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Mesenchymal Stem Cell-Based Therapy

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Abstract

Mesenchymal stem cells (MSCs) are multipotent adult stem cells which have self-renewal capacity and differentiation potential into several mesenchymal lineages including bones, cartilages, adipose tissues and tendons. MSCs may repair tissue injuries and prevent immune cell activation and proliferation. Immunomodulation and secretion of growth factors by MSCs have led to realizing the true potential of MSC-based cell therapy. The use of MSCs as immunomdulators has been explored in cell/organ transplant, tissue repair, autoimmune diseases and prevention of graft vs. host disease (GVHD). This review focuses on the clinical applications of MSC-based cell therapy, with particular emphasis on islet transplantation for treating type I diabetes.

Keywords

Mesenchymal stem cells; immunomodulation; islet transplantation; gene delivery; biomaterials

INTRODUCTION

Stem cells have self-renewal potential and ability to differentiate into one or more specialized cell types.[1] There are two broad types of stem cells, embryonic stem cells (ESCs) and adult stem cells. ESCs are isolated from inner cell mass of blastocyst and can transdifferentiate into cells of all three germ layers.[2] Unlike ESCs, adult stem cells show restricted proliferation and lineage differentiation. Adult stem cells which undergo mesodermal lineage-specific differentiation to osteocytes, adipocytes, and chondrocytes are named as mesenchymal stem cells (MSCs). Friedenstein was the first to show the isolation of clonogenic, proliferating fibroblastic cells from bone marrow and their differentiation into bones and osteocytes.[3] There are two independent adult stem cell populations in bone marrow: hematopoietic stem cells (HSCs) and MSCs.[4]

Although ESCs can proliferate and differentiate into virtually any cell types,[5, 6] danger of teratoma formation and ethical concerns limit their use.[7] In contrast, adult stem cells are safe, do not form teratoma, and can be used for tissue repair and regeneration. Adult stem cells secrete growth factors and immunoprotective cytokines which have been used in the field of organ and cell transplantation. Ease of isolation, hypoimmunogenicity and immunomodulatory properties of MSCs make them ideal candidate for adult stem cell-based therapy and their use as gene carriers.

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MSCs are characterized by their adherence to tissue culture-treated plates and the absence of hematopoietic marker expression. Upon exposure to differentiation media, MSCs undergo differentiation into osteocytes, chondrocytes, and adipocytes. The differentiation potential of MSCs to connective tissues has been exploited for tissue engineering. MSCs have been encapsulated in tissue-specific scaffold and implanted to regenerate damaged cartilages and bones.[8, 9] In addition to their regeneration potential, MSCs have tropic activity to the sites of injured tissues. MSCs secrete various paracrine factors that promote angiogenesis and mitosis and reduce apoptosis. Various animal models have shown that MSCs are useful in repairing or regenerating cardiac tissue, brain tissues and meniscus.[10–12]

MSCs are known to have a functional role in the proliferation of immune cells, as confirmed by various in vitro studies and preclinical studies.[13–15] MSCs have been used as immunomodulators in bone marrow transplantation. Clinical success of MSC therapy in GVHD suggests their potential use in the field of solid organ transplantation.[16, 17] In this review, we discuss the potential use of MSCs as nursing cells for successful organ transplantation, with particular emphasis on islet transplantation for treating type 1 diabetes.

IDENTITY AND LOCATION OF MSCs

MSCs isolated from various tissues including bone marrow, umbilical cord, adipose tissues, liver and pancreas have different differentiation and proliferation potential.[4, 18–21] To achieve consensus, the International Society for Cellular Therapy has defined criteria to identify MSCs. MSCs should proliferate in vitro as plastic adherent cells; be positive for the expression of CD105, CD73, and CD90; and be negative for the expression of hematopoietic cell surface markers [CD34, CD19, CD45, CD11a, HLA DR (human leukocyte antigen)]; and undergo mesenchymal differentiation under in vitro culture conditions.[22] Although MSCs have been defined and characterized by the presence/absence of certain surface marker proteins, questions remain about the location and identity of MSCs.

Location of MSCs within bone marrow is corroborated by their physiological function. MSCs within bone marrow form HSC niches and control the proliferation, differentiation, and release of HSCs and their progeny. Since MSCs have been isolated from almost all the postnatal organs, this has raised questions about their identity and peripheral function. MSCs are naturally found as perivascular cells referred to pericytes, which are released at the site of injury to secrete bioactive factors having immunomodulatory and trophic activity. The trophic activity inhibits ischemia-caused apoptosis and scarring while stimulating angiogenesis and mitosis of tissue intrinsic progenitor cells. The immunomodulation inhibits lymphocyte surveillance of the injured tissue, thus preventing autoimmunity, and allows allogeneic MSCs to be used in a variety of clinical situations. Finally, insights about the perivascular location and identity of MSCs will provide a rationale for designing new therapeutic applications of MSCs.

IMMUNOMODULATION

MSCs have immunomodulatory activity. As illustrated in Fig. 1, MSCs have differential effects on the proliferation and cytokine secretion profile of a subpopulation of immune cells. MSCs do not express class II major histocompatibility complex (MHC) on their cell surface or other classical stimulatory molecules like CD80, CD86, and CD40. Absence of class II MHC and other co-stimulatory molecules makes MSCs immune-privileged cells and allows allogenic transplantation of MSCs. However, there are conflicting reports about the interaction of MSCs with immune cells, depending on the species involved, co-culture conditions, and stimulants used for immune cells.[23–25] It is imperative to understand the role of each subtype of immune cells and the effect of MSCs on these cells for clinical success of MSCs in solid organ transplantation.

MSCs and T cells

MSCs inhibit T cell activation which is independent of the MHC status, allowing the administration of third-party MSCs for immunomodulation. MSCs inhibit the proliferation and maturation of T cells.[13, 26–29] Different mechanisms of T cell inhibition have been proposed. Krampera et al. showed that MSCs inhibit both naive and memory T cells to their cognate antigens. MSC action on T cells was independent of both antigen-presenting cells (APCs) and CD4+/CD25+ regulatory T cells.[28] Contrary to this mechanism, Beyth et al. showed that human MSCs inhibit T cells indirectly by inducing regulatory APCs.[30] Another school of thought believes that MSCs lead to expansion of CD4+CD25+Foxp3+ regulatory T cells that can inhibit T cell activity.[29] Taken together, all these studies indicate that MSCs modulate the intensity of an immune response by inhibiting antigen-specific T cell proliferation and cytotoxicity and by promoting the generation of regulatory T cells.

MSCs and Dendritic cells

Dendritic cells (DCs) are APCs capable of stimulating both naive and memory T cells. MSCs affect the differentiation, maturation, and function of APCs at different levels.[31–33] Human MSCs strongly inhibit the differentiation of both CD14+ monocytes and CD34+ hematopoietic progenitors.[32] MSC co-culture strongly inhibits the initial differentiation of CD14+ monocytes to DCs; however, this effect is reversible at a higher MSC/monocyte ratio (1:10). MSCs also affect the activity of mature DCs. MSCs significantly reduce the expression of CD83, which is a co-stimulatory receptor for T cell activation, leading to a shift to immature status. Furthermore, MSC exposure decreases the expression of presentation molecules (HLA-DR and CD1a) as well as co-stimulatory molecules (CD80 and CD86) and down-regulated IL-12 secretion. These effects of MSCs are consistent with impaired ability of mature DCs to stimulate the naive T cells. Nauta et al. showed that the soluble factors of MSCs affect differentiation and the ability of DCs to stimulate naive T cell proliferation. Transwell co-culture has suggested that IL-6 and macrophage–colony stimulating factor (M-CSF) are partially involved and that other factors also play a role in inhibiting DC differentiation by MSCs.[31] MSCs also alter the cytokine secretion profile of DCs. MSCs coculture causes decreased tumor necrosis factor α (TNF-α) secretion by mature myeloid DCs as well as increased secretion of IL-10 by plasmacytoid DCs.[34] Chiesa et al. showed that MSCs impair Toll-like receptor-4-induced activation of DCs, affect antigen presentation to CD4+ T and CD8+ T cells, inhibit secretion of inflammatory cytokines, and downregulate expression of molecules involved in the migration to lymph nodes.[35] All these studies suggest that MSC administration would affect the antigen presentation ability of DCs and thereby prevent the acute rejection in solid organ transplant.

MSCs and B lymphocytes

B cells are involved in adaptive immunity by producing antibody. Interaction between B cells and MSCs produces different results depending upon the culture conditions and species involved.[36–38] Krampera et al. showed that native MSCs do not affect the proliferation of B cells. However, IFN-γ-treated MSCs inhibited the proliferation of B cells in transwell culture systems. IFN-γ increased the expression of indoleamine 2,3-dioxygenase by MSCs that inhibited the proliferation of B cells.[36] In another report, MSCs arrested the proliferation of B cells by arresting the G0/G1 phase of the cell cycle. This action of MSCs was mediated by soluble factors. MSCs also impaired B cell differentiation, and the production of IgM, IgG and IgA was significantly impaired. MSCs also down regulated the expression of various chemotaxis ligands on the surface of B cells, thereby interfering with the tropism of B cells.[39]

Plasmacytoid DCs play a key role in the maturation of B cells and increases the number of $CD38^{++}/CD138^{++}$ cells, which also display higher levels of cytoplasmic immunoglobulin

and lower levels of CD19, CCR7, and surface immunoglobulin.[37] MSCs inhibited this plasmacytoid DCs mediated maturation of B cells and maintained the B cells in native state.[37] Irrespective of the MSCs in vitro effect on B cells, it should be kept in mind that in vivo MSCs' effect on B cells is mediated by the inhibitory effect of MSCs on T cells and DCs.

MSCs and Natural Killer Cells

Natural killer (NK) cells are key effector cells of innate immunity. Although the primary role of NK cells is the cytolysis of tumor cells and pathogen-infected cells, they play a crucial role in activating innate and adaptive immunity. However, the role of NK cells in acute and chronic graft rejection is not clear. NK cells exert selective cell lysis by expressing activating and inhibitory receptors on the cell surface. Sudeepta et al. showed that co-culture of NK cells with human MSCs led to significant reduction in the secretion of IFN-γ[34] Sotiropoulou et al. showed that, at low NK-to-MSC ratios, MSCs alter the phenotype of NK cells and suppress proliferation, cytokine secretion, and cytotoxicity against mismatched HLA-class I expressing targets.[40] Certain inhibitory effects of MSCs on NK cells require cell-to-cell contact, whereas others are mediated by soluble factors including transforming growth factor (TGF)-β1 and prostaglandin E2.[33] The roles of cell-cell interaction and soluble factors suggest multiple mechanisms for MSC-mediated NK-cell suppression.[33] MSCs are susceptible to lysis by activated NK cells but are resistant to resting NK cells.[40] Spaggiari et al. evaluated the lysis of MSCs by NK cells. MSCs express ulpv, pvr, and nectin-2, which are well known activating ligands for NK cell-mediated cytotoxicity. However, IFN-γ-treated MSCs are resistant to lysis by NK cells.[33] Treatment of MSCs with IFN-γ increases the expression of MHC class I antigen by MSCs but does not alter the expression of PVR (CD155) or nectin-2 (CD122).[41] The significance of interaction between MSCs and NK cells is still not clearly defined and requires further preclinical studies.

Cell and Organ Transplantation

Immunomodulatory properties of MSCs along with clinical success in GVHD have sparked interest in MSC-based immune therapy in allogeneic cell and organ transplantation (Table 1). MSCs have been used in various animal models for solid organ transplantation, but results are ambiguous.[42–45] Casiraghi et al. showed positive outcomes of pretransplant MSC infusion in a partial MHC match heart transplant model. Both donor and recipient MSCs led to protolerogenic effects, attributed to expansion of $CD4^+CD25^+Foxp3^+$ regulatory T cells and impaired anti-donor Th1 activity. Although this study reported in vivo distribution of MSCs, it is difficult to conclude whether MSCs underwent distribution to lymphoid tissues and interacted with regulatory T cells to stimulate their expansion.[46] In another rat heart transplant model, pretransplant infusion of MSCs along with low dose of immunosuppressant mycophenolate mofetil (MMF) induced a long-term allograft acceptance in a completely MHC mismatched model. Injection of MSCs alone 4 days prior to transplantation led to the rejection earlier than in animals receiving no treatment at all. This finding suggests that allogenic MSCs might elicit an immunogenic response leading to allograft rejection. Moreover, third-party MSCs did not prolong allograft survival.[47] This result is contrary to the in vitro findings that MSCs exert immune modulation independent of MHC status.[34, 48] Rejection of allograft by pretransplant infusion of MSCs can be explained by the activation of T cells by MSCs. Pretransplant infusion of allogeneic MSCs resulted in earlier appearance of activated CD4+ and CD8+ cells in secondary lymphatic organs of MSC- and MSCs/MMF-treated animals compared to control animals. It is presumed that MMF preferentially eliminated activated T cells, thereby preventing rejection by MSCs alone.[47] MSCs also affect the activation of $CD86^+$ APCs in secondary organs. MSC treatment significantly reduced the relative frequency of activated APCs in the spleen and abdominal lymph nodes as compared to MMF-treated and control animals. MSC/MMF

co-treatment eliminates MSC-activated T cells, reduces the activation of APCs, and decreases intragraft APCs and T cell trafficking by modulating the donor endothelium.[47]

MSCs can be either inhibitory or stimulating, depending on the presence of pro-inflammatory cytokines. MSCs fail to inhibit the activation of T cells isolated from IFN-$\gamma^{-/-}$ knockout mice. Exogenous addition of IFN-γ primes MSCs for an immunosuppressive role.[49] Renner et al. also showed that the degree of mitogen stimulation in co-culture affects the immunosuppressive action of MSCs.[50] This requirement of priming MSCs is critical in determining the time of administration of MSCs in organ transplant. Along with in vitro studies, animal models have resulted into conflicting information regarding timing, immune status of MSCs, and mechanism of action.

Various animal studies have shown that MSCs co-therapy support islet function, repair islet injuries and prevent immune cell mediated islet rejection (Table 2).[51–56] Since diabetes is an autoimmune disease, islet transplantation offers additional immune challenges. Recipients are characterized by the presence of islet beta cell reactive antibodies and infiltrating host T cells. The host has preexisting antibodies and primed immune cells against β cell antigens and insulin, which participate in graft destruction, in addition to the immune cells that infiltrate in response to nonself-antigens. Host APCs will also react to nonself-proteins originating from the transplanted tissue in case of allo- and xenotransplantation. Therefore, the major obstacles in islet transplantation are graft rejection and poor revascularization of islet grafts.[57] Ease of co-transplantation, secretion of growth factors, and immunomodulatory activity of MSCs led to MSCs as adjunct cell therapy in islet transplantation (Fig. 2). Figliuzzi et al. showed that MSC co-therapy resulted in reduced number of islets needed to achieve glycemic control in diabetic rats.[53] When islets were co-transplanted with MSCs under the renal capsule in immunocompetent Lewis rats, transplanted MSCs expressed a higher level of vascular endothelial growth factor (VEGF) as confirmed by Western blot and real time RT-PCR. Transplantation of islets with MSCs resulted in significant increase in the number of capillaries/mm^2 and promoted revascularization of transplanted islets.[53] However, investigators did not characterize the effect of MSCs on immune cells; therefore, it is difficult to predict the effect of MSCs on the immune system. Another research group reported that co-transplantation of islets with MSCs resulted in a well-preserved islet structure and higher number of capillaries compared to islets without MSCs with poor capillary growth. One week after transplantation, MSCs labeled with Q dot crystals surrounded islets and were found positive for von Willebrand factor (vWF).[52] Co-localization of MSCs and vWF suggests that some of MSCs have been transformed to endothelial cells, thereby contributing to the vascularization of islets. Park et al. also demonstrated a positive impact of MSCs on islet function and viability. Co-culturing of islets with MSCs resulted in lower ADP/ATP ratio, higher glucose-stimulated insulin secretion, and lower apoptosis. Mice receiving islets (200 islet equivalents) co-cultured with MSCs media resulted in better transplantation outcome and enhanced blood vessel formation.[58] Interleukin (IL)-6, IL-8, VEGF-A, hepatocyte growth factor (HGF), and TGF-β secreted by MSCs were indicated to positively influence the transplanted islets. Berman et al. first reported the generation of Treg in MSC allogeneic islet transplant preclinical model.[51] Co-transplantation of allogeneic MSCs with islets in nonhuman primate enhanced islet engraftment and functioning 1 month post-transplant. Post transplantation infusion of donor or third-party MSCs resulted in reversal of islet rejection and improved functioning of the graft. Co-transplantation of MSC infusion was associated with an increase in the percentage and number of FoxP3 T regulatory cells.[51] MSCs enhanced islet engraftment by immunomodulation and secretion of growth factors for revascularization. Systemic administration of MSCs along with islets may be responsible for generating FoxP3 T regulatory cells.[51] Ding et al. suggested a local mechanism of immunomodulation by MSCs in islet transplantation model.[59] Diabetic BALB/c Rag-1$^{-/-}\gamma^{-/-}$ reconstituted with IV

administration of $CD4^+CD25^-$ T cells as effector T cell population was used as the animal model. Co-transplantation of islets and MSCs resulted in prolonged normoglycemia (95 days vs. 30 days), indicating the efficacy of MSCs. Another group of diabetic recipients was transplanted with allogenic islets reconstituted with effector cells and MSCs. However, these animals also received MMP-2 and MMP-9 inhibitors until rejection. All animals in this group became profoundly diabetic within 30 days which suggested a role of the MMP in immunomodulation of MSCs.[59]

Although MSCs have been studied in a number of solid organ and cell transplantation animal models, it would be critical to assess the site of action for MSCs and determine whether MSCs create a local immunosuppressive milieu around the transplanted tissue or whether MSCs undergo trafficking to draining lymph nodes of the target organ to exert an immunomodulatory action. Finally, it is critical to understand whether MSCs can block recurrent autoimmunity and prevent allograft rejection, as the former response is more difficult to impede, because memory T cell responses may be less dependent on IL-2 signaling for effector function. Answers to these questions are critical to carry forward the immunomodulatory action of MSCs in prevention and treatment of diabetes as well as other autoimmune diseases.

Graft vs. Host Disease

HSC transplantation is the potential therapeutic approach for treating various malignant and nonmalignant hematological disorders. Allogenic transplantation of HSCs as compared to solid organ transplant presents different immunological challenges. In the case of solid organ transplant, transplanted organs contain only few active immune cells, and therefore, rejection of the graft is of major concern. However, allogenic HSCs contain many donor reactive immune cells and show reactivity against normal host tissue. This activity of donor-derived immune cells against the host tissue results in a clinical situation known as GVHD. Clinical success of HSC transplantation is limited by the morbidity and mortality associated with GVHD. Almost 30% of the recipients of stem cells or bone marrow transplantation from HLA identically related donors develop acute GVHD.[30] Corticosteroids are the mainstay therapy for the prophylaxis and treatment of GVHD after HSC transplantation. However, adverse effects associated with corticosteroid therapy and development of chronic GVHD despite steroid therapy has limited the positive outcome of corticosteroid therapy.

Immunomodulatory properties of MSCs have resulted in a promising clinical strategy to promote engraftment of HSCs and prevent/treat GVHD. Omer et al. published the first report showing a positive impact of MSC infusion on the engraftment of blood progenitor cells after infusion.[60] The infusion of culture-expanded autologous MSCs along with blood progenitor cells led to the rapid hematopoietic recovery and was devoid of toxicity. Blanc et al. reported complete remission of grade IV acute GVHD with an infusion of MSCs.[17] Upon the receipt of blood stem cells, patients with lymphoblastic leukemia developed severe grade IV GVHD and did not respond to any conventional therapies. On administration of multiple infusions of MSCs, complete resolution of the symptoms of GVHD with no remission of lymphoblastic leukemia was observed. To avoid alloreactivity of a patient's lymphocytes against infused MSCs, culture experiments were performed prior to the first infusion and thereafter on several occasions to ensure the safety of MSC infusions. MSCs from the donor and controls inhibited patient lymphocyte proliferation by 90% before and after transplantation, thereby showing the efficacy of MSCs on lymphocyte proliferation.[17] In another study, patients with steroid-resistant, severe, acute GVHD were treated with infusions of MSCs. This study reported that more than half of the patients with steroid-refractory acute GVHD responded to treatment with MSCs with no associated toxicity.[16]

Infusion of MSCs appears to be safe and not associated with long-term side effects. However, immunosuppression by MSCs may abrogate the benefits associated with HSC transplantation in hematological malignancies. Although co-transplantation of MSCs and HSCs prevented the development of GVHD, the relapse rate of hematological malignancies was higher in the MSC group.[61] Therefore, cotransplantation of MSCs along with HSCs may prove to be a double-edged sword in case of hematological malignancy. Additionally, large-scale randomized clinical trials are needed to evaluate the risk of graft vs. leukemic effect in co-transplantation of MSCs with HSCs in hematological malignancies.

REGENERATIVE MEDICINES

ESCs have the ability to differentiate into all human cell types and can be clinically used to generate various organs. Insulin and other pancreatic endocrine hormone producing cells can be generated from ESCs.[62, 63] ESCs have been demonstrated to self-assemble to form glucose-responsive three-dimensional clusters similar to normal pancreatic islets. Despite the mesodermal-restricted differentiation of MSCs, surprisingly many studies have shown that MSCs can undergo differentiation to cells of endodermal and ectodermal origins.[64] Such high plasticity of MSCs can be attributed to the expression of ESC markers such as Oct 4, Nanog, alkaline phosphatase, and Sox-2.[65, 66] Moreover, characteristic gene expression of three germ layers is not silenced in MSCs and can be unregulated by exposure to certain growth factors and specific culture conditions.[67] This transdifferentiation of MSCs provide a unique platform for generating tissue-specific cells. MSCs have been reported to generate islet β cells, myocytes and neurons.[68–70]

Transdifferentiation of MSCs to β cells has been reported and can be used to treat type I diabetes. Successful application of islet transplantation to treat type I diabetes is restricted by limited number of donors and the viability of transplanted islets.[71] Xenogeneic islets have been explored to meet the growing demand of islets, but the approach has resulted in limited success. Transdifferentiation of autologous MSCs to functional islet β cells can improve the current therapeutic treatments for type I diabetes.

Genetic modifications of MSCs have been reported to generate insulin-expressing cells. Transduction of MSCs with adenoviruses expressing transcription factors has been demonstrated to increase expression of islet genes. Irrespective of culture conditions, expression of transcription factors has resulted in insulin gene expression.[68] Zulewski et al. induced differentiation of human adipose tissue-derived MSCs into a pancreatic endocrine phenotype by modulating the culture conditions.[72] Differentiation medium altered the genetic makeup of MSCs and resulted in a profound increase in gene expression of insulin, glucagon, and somatostatin. In another study, researchers reported genetic manipulation of MSCs with pancreatic duodenal homeobox 1 (Pdx1) and the differentiation of MSCs toward β cell phenotype. Transplantation of differentiated β cells into streptozotocin induced diabetic immunodeficient mice further enhanced differentiation, including the induction of neurod1, and reduction of hyperglycemia.[73] The microenvironment of MSCs has also been manipulated to induce transdifferentiation to beta cell phenotype.[64] Considering the limited number of donors and immunogenicity with islet transplantation, transdifferentiated β cells could be a potential source of functional islets.

Myocyte lose in ischemic conditions irreversibly impair cardiac function. Several cell therapies including hematopoietic stem cells, endothelia progenitor cells, and MSCs have been shown to reduce apoptosis and improve myocardial injury.[74] However, to regain/repair damage to cardiac tissue, it is essential to identify a source of somatic cells that are able to regenerate cardiac myocytes. MSCs have demonstrated a high degree of plasticity and have undergone differentiation to cardiac tissues.[70, 75, 76] These studies have shown that de novo

generation of myocardium improved cardiac function. Moreover, findings of these studies prompted to generate ready-to-transplant cardiac myocytes from MSCs. In vitro differentiation of MSCs to cardiac myocytes has confirmed the phenotype, pharmacology, and electrophysiology of differentiated myocytes.[77–79] However, therapeutic efficacy of in vitro-generated cardiac myocytes from MSCs needs to be established in the animal models before moving to human clinical trials.

Ectodermal differentiation of MSCs has shown to attenuate the symptoms of central nervous system (CNS) disorders. Neurospheres, neurons, and astrocytes have been generated from MSCs by using defined culture conditions.[69, 80, 81] Transplantation of MSC-derived neurons led to functional improvement in a 6-hydroxy dopamine rat model of Parkinson's disease.[69, 82] The neuroregenerative potential of MSCs has also been tested in animal models of amyotrophic lateral sclerosis and Alzheimer disease.[83, 84] To summarize, MSCs have shown high plasticity and have undergone differentiation to tissues of ectoderm and endoderm. Transdifferentiation of MSCs can be used to treat diabetes, cardiovascular disorders, and neurological disorders.

GENE CARRIERS

MSCs can be used as gene carriers due to the ease of their transformation, nonimmunogenicity, and tropism to injured tissues. MSCs have been transduced with different vectors to optimize transgene expression.[85] Nonviral vectors, which require the use of cationic polymers and liposomes for condensation are plagued with the problem of poor transfection efficiency.[86] Although Adv transduction of MSCs has been demonstrated, transduction efficiency is poor because of low expression of coxsackievirus and adenovirus receptor (CAR) by MSCs.[87] Adv vectors have been modified to target integrin overexpressing on MSCs. RGD-modified adenovirus led to enhanced expression of BMP2 gene as compared to conventional Adv vector.[83] To further enhance transduction efficiency of Adv vectors, knobs were modified and compared for their transduction efficiency in MSCs.[88] As Adv transduction is associated with transient overexpression, it would be more suitable for MSC-based ex vivo gene delivery. However, in certain cases (e.g., treatment of life-long ailments), permanent transduction of MSCs may be desired, which can be achieved by modifying MSCs with retroviruses with high efficiency to obtain long-term expression.[89] However, transduction with retroviruses requires caution, as retroviruses integrate their genome with the host genome, and this action may render retroviral vectors unsafe for human clinical applications.[90]

Genetic modifications of MSCs have been performed to improve the MSC efficacy in tissue repair/regeneration, to modulate or promote MSC differentiation, and to improve the targeting of MSCs. MSCs have been genetically modified to improve their viability in acute cardiovascular disease.[91–93] Rat MSCs have been genetically modified to express anti-apoptotic protein Akt-I.[93] Ex vivo transduction of MSCs with retrovirus has improved the survival of injected MSCs, resulting in improved cardiac performance. MSCs have been transfected with polyethyleneimine (PEI)/plasmid complexes to improve the survival of MSCs in myocardial infarction. MSCs were genetically engineered with an anti-apoptotic *Bcl-2* gene and were evaluated for apoptosis, secretion of growth factors, engraftment, and cardiac function. Overexpression of Bcl-2 was associated with significant improvement of MSC survival in hypoxia. Enhanced survival of MSCs resulted in the improvement in infarct symptoms and cardiac function.[92]

Secretion of growth factors from MSCs has been supplemented by disease-specific proteins by using gene delivery to augment the tissue repair process. MSCs overexpressing neurotropic factors have demonstrated improvement in functional outcomes of amylotropic

lateral sclerosis.[4, 94] Overexpression of HGF, BDNF, IGF-1 and VEGF by MSCs resulted in functional improvements in disease models of liver transplantation, spinal cord injury, myocardial infarction, and hind limb ischemia, respectively.[18–21] All these studies have shown that overexpression of these growth factors confer additional cytoprotective effects and lead to tissue repair.

Islet destruction due to hypoxia, inflammatory and immune reaction limits successful application of islet transplantation. Gene therapy has been utilized to promote islet vascularization and prevent islet apoptotic death. As islet is a cluster of 1000 non-dividing cells, most non-viral approaches including cationic liposomes were ineffective in transfecting islets and were toxic at higher doses.[95, 96] Therefore, we constructed replication deficient (E1-, E3-deleted) adenoviral (Adv) vectors to improve the transduction efficiency.[97] We have previously used bipartite plasmid and Adv vectors expressing growth factors and cytokines receptor antagonists to abrogate cytokine-mediated insult and hypoxic milieu of transplanted islets.[98–100] However, one major concern with Adv-based gene therapy is the potential immunogenicity of Adv particles and the increased rejection of islets at higher multiplicity of infection (MOI). To avoid the transduction of islets with Adv vectors, we transduced MSCs to express different genes to improve islet transplantation. We demonstrated that transduction of human bone marrow derived mesenchymal stem cells (hBMSCs) with bipartite Adv vector co-expressing hHGF and hIL-1Ra improved islet transplantation (Fig. 3). Transduction of hBMSCs at low MOIs did not adversely affect the differentiation potential and secretion of growth factors by hBMSCs.[101] MSCs overexpressing hHGF and hIL-1Ra promoted revascularization, protected islet viability, and reduced islet mass required to restore euglycemia. These studies have shown that MSCs can be genetically modified to enhance their survival and differentiation and to augment the secretion of growth factors and immunomodulation to meet disease-specific requirements.[91–93, 101]

ROLE OF BIOMATERIALS IN MSC-BASED CELL THERAPY

Successful translation of MSCs from bench to bedside will depend upon efficient delivery and retention of viable MSCs at the site of injury. Material engineering approaches have been exploited to improve the retention and viability of transplanted MSCs. However, designing synthetic and natural polymers for systemic delivery of MSCs requires critical understanding of MSCs' in vivo niche of a highly hydrated network of insoluble proteins, growth factors, cytokines, chemokines, and ligands of surrounding cells.[102] This microenvironment provides extrinsic and intrinsic physicochemical signals controlling the replication and differentiation of MSCs.

Both synthetic and natural polymers have been used as biomaterials for MSCs. Natural extracellular matrix (ECMs) like collagen and fibrin confer inherent advantages like presentation of inherent ligands, elastic properties, and susceptibility to proteolytic degradation. However, complexities associated with purification, immunogenicity, and need for custom-made matrices for tissue-specific application have restricted the use of natural ECMs. This situation has led to synthesizing biomimetic polymeric scaffolds for tissue engineering. The key traits for polymeric scaffold include substrate elasticity, density, pore size, fiber dimensions, and substrate composition. Scaffolds generated from nano/microfibers and polymeric hydrogels have been used for tissue engineering. Electrospinning has allowed the generation of nanofibers down to 10 nM.[103] Nanofibers resemble the fibers of ECM components and provide high surface area for attachment and growth of MSCs. Nanofibers generated from polycaprolactone and poly(lactide co-glutamic acid) have been used for bone tissue engineering.[103, 104] Cross-linked hydrogels have also been used for engrafting MSCs.[105, 106] These cross-linked hydrogels simulate tissue-like features of high

water content, diffusion of fluids, and interstitial flow characteristics. Mild reaction schemes for cross-linking polymers to maintain the viability of encapsulated cells have allowed in situ generation of hydrogels. Cross-linked hydrogels have been modified to include cell-interacting ligands, enzyme-susceptible crosslinking, and manipulation of mechanical characteristics.[106, 107]

Polymeric scaffolds have been modified to provide localized delivery of growth factors to encapsulated MSCs. Nano/microparticles loaded with growth factors have been encapsulated along with MSCs to enhance the differentiation and viability.[108] TGF-β1-loaded microparticles have enhanced the osteocyte differentiation of encapsulated MSCs.[108] Biomimetic polymers surfaces have been created by covalent modification to present epidermal growth factors (EGF). Surface-tethered EGF promoted both cell spreading and survival more strongly than did saturated concentration of soluble EGF.[109] However, implantation of scaffold is an invasive procedure and requires customization for each application. For the delivery of MSCs, the ideal approach is an injectable system that would undergo transition at the physiological milieu to provide delivery and retention of MSCs. Modified chitosan has been reported to undergo thermosensitive sol-to-gel transition. Hydroxybutyl chitosan forms a gel at physiological temperature and provides a matrix for encapsulating MSCs. However, the extent of chitosan modification and polymer concentration needs to be tailored to manipulate matrix elasticity and mechanical strength.[110]

Molecular self-assembly is an exciting approach to generating novel supramolecular structures. Generating these structures is based on weak, noncovalent interaction. Self-assembling peptides have been reported to generate nanofiber microenvironments within myocardium.[111] Although self-assembling peptides did not have the biological signal for recruiting endothelial cells, nanofiber microenvironments recruited both endothelial markers expressing progenitor and vascular smooth muscle cells. This behavior could be attributed to the diffusion and selective binding of chemotactic factors in the peptide microenvironment. Transplantation of neonatal cardiomyocytes along with self-assembling peptides resulted in the survival of the cardiomyocytes in surrounding microenvironment and also augmented endogenous cell recruitment.[111] Haines-Buttreick et al. reported the synthesis of a 20-residue peptide that undergoes self-assembly to give mechanically rigid peptides.[112] These hydrogels undergo sheer thinning upon stress, and this property of peptides allows precise delivery of gel/cell through a syringe. Homogeneity of cell distribution and cell viability is unaffected by the injection process, and gel/cell constructs stay fixed at the point of introduction, rendering these gels useful for the delivery of cells to target biological sites in tissue regeneration efforts. In summary, successful application of material science in tissue regeneration/therapeutics will depend upon the knowledge of MSCs' in vivo niche; this knowledge will drive the synthesis and modification of polymers to control the proliferation, differentiation, and delivery of MSCs to targeted tissues.

CHALLENGES TO MSC-BASED THERAPY

Mixed results from preclinical animal studies have thrown new challenges in the field of MSC-based cell therapy. There is general agreement that poor delivery of MSCs to the target tissues, spontaneous malignant transformation of MSCs, unwanted differentiation of transplanted MSCs, and uniformity of biological properties of ex vivo-expanded MSCs are the potential challenges associated with successful MSC-based cell therapy (Table 3). To date, no one has reported adverse effects associated with systemic MSC infusion; however, considering the proliferation capacity and immunosuppressive ability of MSCs requires caution.[113–115]

Tumorigenicity

Although there are no reports of tumor formation in humans after MSC infusion, the risk of cancer with MSC therapy remains. There are conflicting reports of the spontaneous transformation of MSCs owing to different species, source, culturing techniques, and duration of ex vivo expansion.[26, 89, 90]

MSCs undergo aging on in vitro cultivation, thereby preventing spontaneous malignant transformation. Bernardo et al. showed that MSCs can be safely expanded ex vivo and that, upon long-term in vitro culture, they retained their morphologic, phenotypical, and functional characteristics.[26] Moreover, MSCs propagated in culture for 44 weeks maintained a normal karyotype, without showing expression of telomere maintenance mechanisms. MSCs expanded ex vivo presented telomere shortening as indicated by a progressive reduction in the mean telomere restriction fragment (TRF) length or appearance of shorter TRFs. Furthermore, Wagner et al. elucidated that replicative senescence of human MSCs is a continuous and organized process. Upon culturing, MSCs acquire morphological abnormalities, attenuated expression of surface markers, and altered differentiation potential. Gene expression profiling of MSCs on each passage has shown that genes involved in cell cycle, DNA replication, and DNA repair are significantly down regulated in late passages.[116]

Following systemic administration, murine MSCs have been shown to undergo tumorigenic transformation embolized in lung capillaries and invaded the lung parenchyma, forming tumor nodules.[89, 90] One million MSCs of passage 6 were injected intramuscularly and intravenously and animals were euthanized after 35 days to characterize the tumor development.[90] Immunohistochemical characteristics of cells from lesions displayed the characteristics of immature bone and cartilage, suggesting the role of trapped MSCs. Unlike human MSCs, MSCs from mouse can acquire chromosomal abnormalities after only a few in vitro passages. Moreover, other parameters such as mouse strain might also play a role in the induction of tumors.[117] In another study, murine MSCs were shown to promote a tumorigenic process in surrounding cells.[118] Host-derived sarcomas developed upon implantation of MSC/bioscaffold constructs into syngeneic and immunodeficient recipients, but not in allogeneic hosts or when MSCs were injected as cell suspensions. Bioscaffold provided a three-dimensional support for the aggregation of MSCs, thereby producing the stimulus for triggering the process eventually leading to the transformation of surrounding cells and creating a surrogate tumor stroma. However, it is critical to note that MSCs themselves had not undergone malignant transformation and immunomodulatory action of MSC-expanded clones of CD41CD251 T regulatory lymphocytes suppressed the antitumor immune response of the host.[118]

Another fundamental safety concern with MSC administration is that transplanted MSCs may undergo transformation to promote tumor growth, vascularization, and metastasis. Upon exposure to tumor-conditioned media for a prolonged period, MSCs acquired a carcinoma-associated fibroblast (CAF) phenotype. These MSC-derived CFAs promoted tumor progression after transplantation.[119] Although human MSCs compared to murine MSCs show poor susceptibility for oncogenic transformation, two recent studies described the capacity of MSCs to accumulate chromosomal instability and give rise to carcinoma in immunocompromised mice after long-term culture.[120] Some transient and donor dependent recurring chromosomal abnormality of MSCs was detected in vitro, independently of the culture process. In spite of chromosomal instability, MSCs showed progressive growth arrest and entered senescence without evidence of transformation either in vitro or in vivo.[120] Taken together, there are no reports of ex vivo and/or in vivo malignant transformation of human MSCs, even though there are reports of malignant transformation of murine MSCs.

Unwanted Differentiation

Unwanted differentiation of MSCs poses a major challenge for MSC-based cell therapy. Thirabanjasak et al. reported multiple angioproliferative and myeloproliferative lesions in a patient treated with autologous stem cells. Multiple percutaneous injections of stem cells in the presence of VEGF may be responsible for the lesions.[121] MSCs have been reported to generate hepatocytes in vitro and in vivo. However, transplantation of MSCs in mice with liver injury resulted in MSCs with myofibroblast phenotype. MSC differentiation to profibrogenic myofibroblasts instead of hepatocytes is unwanted and leads to worsening of liver injury.[122]

Systemic Delivery of MSCs

MSC-based clinical therapy relies on the tropism and engraftment of infused MSCs to the organ of interest. In vivo cell trafficking of infused MSCs has shown their entrapment in lungs, liver and spleen.[123, 124] Passive entrapment of MSCs has been associated with poor clinical outcome as compared to local infusions in certain myocardial ischemia (MI) models.[125, 126] Mounting evidence indicates that host MSCs as well as transfused MSCs actively home to the site of the inflammation or injury. MSC adherence to inflamed tissue is a multistep process involving distinct receptors and ligands. MSCs roll and tether primarily by integrins and selectin. MSCs undergo adhesion and firm attachment using integrin receptors VLA-4/VLA-5 and chemokines.[127] Chemokines contribute to the activation and upregulation of integrins, thereby contributing to the attachment of MSCs to ECs. However, there is not enough clarity regarding the transendothelial migration of MSCs. Chemokines and proteases have been shown to promote transendothelial migration of MSCs.[128]

Adhesion molecules and chemokine receptors play an important role in homing and engraftment of MSCs. This strategy can enhance the recruitment of transfused MSCs to tissues. With the goal of devising a noninvasive cell therapy, various approaches have been used to overexpress the chemokine receptors and adhesion molecules on the surface of MSCs.[129, 130] Chemical as well as genetic modification of MSCs has been carried out to improve the targeting of MSCs to tissues and prevent their passive entrapment in the lung and spleen. Although these studies have shown that surface engineering of MSCs can be carried out to enhance their delivery to the target tissues without affecting the viability or multipotency of MSCs, further work needs to be carried out.

CONCLUSION

MSCs are one of the most promising stem cells for improving the outcome of tissue engineering and organ/cell transplantation. Although MSCs have been used for tissue regeneration, their effect on immune cells and secretion of growth factors has opened new clinical avenues. MSCs as cell therapy have been successfully explored in animal models of organ/cell transplantation, treatment of autoimmune disease, and tissue repair. In spite of all the positive outcomes in preclinical models, certain key questions, including MSC identity, niche and normal physiological function, remain to be answered to maximize the clinical potential of MSCs. Isolation of MSCs from different organs has raised concerns regarding the peripheral presence of MSCs. It is critical to understand the physiological function of tissue-resident MSCs as it will help to determine whether MSCs act locally or systemically. Determining the site of action of MSCs in various disease models will help us to modify in vivo trafficking of MSCs.

Consensus is lacking regarding the timing, dose, immune status, and route of administration of MSCs in solid organ/cell transplantation disease models. It is important to understand whether MSCs distribute to lymphoid tissue or act locally to control the proliferation of

immune cells. Understanding the effect of immune cell activation and cytokine release on the immunomodulatory action of MSCs will help to address their administration pre- or post-transplantation.

Finally, the safety of MSC therapy remains the major concerns. Although no adverse effects has been reported with MSC-based therapy, their long-term effect on immunogenicity and tumorigenicity need to be considered. Furthermore, the effects of MSCs isolated from different organs and inconsistent culturing conditions on MSC biology need to be ascertained to prevent ectopic differentiation of administered MSCs. Path breaking work has been done in the field of stem cell biology since Friedenstein first reported the isolation of MSCs, however answers to these questions will herald new vistas in MSC-based cell therapy.

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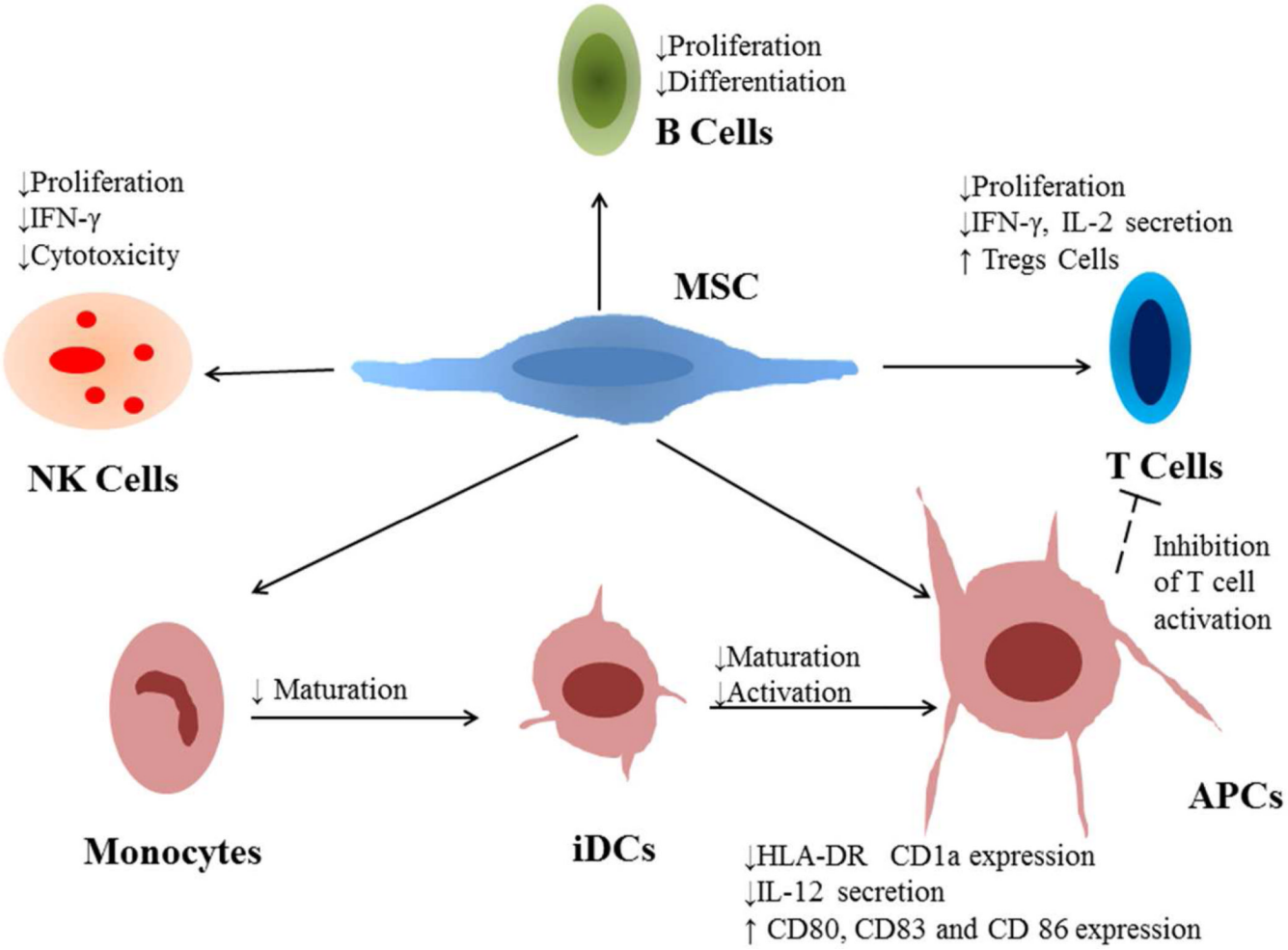

Figure 1. Immunomodulatory effect of MSCs

MSCs effect proliferation, maturation and cytokine secretion by different immune cells. NK cells, Natural Killer Cells; iDCs, Immature Dendritic Cells; APCs, Antigen Presenting Cells.

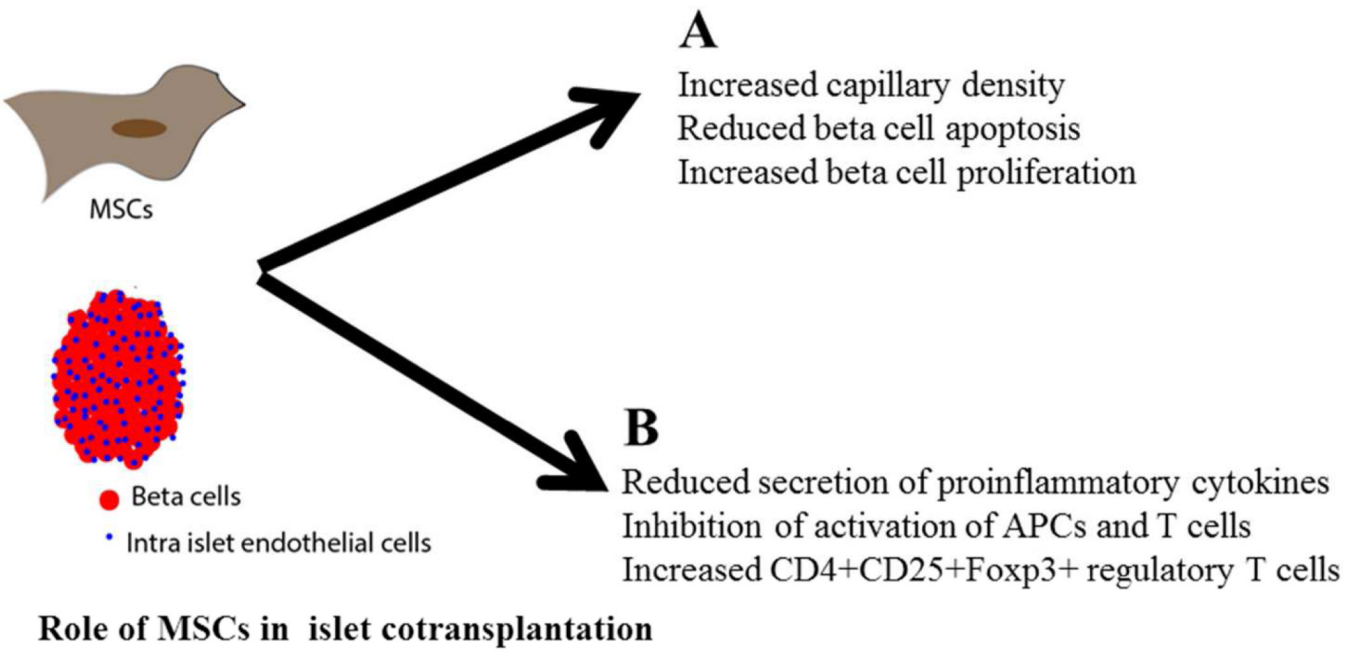

Figure 2. MSCs therapy to promote vascularization and reduction of immune rejection of transplanted islet

(A) Secretion of growth factors promotes proliferation, migration of endothelial cells, and revascularization of transplanted islet. MSCs promote beta cell proliferation and reduce apoptosis of graft. (B) MSCs inhibit the secretion of proinflammtory cytokines and activation of graft infiltrating immune cells.

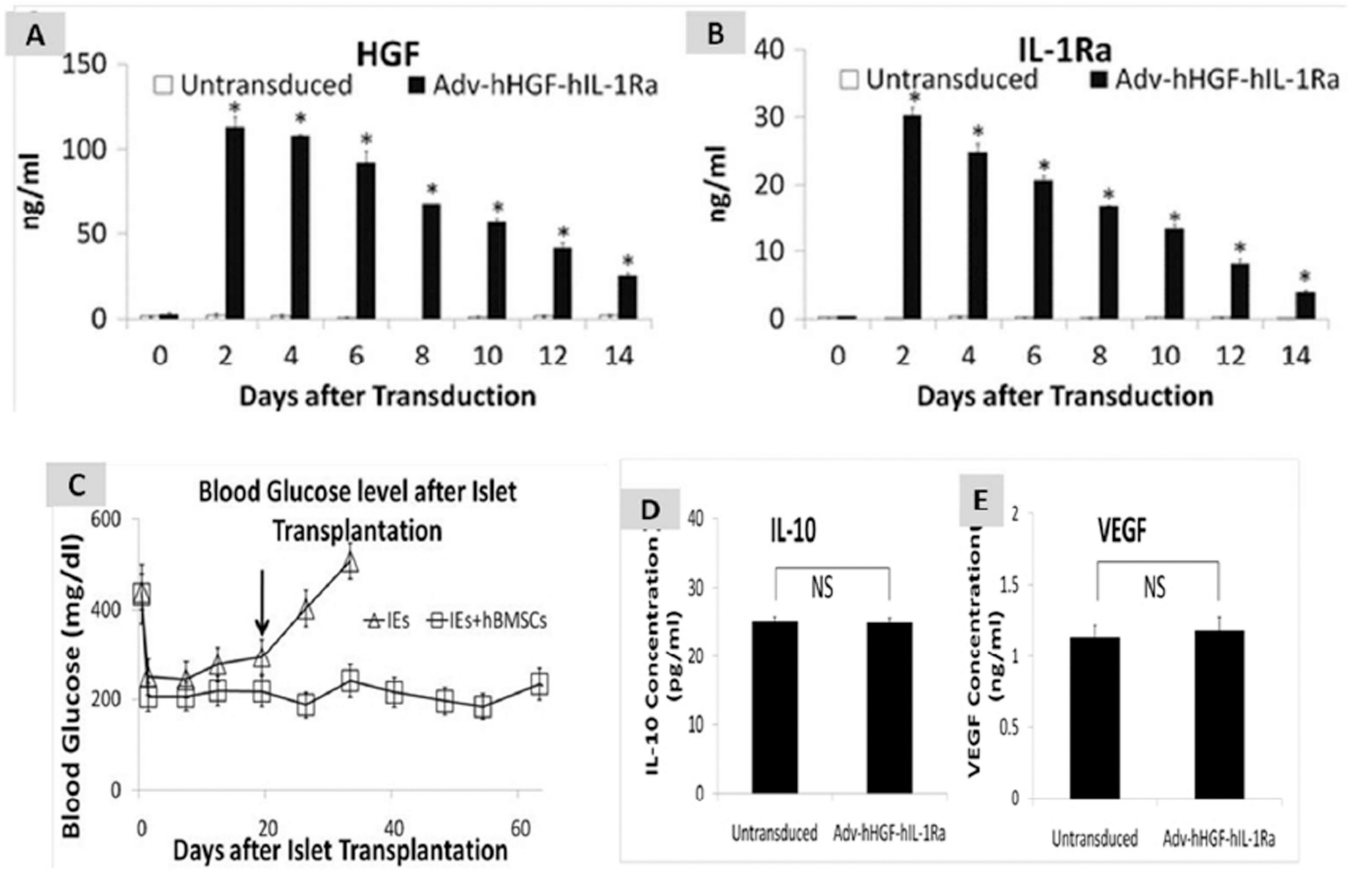

Figure 3. MSCs as gene delivery vehicles to promote human islet transplantation in streptozotocin induced diabetic mice

(A) Overexpression of hepatocyte growth factor by Adv-hHGF-hIL-Ra transduced MSCs. (B) Overexpression of human interleukin 1 receptor antagonist by Adv-hHGF-hIL-Ra transduced MSCs. (C) Genetically modified MSCs enhanced the islets revascularization and prevented cytokines injury and inflammatory reactions as evident in prolonged normoglycemia in islets + adenovirus transduced MSCs group. (D) Transduction of MSCs with adenovirus did not affect differentiation ability and secretion of growth factors as measured by levels of VEGF and IL-10

Table 1

MSC as immune therapy in solid organ transplant.

Disease Model	Experimental Design	Therapeutic Outcome
Rat heart transplantation model in fully MHC mismatched model	Donor/recipient/ third party mismatched MSCS were injected pre transplant with concurrent administration of MMF	Long term graft acceptance only with concurrent MMF therapy. MSCs injection alone lead to rejection of graft.[45]
Rat heart transplantation model in fully MHC mismatched model	Donor MSCS were injected with concurrent administration of cyclosporine	MSCs administration did not prolong graft survival.[43]
Mouse heart transplantation model in fully MHC mismatched model	Tail vein or portal injection of donor or recipient derived MSCs prior to transplantat in an unconditioned recipient mice	Peri transplant infusion of MSCs was less effective than pre transplant infusion.[44]
Human kidney transplant model	Induction regimen with maintenance immunosuppression with cyclosporine and MMF	Increase in number of Treg in peripheral blood, and reduced memory $CD8^+$ T cell function. Established safety and clinical feasibility of autologous MSC approach in human.[44]
Human GVHD	Infusion of HLA-identical sibling donors, haploidentical donors and third-party HLA-mismatched donors	Complete and partial improvement in GVHD. Response rate was not related to donor HLA-match.[16]
Skin grafts in baboon	Intravenous infusion of donor MSC on the day of transplant	Prolongation of skin graft survival in comparison to control animals.[13]

Table 2

MSCs as cell therapy to promote engraftment of transplanted islets for the treatment of type 1 diabetes.

Cell Source	Route/site of administration	Therapeutic outcome
Allogeneic bone marrow MSCs	Intraportal co-transplantation followed by IV infusions	Increased numbers of regulatory T-cells resulted in reversal of rejection episodes and prolongation of islet function.[51]
Allogeneic bone marrow MSCs	Intraportal co-transplantation	Improvement of islet graft morphology and graft revascularization. Transdifferentiation of MSCs to endothelial cells promoted revascularization.[52]
Allogeneic bone marrow MSCs	Renal capsule transplantation	Secretion of VEGE and growth factors promoted islet vasculatization and improved glycemic control.[53]
Autologous bone marrow MSCS	Omental pouch transplantation	Autologous MSCs improved graft survival through combination of grouth factors and increase of IL-10 secreting CD4+ T cells.[52]
Syngeneic and Allogeneic MSCs	Intraportal /systemic administration	Prolonged graft survival and reduced rejection of islet mass.[55]
Umbilical cord MSCs	Intra portal administration of differentiated pancreatic cells from umbilical cord MSC	Reduced blood sugar level by transdifferentiation of MSCs into insulin producing cells.[56]
Cocultue of mice islet with human cord blood MSCs	Renal capsule transplantation	Culturing of islet with MSCs promoted islet islet viability and insulin secretory function.[58]
Autologous MSCs	Renal capsule transplantation	Improved glycemic control by blocking CD25 T cell activation and IL-2 signaling.[59]

Table 3

Potential challenges to MSC-based islet transplantation.

- Safety profile of ex-vivo expanded MSCs
- Local vs. global site of action
- Mechanism of action of MSC in islet transplantation (immunomodulation vs. revascularization and proliferation of beta cells)
- Ex vivo transdifferentiation of MSCs to functional beta cells
- Optimization of dose, timing and co-immune therapy for islet transplantation
- Autologous vs. allogenic MSC therapy