneic rodent islets, grafted under the kidney capsule, occurs when co-transplanted in diabetic immune competent rodents with rodent Sertoli cells (126-128). However, after intraportal transplantation, mouse Sertoli cells tend to segregate from islets and embolize in smaller hepatic venules due to their smaller size compared to islets (129). This hurdle to potential clinical use was circumvented by generation of co-aggregates in a

hanging drop, resulting in long-term graft survival in the absence of immune-suppressive therapy in immune competent diabetic mice (129).

IV. POTENTIAL ROADMAP FOR THE USE OF ACCESSORY CELLS DEPENDING ON THE TRANSPLANTATION SITE

The ideal islet transplantation site is characterized by: (i) minimized risk for IBMIR, (ii) rapid and adequate revascularization and reinnervation, (iii) physiological insulin drainage, (iv) protection against auto- and allospecific graft rejection and (v) easy accessibility with the potential of full graft retrieval, considering the possibility of transplanting tumorigenic stem cell-derived beta(-like) cells. Unfortunately, none of the current clinical islet transplantation sites meet all of these criteria (**Table 3**). Taken into account the requirements of an ideal transplantation site, current site-specific disadvantages and the characteristics of accessory cells for islet transplantation, a roadmap for the clinical use of a particular accessory cell type or combination thereof per transplantation site can be envisioned (**Figure 2**).

An important disadvantage of *intra-portal delivery* of islets is the occurrence of IBMIR; a non-specific innate immune reaction triggered by graft-derived tissue factor and pro-inflammatory mediators (13). Subsequent activation of the coagulation- and complement cascades results in thrombosis and insulitis which inflicts acute and severe islet damage (reviewed in (130)). Islet-coating with endothelial cells prevents IBMIR (76, 83) and could therefore be suggested as a strategy to improve the outcome of clinical, intra-portal islet transplantation.

In an attempt to circumvent IBMIR, *extra-vascular transplantation sites* are explored such as the gastric submucosal space, spleen, pancreas, kidney, genitourinary tract, omentum, subcutis, bone marrow, peritoneum and muscle, the latter five in human clinical trials (reviewed in (4)). While these sites are characterized by a minimal risk for IBMIR thanks to their extra-vascular location, they are inherently hampered by insufficient graft revascularization and innervation as well as by the risk of graft rejection due to allo- and recurrent/persistent autoimmunity. Similar to the intra-portal transplantation site, these sites would therefore benefit from the use of

accessory cells with angiogenic, neurogenic and immune-modulatory properties. In this context, MSCs, ASCs, EPCs/BOECs, NCSCs and tolerogenic cell types such as Tregs could be envisioned. Of note, combining the beneficial islet-supporting properties of different accessory cells might even prove superior to monotherapy. For example, rat islet coating with rat endothelial cells combined with Sertoli cell/islet co-culture or Sertoli cell infusion prior to islet transplantation in diabetic rats creates an active surface on islets that inhibits IBMIR, stimulates endothelial cell proliferation and survival, reduces immunogenicity and subsequently promotes graft survival and revascularization in the renal subcapsular space (130).

Finally, to bypass immune-rejection, *immune-privileged sites* such as testis (131), brain(132), thymus (133, 134) and the ocular space (135) have been investigated. While being deprived from IBMIR and immune-rejection, these sites are still likely to benefit from trophic stimuli derived from graft-supporting cells such as MSCs, ASCs, EPCs/BOECs and NCSCs.

V. POTENTIAL (REGULATORY) BARRIERS TO THE USE OF ACCESSORY CELLS

Although promising experimental results have been obtained by the use of accessory cells in islet transplantation, several important barriers need to be considered prior to clinical translation (136). Since most of the islet transplantation sites are size-restricted, rigorous preclinical dose titration experiments (including infusion frequency) need to be performed for accessory cell transplantation to support rather than preclude transplantation of a sufficient beta cell mass. As with islet transplantation, the potential clinical benefit of accessory cell transplantation should outweigh its risk. Features that contribute to this risk include a potential of prolonged, deleterious biological activity after a single administration as well as immunogenicity of the accessory cell type. Preclinical safety trials should thus be performed in appropriate animal species to evaluate tumorigenicity and migration potential of a particular accessory cell type (in particular stem cells). If possible, autologous rather than allogeneic accessory cells should be used as the latter might elicit adverse immune reaction and jeopardize the outcome of future allogeneic

transplantations due to antibody formation. Accessory cell types that could easily be obtained for autologous transplantation include MSCs, ASCs, EPCs, BOECs, NCSCs, Tregs, Tol-DCs and MDSCs. For clinical trials using these cells, it is important to have clinical grade cell isolation, expansion and/or cryopreservation protocols available that are compliant with Good Manufacturing Practice (GMP) regulations. If prior culture of these cells is needed, long-term expansion should be avoided to prevent cellular senescence, genomic alterations and loss of viability, homing capacity and function (37). Topical rather than systemic delivery can be envisioned to prevent side-effects of cellular therapy in off-target organs or tissues and to overcome insufficient extravasation and homing to the target site. To circumvent some of these potential problems, accessory cells with proliferative and immunogenic potential could be grafted, enclosed in a retrievable device as currently proposed in the first Phase I/II clinical trial with ESC-derived beta-like cells (Clinical Trial, Registration number NCT02239354, clinicaltrials.gov).

VI. CONCLUDING REMARKS

Over the past decade, substantial progress has been made in the beta cell therapy field. Yet, several important hurdles still need to be overcome. Optimization of islet isolation from selected donor organs and development of alternative beta cell sources would allow for a significant surge in the currently available cell mass for transplantation. When combined with approaches that significantly improve graft survival and function, a realistic and universally applicable cure for both T1D and a subset of T2D patients would ensue. Compared to genetic modulation and (single) growth factor application, multi-modal effects of accessory cells make them an interesting and likely superior tool to improve islet transplantation outcome and alleviate donor organ burden. Nonetheless, additional research is advocated to determine stability, safety, efficacy and optimal dosing. We speculate that autologous MSCs will be the first accessory cell type to reach clinical translation as they were recently shown to arrest disease progression in early-onset T1D patients.

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Table 1.

Acc. Cell Type	Islet origin	Acc. cell origin	Recipient Species	Recipient Immune Status	Recipient Metabolic Status	Transplant Site	Transplant type	Beneficial Effects of Co-Transplantation	Ref	
MSCs	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Kidney capsule	Syngeneic	Graft survival, glucose homeostasis, revascularization rate	Borg DJ *et al.*, 2014	(25)
				Normal	Diabetic: C57BL/6-Ins2Akita/J	Hepatic vein	Syngeneic			
				Immune deficient	Non diabetic	AC eye	Syngeneic			
	Rat	Rat	Rat	Normal	Diabetic: STZ	Omentum	Syngeneic	Graft survival, glucose homeostasis	Solari MG *et al.*, 2009	(26)
				Normal / Immune suppressed		Omentum	Allogeneic	Graft survival, glucose homeostasis, immunomodulation		
	Mouse	Mouse	Mouse	Normal with isolated graft	Diabetic	Intraperitoneal	Syngeneic	Graft function	Kerby A *et al.,* 2013	(27)
	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Kidney capsule	Syngeneic	Glucose homeostasis, preserved islet structure, vascularization	Rackham CL *et al*, 2011	(28)
	Nonhuman primate	Nonhuman primate	Nonhuman primate	Normal	Diabetic: STZ	Intraportal	Allogeneic	Islet engraftment and function	Berman DM *et al*, 2010	(29)
	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Kidney capsule	Syngeneic	Glucose homeostasis, vascularization	Sakata N *et al,* 2010	(30)
	Rat	Rat	Mouse	Immune deficient	Diabetic: NOD SCID	Kidney capsule	Xenogeneic	Glucose homeostasis, preserved islet structure, vascularization	Ito T *et al*, 2010	(31)
	Rat	Rat	Rat	Normal	Diabetic: STZ	Kidney capsule	Syngeneic	Glucose homeostasis, vascularization	Figliuzzi M *et al*, 2009	(32)
	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Intramuscular	Syngeneic	Immunomodulation	Yoshimatsu G *et al*, 2015	(36)
ASCs	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Kidney capsule	Allogeneic islets Syngeneic acc. cell	Graft survival, glucose homeostasis, revascularization, immunomodulation	Ohmura Y *et al.,* 2010	(67)
	Rat	Human	Mouse	Immune deficient	Diabetic: STZ	Subcutaneous in fibrin gel	Xenogeneic	Graft survival, glucose homeostasis, revascularization	Bhang S *et al*., 2013	(69)
	Rat	Human	Rat	Normal	Diabetic: STZ	Portal vein	Syngeneic islets Xenogeneic acc. cell	Glucose homeostasis, revascularization	Cavallari G *et al*., 2011	(70)
E(P)Cs	Rat	Rat	Rat	Normal	Diabetic: STZ	Kidney capsule	Syngeneic	Graft Survival, glucose homeostasis, revascularization	Song HJ *et al*., 2010	(74)
	Pig	Human	Mouse	Immune deficient	Diabetic: STZ	Kidney capsule	Xenogeneic	Graft survival, glucose homeostasis, revascularization, beta cell proliferation, protect against IBMIR	Kang S *et al*., 2012 Kim JH *et al*., 2011	(75) (76)
	Rat	Rat	Rat	Normal	Diabetic: STZ	Kidney capsule	Allogeneic	Graft survival, glucose homeostasis, vascularization, increased insulin secretion.	Li Y *et al.,* 2013	(130)
BOECs	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Kidney capsule	Syngeneic	Graft survival, glucose homeostasis, increased insulin secretion, revascularization	Oh BJ *et al*., 2013 Penko D *et al*., 2015	(77) (78)
	Rat	Human	Mouse	Immune deficient	Diabetic: ALX	Kidney capsule	Xenogeneic	Graft survival, glucose homeostasis, reduced beta cell death, beta cell proliferation	Coppens V *et al.,* 2013	(87)
NCSCs	Mouse	Mouse	Mouse	Normal	Diabetic: ALX	Portal vein	Syngeneic	Glucose homeostasis, revascularization, reinnervation	Lau J *et al*., 2014	(93)
	Mouse	Mouse	Mouse	Normal	Diabetic: ALX	Kidney capsule	Syngeneic	Graft survival, glucose homeostasis, beta cell proliferation	Olerud J *et al*., 2009	(95)
	Human	Mouse	Mouse	Immune deficient	Normal	Kidney capsule	Xenogeneic islets Syngeneic acc. cell	Beta cell proliferation, revascularization, reinnervation	Grapensparr L *et al*., 2015	(96)
Tregs	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Portal vein	Allogeneic islets Syngeneic acc. Cell	Graft survival, glucose homeostasis,	Takemoto T *et al., 2015*	(103)
MDSCs	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Kidney capsule	Allogeneic islet	Graft survival, glucose homeostasis, Treg	Chou HS *et al*., 2012	(107)

							Syngeneic acc. cell	expansion	Arakawa Y *et al*., 2014	(109)
HepSCs	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Kidney capsule	Allogeneic	Graft survival, glucose homeostasis, prevent infiltration of innate immune cells.	Zhang ZY *et al.,* 2014 Chou HS *et al*., 2011	(114) (115)
Tol-DCs	No co-transplantation experiments only adoptive infusion of DC prior to islet graft transplantation								Baas MC *et al., 2014*	(122)
Sertoli cells	Rat	Rat	Rat	Normal	Diabetic: STZ	Kidney capsule	Allogeneic	Graft survival, glucose homeostasis, reduced infiltration of innate immune cells.	Korbutt GS *et al.1997*	(126)
	Mouse	Mouse	Mouse	Normal	Diabetic: NOD	Kidney capsule	Syngeneic	Graft survival, glucose homeostasis	Suarez-Pinzon W *et al*., 2000	(128)
	Mouse	Mouse	Mouse	Normal	Diabetic: STZ	Portal vein	Allogeneic	Graft survival, glucose homeostasis	Takemoto N *et al.,* 2014	(129)

Table 2.

Acc. Cell Type	Function	Mechanism	Responsible factors and molecules	Ref	
MSCs	Immunomodulation	Decreased activation and proliferation of T, B, DC and NK cells.	PD-(L)1	Halvorsen T *et al.*, 2000	(37)
			PGE2	Tse WT *et al.*, 2003	(38)
				Chen L *et al.,* 2007	(39)
				Sotiropoulou PA *et al.*, 2006	(40)
			IDO	Krampera M *et al.,* 2006	(41)
			TGF-beta	Di Nicola, M *et al.*, 2002	(42)
				Aggarwal, S *et al.*, 2005	(43)
				Casiraghi, F *et al.*, 2008	(44)
			NO	Sato K *et al.,* 2007	(45)
			MMPs	Ding Y *et al.,* 2009	(46)
			Galectins	Sioud M *et al.*, 2011	(47)
			HLA-G5	Selmani Z *et al.*, 2008	(48)
			IL-6	Jiang XX *et al.*, 2005	(49)
				Engela AU *et al.*, 2013	(50)
				Crop MJ *et al.,* 2010	(51)
			HGF	Di Nicola M *et al., 2002*	(52)
				Sotiropoulou PA *et al.*, 2006	(40)
		Modulation of neutrophil and B cell function, differentiation and chemotaxis	Downregulation of chemokine receptor CXCR4, CXCR5, and CCR7	Corcione A *et al*., 2006	(53)
			IL-6, TGF-beta	Raffaghello L *et al., 2008*	(54)
		Treg cell activation and IL-10 production	TGF-beta	Peng Y *et al.,* 2004	(55)
			IL-10	Engela AU *et al.,* 2013	(50)
				Crop MJ *et al*, 2010	(51)
			Galectins	Sioud M *et al*, 2011	(47)
	Pro-angiogenic	Secretion of angiogenic factors	VEGF-A, IL-6, IL-8, HGF, PDGF,	Sakata N *et al*., 2010, Burlacu A *et al*., 2013	(30, 34)
		Secretion of matrix metalloproteinases	MMP-2, MMP-9		
		Differentiation into pericytes	TGF-beta1	Au P *et al*., 2008	(35)
	Anti-apoptotic	Increased expression of anti-apoptotic signaling molecules, a.o. XIAP, Bcl-2, Bcl-xL	HGF, TGF-beta,	Yeung TY *et al*.,2012	(56)
ASCs	Pro-angiogenic	Secretion of angiogenic factors	VEGF-A, HGF, EGF	Rehman J *et al.,* 2004, Kuo YR *et al*., 2015	(63) (64)
		Differentiation into endothelial cells and pericytes		Merfeld-Clauss M *et al*.,2014	(65)
	Anti-apoptotic	Secretion of anti-apoptotic factors	HGF, TGF-beta, GM-CSF	Rehman J *et al.,* 2004	(63)
	Immunomodulation	Decreased activation and infiltration of CD4+ and CD8+ T cells	TSG-6	Kato T *et al*., 2014	(68)
E(P)Cs	Prevention of IBMIR	Lower platelet consumption and C3a level.	IL-8	Kim JH *et al*., 2011	(76)
		Protection against complement-mediated lysis & activation of coagulation			
	Pro-angiogenic	Direct differentiation into vasculature, incorporation into vascular network and release of angiogenic factors	HGF	Kang S *et al*., 2012	(75)
			VEGF-A, Ang-2, PlGF, PDGF-B, MMP-1,	Oh BJ *et al*., 2013, Hendrickx B *et* al.,	(77)
			MMP-9, MMP-14	2010, Coppens V *et al*., 2013	(85)
					(87)
			microRNAs (MiR-126, MiR-296)	Cantaluppi V *et al*., 2012	(82)
	Stimulation of beta cell proliferation	Production of basement membrane	HGF	Kang S *et al*., 2012	(75)
	Increased beta cell function	Modulation of connexin 36 expression	Connexin 36	Penko D *et al*., 2015	(78)
		Thrombospondin-1-mediated activation of TGF-beta1	Thrombospondin-1	Olerud J *et al*., 2011	(80)
NCSCs	Beta cell proliferation	Production of trophic factors	GDNF	Grouwels G *et al*., 2012	(94)
	Anti-apoptotic	Production of protective factors	GDNF	Mwangi SM *et al*., 2011	(90)
	Pro-angiogenic/-neurogenic	Release of angiogenic and neurotrophic factors.	VEGF-A, MMP-2, MMP-9	Lau J *et al*., 2015	(93)
Tregs	Immunomodulation	Suppress activation, proliferation and function of CD4+ and CD8+ T cells.	TGF-beta , IL-10, IL-5	Battaglia M *et al*., 2006	(102)
		Inhibition of immune infiltration by downregulation of islet graft-derived CCL2.	CTLA-4, IL-2	Xiao F *et al*., 2014	(105)

MDSCs	Immunomodulation	APC function and induction of Tregs Suppression of T cell function and proliferation	MHC class II iNOS and Arg1	Yin B *et al*., 2010 Arakawa Y *et al*., 2014	(108) (109)
HepSCs	Immunomodulation	Suppression of T-lymphocytes T cell apoptosis Promote Treg induction MDSC induction	TGF-beta TRAIL, PD-(L)1 RA, TGF-beta IFN-γ	Zhang ZY *et al*., 2014 Yang HR *et al*., 2010, Yu MC *et* al., 2004, Dunham RM *et al*., 2013 Chou HS *et al*., 2011	(114) (112, 113) (110) (115)
Tol-DCs	Immunomodulation	Deletion of CD4+ and CD8+ T cells, induction of anergic T cells and Tregs.	Decreased MHC II and costimulatory signals	Turnquist HR *et al*., 2007, Baas MC *et al*., 2014, Thomas DC *et al*., 2013,	(119, 122) (124)
Sertoli cells	Immunomodulation Protect beta cells from cell death	Production of immune-suppressive factors Production of protective factors	FAS-L, TGF-beta TGF-beta	Korbutt GS *et al*., 1997, Li Y *et al*., 2013Suarez-Pinzon W *et al*.,2000	(126, 130) (128)

Table 3.

IDEAL SITE REQUIREMENTS :	LIVER	OMENTUM	BONE MARROW	SUBCUTIS	MUSCLE
1. MINIMAL RISK FOR IBMIR	-	+	+	+	+
2. PHYSIOLOGICAL INSULIN RELEASE	+	+	-	-	-
3. VASCULARIZATION	+/-	+	+	+/-	+
4. IMMUNOPROTECTION	-	+/-	-	-	-
5. ACCESSIBILITY & RETRIEVABILITY	-	+	+/-	+	+