Stem Cells and Cloning: Advances and Applications

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REVIEW

Potential clinical applications of adult human mesenchymal stem cell (Prochymal®) therapy

This article was published in the following Dove Press journal:
Stem Cells and Cloning: Advances and Applications
16 November 2011
Number of times this article has been viewed

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Abstract: In vitro, in vivo animal, and human clinical data show a broad field of application for mesenchymal stem cells (MSCs). There is overwhelming evidence of the usefulness of MSCs in regenerative medicine, tissue engineering, and immune therapy. At present, there are a significant number of clinical trials exploring the use of MSCs for the treatment of various diseases, including myocardial infarction and stroke, in which oxygen suppression causes widespread cell death, and others with clear involvement of the immune system, such as graft-versus-host disease, Crohn's disease, and diabetes. With no less impact, MSCs have been used as cell therapy to treat defects in bone and cartilage and to help in wound healing, or in combination with biomaterials in tissue engineering development. Among the MSCs, allogeneic MSCs have been associated with a regenerative capacity due to their unique immune modulatory properties. Their immunosuppressive capability without evidence of immunosuppressive toxicity at a global level define their application in the treatment of diseases with a pathogenesis involving uncontrolled activity of the immune system. Until now, the limitation in the number of totally characterized autologous MSCs available represents a major obstacle to their use for adult stem cell therapy. The use of premanufactured allogeneic MSCs from controlled donors under optimal conditions and their application in highly standardized clinical trials would lead to a better understanding of their real applications and reduce the time to clinical translation.

Keywords: regeneration, immunomodulation, tissue engineering, allogeneic, mesenchymal stem cells

Introduction

Mesenchymal stem cells (MSCs) are multipotent adult cells that were first isolated and characterized from bone marrow. They were further identified by their ability to attach to the plastic of tissue culture dishes.[1] In the bone marrow, the multipotent stromal mesenchymal cells that have been isolated are part of the marrow microenvironment, together with endothelial and reticular cells, adipocytes, osteoblasts, and macrophages.[2,3] In this context, MSCs are involved in many key events related to hematopoiesis, immune cell generation and activation, immunomodulation, and immune tolerance.[4] These processes are mediated by physical and chemical signals to which MSCs are responsive through phenotypic changes and growth factor production and secretion.[5] Furthermore, they are distributed in an undifferentiated state in their primary location throughout the bone marrow.[6] Most of the basic scientific and preclinical studies have been done using MSCs isolated from bone marrow. However, many different sources can be used, and fat could be especially relevant. Stem cells have been isolated from bone marrow aspirates, fat, striated, smooth, and cardiac

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muscle, the spleen, placenta, and umbilical cord blood, and may perhaps be isolated from other sources in which they are resident components or part of the reticular tissue associated with the vasculature.[7–17] Human MSCs do not express the hematopoietic markers CD11a/lymphocyte function-associated antigen 1, CD14, CD31, CD34, or CD45, or the costimulatory molecules CD80, CD86, and CD40. However, they do express CD44, CD49, CD54/CD102, CD71, CD73, CD90, CD105, CD166, and CD271, among other cytokines and receptors that define their behavior under different conditions[18–20] (Table 1). Given optimal stimuli, MSCs can differentiate into phenotypes of many different types of mesenchymal cells including, but not limited to, the connective tissue of different organs, stroma, fat, muscle, bone, cartilage, and tendon, along with endothelial and neural lineages.[15] It has been observed that the beneficial effects of MSCs are not restricted to a unique tissue source. Human MSCs derived from adipose tissue have been shown to have an effect similar to those of the bone marrow in a murine model of graft-versus-host-disease (GVHD).[21] MSCs can be isolated, expanded in culture, and characterized in vitro.[6,22] Due to these characteristics, MSCs have been used quickly in clinical trials and treatments. Autologous human MSCs were first infused in cancer patients, after a long culture period, without evidence of adverse effects.[23]

The goal of cell-based therapies is to use a strategy that includes a combination of activities that are aimed overall to replace, repair, or enhance the function of a cell type, tissue, organ, or system, using intact, amplified, or modified autologous or allogeneic cells. From the philosophical point of view, adult MSCs have a clear advantage over embryonic or fetal stem cells, in terms of basic biological aspects related to immunotolerance, differentiation, and transformation. MSCs are immunologically competent cells.[24] They are fully capable of undertaking an immunological response, and modulate some of the most important mechanisms in this complex response, and so have been used widely in a variety of alternatives for cell therapy.

Table 1 Surface markers for isolation and characterization of bone marrow mesenchymal stem cells

Positive	Negative
CD13	CD11a,b
CD29	CD14
CD44	CD18 integrin β2
CD49b integrin α2	CD31 PECAN
CD49e integrin α5	CD34
CD54 ICAM1	CD38
CD71 transferrin receptor	CD45
CD73	CD49d integrin α4
CD90	CD50 ICAM3
CD105	CD62E E-selectin
CD106	CD117
CD146	CD133
CD166	CD135
Nestin	HLA-DR
p75 LNGR	
HLA-ABC	

Allogeneic or syngeneic MSCs

Clinical use of allogeneic MSCs for preventing or treating acute GVHD dates back to the 1970s.[25] While more limited application of allogeneic MSCs has been undertaken in tissue engineering and reparative medicine. In this therapeutic alternative, in which syngeneic cells are routinely used, the use of allogeneic MSCs is mostly restricted to regeneration of bone and restoration of the myocardium. Bone marrow MSCs are mobilized in response to tissue injury, such as hypoxia, ischemia, or necrosis, along with a variety of mediators associated with tissue damage.[26–29] These cells migrate and settle in the damaged tissues and interact with resident cells and the stroma, secreting very important bioactive molecules that modify the redox potential, modulate apoptosis, induce cell proliferation, and recruit other cells, both stem and nonstem, which continue the reparative process and regulate the local immune response.[30–32] This complex chain of events results mostly in incomplete tissue regeneration, but is the basis of resolution of tissue damage. In any case, there are two major mechanisms that support the rationale for use of MSCs, ie, replacement of damaged cells and local delivery of bioactive molecules.[33] Replacement of cells is the goal of cell therapy for regenerative medicine, and the release of biological signals, as mediators and receptors, is the basis of many cellular processes, including immunomodulation induced by stem cells (Figure 1). These two mechanisms are clearly attributed to the effects reported after infusion of allogeneic MSCs. Meanwhile, additional immune effects are not clearly described in clinical trials for regenerative purposes using syngeneic cells. It is very likely and expected that both mechanisms are involved in the ideal reparative process.

Controversial data concerning the effects of MSCs on regulation of tumor growth have been reported for animal and in vitro models.[34–37] However, no tumors have been found in human recipients of MSCs thus far, and remarkably, even aneuploidy MSCs have not given rise to tumors.[36] It remains controversial as to whether MSCs stimulate growth of other tumors, but there is no clinical validation of this as yet.[37–39] The

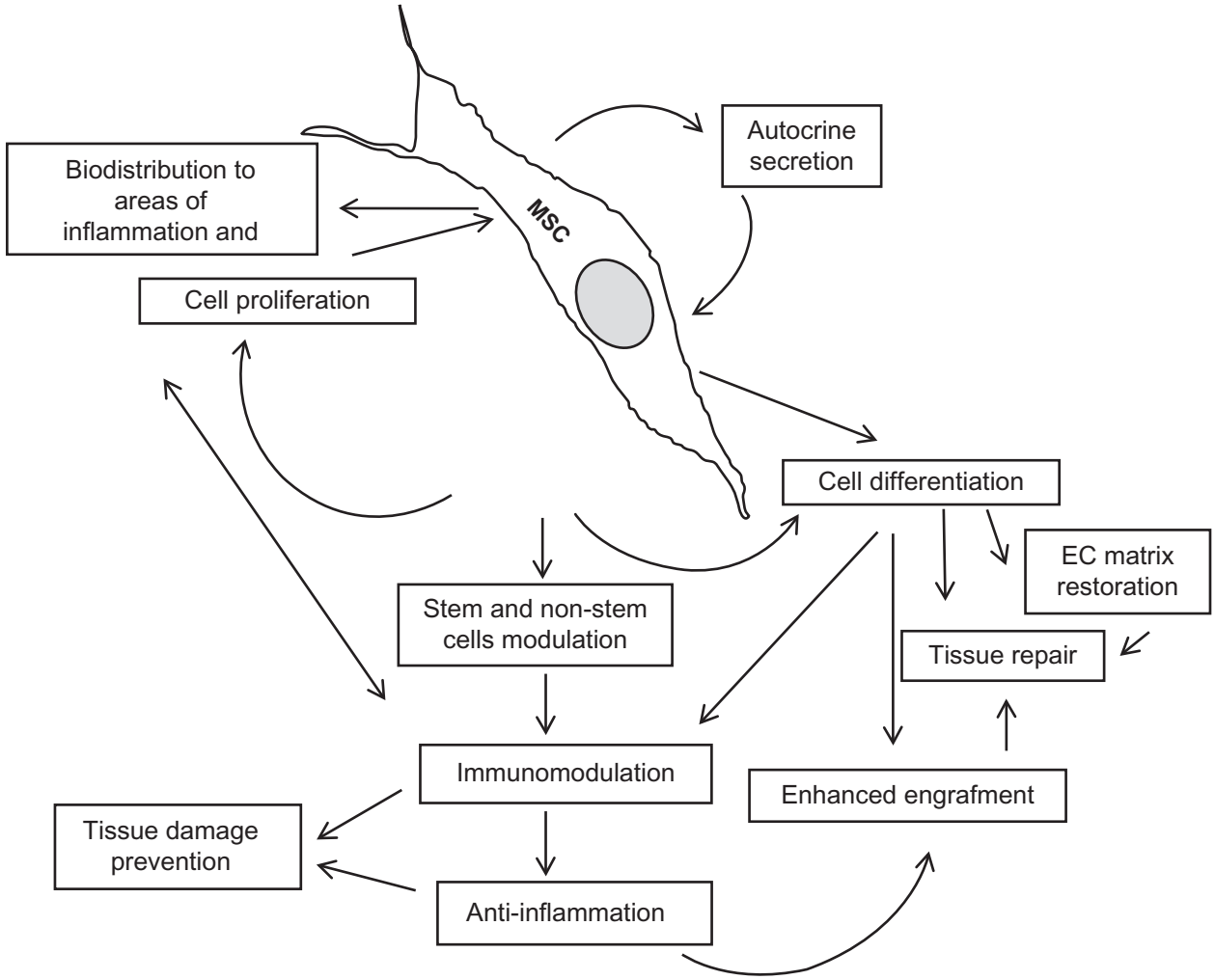

Figure 1 Key mechanisms involved in beneficial effects of cell therapy using allogeneic mesenchymal stem cells.

immunosuppression described in association with allogeneic MSC should be carefully evaluated in cancer patients.

Autologous MSCs have a clear advantage in that they constitute a closed therapeutic system. Their clinical use has been promoted based on avoidance of undesirable immune reactions and lack of contamination by unknown pathogens. In fact, the enormous amount of data generated for MSCs in regenerative medicine is based mainly on the use of autologous cells.

However, autologous MSCs have some obvious limitations. Their procurement requires surgical, albeit minimal, intervention via bone marrow aspiration in sometimes highly compromised patients. Many patients who could benefit from MSC infusion are elderly, in poor nutritional condition, and/or suffering from disorders associated with critical oxidative stress. With advancing age, significant changes in the function and composition of mature blood cells are observed. It has been reported that age-related changes also occur in the human hematopoietic stem cell system.[38] Similar results have been found in the MSC population obtained from bone marrow in animals.[39,40] These issues might also affect the conclusions and validity of many clinical trials, the results of which are difficult to compare because of the characteristics of the patients included. Another limitation to the use of autologous cells is pre-existence of a proliferative or degenerative disease of the bone marrow, which will not only limit the amount of available cells, but may contribute to the original disease. Cultured MSCs derived from multiple myeloma patients have a distinctive array comparative genomic hybridization profile from that observed in their normal counterparts.[41] This may explain why MSCs from myeloma patients show an altered functional pattern with potential involvement in worsening of disease.[42,43] A similar phenomenon can be seen in autoimmune diseases.[44] Further, the limited number of available autologous MSCs represents a major obstacle to their application. For a long time, it was

assumed that MSCs were a stromal cell population with a high capacity for in vitro expansion. In reality, this assumption included three critical issues that needed to be addressed, ie, use of a heterogeneous cell population, the culture period, the cell duplications required to achieve the desired number of cells, and the use of xenogeneic supplements and/or potent cytokines. Culture conditions could have important effects on the phenotype of MSCs, affecting their efficiency, potency, and safety via immunogenicity, sensitizing to animal antigens, allergic reactions, or chromosomal stability.[45–47] These effects can be avoided using defined media or autologous serum. Both procedures can prevent the formation of xenoantibodies and some of the undesirable reactions associated with administration of these cells.[48,49] Unfortunately, defined media requires addition of cytokines, the long-term effects of which, on MSCs in culture is not well understood, and autologous serum collection has obvious limitations.

In contrast with the aforementioned difficulties associated with autologous MSCs, allogeneic MSCs have evident advantages, ie, immediate availability, no limitation of amount, the ability to do a donor selection based on different parameters (including age), the small time frame needed to perform complex quality control, and product stability. In addition, but with special importance, allogeneic MSCs are natural immune-privileged cells, as demonstrated by their persistence in maternal blood.[50] However, it is necessary to have a careful balance between the advantages of using an "off-the-shelf" product and the characteristics of each particular case, the preferences of the patient, the necessity for a Good Manufacturing Practice facility, and extensive preclinical evaluation prior to their application.

Allogeneic MSCs in human therapy

As previously mentioned, allogeneic MSCs are immune-privileged cells. Perhaps for this reason, allogeneic MSCs have been more widely used in the area of immunotherapy than in regenerative medicine. Encouraged by the early application and promising results using autologous bone marrow cells in clinical trials, the most studied model of tissue regeneration using allogeneic MSCs has been myocardial infarction.[51] Allogeneic MSCs have also been used in this model for regenerative purposes. Intramyocardial injection of allogeneic swine MSCs three days after myocardial infarction stimulated cardiac regeneration, decreasing infarct size. The animals showed remarkable improvement in ejection fraction and near normalization, without any associated immune events.[52] No arrhythmogenic events were observed, even when multiple doses were used.[53] Allogeneic MSCs are able to migrate and persist in the infarcted area, and act in a dose-dependent manner when they are administered intravenously.[54] In an acute myocardial infarct model using a combined single-photon emission/computed tomography scanner for imaging of the labeled cells, both focal and diffuse uptake of allogeneic MSCs in the infarcted myocardium was visible in the first 24 hours after intravenous injection and persisted until seven days after injection.[55] In another swine model, allogeneic MSCs showed the capacity to survive and engraft when injected into the affected myocardium 12 weeks following induced infarction. In female swine that received catheter-based transendocardial injection of male allogeneic MSCs, Y chromosome-positive cells expressed GATA-4, Nkx2.5, and alpha-sarcomeric actin. Some of the cells showing a vascular smooth muscle and endothelial phenotype appeared to contribute to local angiogenesis.[56] Induction of angiogenesis in the long-term post infarction area has been confirmed in fully immunocompetent pigs, but limited to viable myocardium adjacent to the infarct.[57,58] Clearly, homing of allogeneic MSCs is associated with cooperative morphological and functional change in the restoration of damaged tissue.

The safety of intravenous injection of allogeneic MSCs has been reported in a randomized, double-blind, placebo-controlled, dose-escalation study using Prochymal® (Osiris Therapeutics Inc, Columbia, MD) after acute myocardial infarction. Prochymal is a premanufactured, universal donor formulation of human MSCs from different donors screened and tested according to US Food and Drug Administration requirements and processed under Good Manufacturing Practice guidelines in a scaled adaptation of the method described by Pittenger et al.[6] The ex vivo cultured MSC manufacturing process requires a total of five cell passages according to Food and Drug Administration Good Manufacturing Practice.[59] Global symptom score and ejection fraction was significantly improved in treated patients. After six months, a cardiac magnetic resonance imaging substudy showed that MSC treatment, but not placebo, increased left ventricular ejection fraction and led to reverse remodeling. There was also improvement in pulmonary function tests and a reduction in ventricular arrhythmias.[60]

The beneficial effects of allogeneic MSCs in cardiac repair should be evaluated further, considering the particular immune capacities of these cells. Inflammation is a critical factor in evolution of myocardial ischemia, and the immunomodulation exerted by allogeneic MSCs could be a major factor in the generation of a favorable microenvironment for muscle protection and repair.[61] In addition, allogeneic MSCs

could cooperate with local cells and provide the antiapoptotic activity necessary to limit the damage and sequelae of ischemic events. This activity is exerted by MSCs in the bone marrow, at the hematopoietic stem cell niche, where MSCs control the proliferation and differentiation needed to avoid the apoptotic events associated with quiescent cells.[18,62]

The capacity of allogeneic bone morphogenetic protein-2 (BMP-2)-engineered allogeneic MSCs to facilitate bone healing was studied in rats with a femoral segmental defect. The results showed that BMP-2-engineered allogeneic MSCs repaired bone defects to the same degree as in rats treated with BMP-2-engineered autologous MSCs. It was also demonstrated that allogeneic gene-transferred MSCs are directly involved in bone repair, in addition to acting as gene deliverers. The positive clinical benefits of allogeneic MSCs were dependent on their immunosuppressive and regenerative properties.[63] Nevertheless, another study using allogeneic MSCs loaded on hydroxyapatite-tricalcium phosphate implants enhanced, the repair of a critical-sized segmental defect in dog femurs without the use of immunosuppressive therapy. In this case, no adverse immune response was detected.[64] Furthermore, the absence of immunogenicity of allogeneic MSCs in orthopedics is an advantage for the clinical application of preconstructed tissue-engineered bone.[65] This lack of induction of an immune response should be considered a unique advantage in the use of genetically modified MSCs as carriers of therapeutic agents. The use of nonautologous genetically modified cells is beyond the scope of this review.

Allogeneic MSCs as immunomodulators

The first reported immunomodulatory activity of allogeneic MSCs was inhibition of T cell proliferation in vitro and in vivo.[66,67] As with their reparative activity in tissues, allogeneic MSCs act through intercellular interaction and release a myriad soluble bioactive factors. Allogeneic MSCs have an inhibitory effect on the activity of antigen-presenting cells which impacts on T cell function.[68] The immunosuppressive activity of allogeneic MSCs is exerted via inhibitory mediators, such as prostaglandin E2, transforming growth factor beta-1, hepatocyte growth factor, and the human leukocyte antigen G isoform.[67–70] Allogeneic MSCs induce upregulation of the indoleamine 2,3 intracellular pathway, dioxygenase expression, and inducible nitric oxide synthetase and heme oxygenase-1 that contribute to immune suppression.[71–73] MSCs inhibit $CD4^+$ and $CD8^+$ T cells, the cytotoxic function of resting natural killer cells, and generation of innate and adaptive immune regulatory cell populations. MSCs upmodulate secretion of interleukin-10 by dendritic cells, that acts on T cells and downregulates production of interferon-gamma and interleukin-2. It has also been suggested that MSCs suppress the B cell proliferative response with regard to generation of antibodies.[69]

Clinical application of allogeneic MSCs

In addition to the use of allogeneic MSCs in regenerative medicine, there has been a large amount of data generated in preclinical models of disease treated with allogeneic MSCs, mostly taking advantage of their capacity to modulate the local immune reaction. The first report of allogeneic MSCs acting as useful immunosuppressive agents demonstrated their capacity to prolong skin graft survival.[66] There is also diverse literature supporting the efficiency of allogeneic MSC infusion to enhance hematopoietic stem cell engraftment and prevention of GVHD.[74] Patient survival is poor when GVHD is unresponsive to steroid therapy. The successful use of allogeneic MSCs for the treatment of GVHD has been reported in isolated cases and multicenter nonrandomized trials.[75–77] Prochymal in combination with steroids has been used in patients with Grade II–IV GVHD. Study endpoints included safety of Prochymal administration, induction of response, and overall response of GVHD by day 28, as well as long-term safety. Ninety-four percent of patients had an initial response to Prochymal (77% complete response and 16% had a partial response). No infusional toxicity or ectopic tissue formation was reported. There was no difference with respect to safety or efficacy between low and high Prochymal doses.[78] Further, a beneficial effect of Prochymal infusion was observed in pediatric patients, and more than 50% of treated patients responded after only one dose of cells.[59] The response rate varies in different studies, and without a clear explanation for these different responses. As previously mentioned, the methodology to expand the cells appears to be a critical step in the generation of allogeneic MSCs as a therapeutic tool. It is clear that allogeneic MSCs are potential candidates for the treatment of other conditions in which immune disorders, such as autoimmunity, are part of the pathogenesis. For example, Prochymal cells are been used in clinical trials for Crohn's disease and osteoarthritis.[79]

MSCs have been shown to pass through the blood–brain barrier and migrate throughout the forebrain and cerebellum without disrupting the host brain architecture.[80] Administration of allogeneic MSCs to mice with pre-established experimental autoimmune encephalomyelitis led to a significant

decrease in the disease score over time comparable with that achieved with syngeneic MSCs. It was correlated with a blunting of immune cell infiltration in the spinal cord and reduced circulating levels of interferon-gamma and interleukin-17.[81] Human MSCs administered to mice with proteolipid protein-induced experimental autoimmune encephalomyelitis resulted in a reduction in disease severity, and this correlated well with an increase in axonal density and cells expressing nerve growth factor in association with immune modulatory events.[82,83] MSCs appear to act in the central nervous system according to two general mechanisms, ie, replacement of cells and intense paracrine activity. In a focal ischemia animal model of middle cerebral artery occlusion in the rat, xenotransplantation of human MSCs induced functional improvement, reduced infarct volume, and conferred neuroprotection, possibly by providing insulin-like growth factor-1 and inducing vascular endothelial growth factor, epithelial growth factor, and basic fibroblast growth factor in the host brain.[84] Other authors report upregulation of interleukin-10 and downregulation of tumor necrosis factor-alpha, and an even earlier decrease of infarct volume.[85] In a similar experiment, significant increases in brain-derived neurotrophic factor and nerve growth factor were detected, while the number of apoptotic cells was significantly reduced in the ischemic area. Exposure of neurons to brain-derived neurotrophic factor increased activation of Akt pathways and protected neurons from trophic factor withdrawal. Treated animals showed proliferation of lymphocytes without induction of cytotoxic T lymphocytes.[86,87] Beneficial effects in the treatment of neurological disorders are augmented by the high in vitro culture passages of MSCs, again stressing the relevance of culture technique and environment.[88]

Multiple sclerosis is a major neurological and autoimmune problem in medicine, in which anti-inflammatory treatments have been used in the repair of damaged tissue without major success. It has been assumed that the central nervous system lesions are irreversible. Cell-based therapies have the potential to provide an alternative approach, according to data obtained from animal models of inflammatory nervous disease. The results from an animal model of experimental autoimmune encephalomyelitis give the rationale for use of MSCs in multiple sclerosis. MSCs apparently have an immunoregulatory or immunosuppressive action, but also stimulate the repair of neural structures. Under experimental conditions, MSCs induce immune tolerance, production of neurotrophins, and inhibit production of myelin-specific antibodies.[83,89,90] This capacity of MSCs has interested neurologists for more reasons than just the above-mentioned transdifferentiation that can occur in them under certain circumstances.[91,92] Both autologous and allogeneic MSCs have been administered to a limited number of patients with multiple sclerosis. Preliminary data suggest the absence of major complications or toxicity with autologous expanded MSCs in patients with multiple sclerosis and amyotrophic lateral sclerosis.[93,94]

Based on a similar rationale, MSCs have been tested in different models of lung disease. Human allogeneic MSCs have been shown to restore alveolar epithelial fluid transport and the lung fluid balance from acute lung injury in an ex vivo perfused human lung preparation injured by *Escherichia coli* endotoxin. The treatment reduced extravascular lung water, improved lung endothelial barrier permeability, and restored alveolar fluid clearance. Of note, the authors refer to similar results using culture media conditioned by MSCs, and identify keratinocyte growth factor as essential for the beneficial effects.[95] Attenuation of obliterative bronchiolitis associated with trachea transplantation was observed in mice treated with MSCs. This effect was associated with a significant increase in secretion of interleukin-10 and a decrease in the expression of transforming growth factor-beta.[96] It has been reported that systemic injection of allogeneic MSCs protected the airway from allergen-induced pathology by reduction of IgE. This effect was associated with an increase in interleukin-10 and a decrease in interleukin-4 in bronchial fluid, and appears to be mediated by induction of regulatory T cells and secretion of immunosuppressive molecules, such as hepatocyte growth factor, which negatively regulates allergic airway inflammation and hyper-responsiveness.[97] The above data show the potential therapeutic use of allogeneic MSCs in many respiratory diseases, including chronic asthma.[98]

The regenerative and immunomodulatory properties of allogeneic MSCs make them natural candidates for the treatment of diabetes.[99] Ongoing clinical trials are evaluating the effects of bone marrow-derived (in most cases autologous) MSCs for engraftment and survival of transplanted islets as well as possibly halting the complications of type 1 and type 2 diabetes.[100]

Islets are destroyed in type 1 diabetes by an autoimmune process against beta cells, whereas in diabetes type 2, the islets have a functional alteration that results in inadequate glycemic control. Current clinical therapy using insulin and oral antidiabetic agents does not achieve complete metabolic control or avoid the complications associated with the disease. In this scenario, a genuinely substitutive therapy

Table 2 Clinical evaluation of autologous and allogeneic MSCs*

Trial ID	Sponsor	Condition	Intervention	Phase	Status
NCT00294112	Osiris Therapeutics	Crohn's disease	Prochymal™ adult human mesenchymal stem cells	II	Completed
NCT00136903	Osiris Therapeutics	Graft versus host disease	Prochymal, 2 and 8 million cells	II	Completed
NCT00543374	Osiris Therapeutics	Crohn's disease	Adult human mesenchymal stem cells	II	Completed
NCT00826046	Osiris Therapeutics–Quintiles	Graft versus host disease	Prochymal adult human mesenchymal stem cells	NV	NV
NCT00482092	Osiris Therapeutics	Crohn's disease	Adult human mesenchymal stem cells and placebo	III	Recruiting
NCT00683722	Osiris Therapeutics	Chronic obstructive pulmonary disease, emphysema Chronic bronchitis	Prochymal adult human mesenchymal stem cells and placebo	II	Ongoing
NCT00690066	Osiris Therapeutics and Juvenile Diabetes Research Foundation	Type 1 diabetes mellitus	Prochymal adult human mesenchymal stem cells and placebo	II	Ongoing
NCT00366145	Osiris Therapeutics	Graft versus host disease	Adult human mesenchymal stem cells and placebo	III	Completed
NCT00395200	University of Cambridge	Multiple sclerosis	Adult human mesenchymal stem cells	I and II	NV
NCT01233960	Osiris Therapeutics	Crohn's disease	Adult human mesenchymal stem cells	III	Recruiting
NCT00759018	Osiris Therapeutics	Graft versus host disease in pediatric patients	Prochymal adult human mesenchymal stem cells		Expanded access
NCT00284986	Osiris Therapeutics	Graft versus host disease	Prochymal adult human mesenchymal stem cells	II	Completed
NCT00877903	Osiris Therapeutics	Myocardial infarction	Prochymal adult human mesenchymal stem cells and placebo	II	Ongoing
NCT00702741	Osiris Therapeutics	Recovery following partial medial meniscectomy	Cultured adult human mesenchymal stem cells	I and II	Ongoing
NCT01087996	NHLBI	Chronic ischemic left ventricular dysfunction	Autologous and allogeneic adult human mesenchymal stem cells	I and II	Recruiting
NCT00587990	NHLBI	Chronic ischemic left ventricular dysfunction	Low and high doses of autologous human mesenchymal stem cells	I and II	Ongoing
NCT00114452	Osiris Therapeutics	Myocardial infarction	Ex vivo cultured adult human mesenchymal stem cells (Provacel™)	I	NV
NCT00768066	University of Miami	Left ventricular dysfunction	Autologous human mesenchymal stem cells, bone marrow cells, and placebo	I and II	Recruiting
NCT01206179	Royan Institute	Bone defects	Autologous human mesenchymal stem cells	I	NV
NCT00827398	UMC Utrecht	Graft versus host disease	Mesenchymal stem cells expanded with human plasma and platelet lysate	I and II	Recruiting
NCT00956891	Sun Yat-Sen University	Liver failure	Autologous bone marrow mesenchymal stem cells	I and II	Completed
NCT01318330	HomeoTherapy Co, Ltd	Graft versus host disease	Ex vivo cultured adult human clonal mesenchymal stem cells	I	Recruiting
NCT00885729	Oslo University Hospital	Cartilage defect of the knee	Autologous mesenchymal stem cells	I	Recruiting
NCT00767260	Fuzhou General Hospital	Type 2 diabetes mellitus	Autologous mesenchymal stem cell and bone marrow stem cells	I and II	NV
NCT00749164	Hadassah Medical Organization	Graft versus host disease	Allogeneic mesenchymal stem cells	I and II	Recruiting

(*Continued*)

Table 2 (*Continued*)

Trial ID	Sponsor	Condition	Intervention	Phase	Status
NCT00221130	Translational Research Informatics, Japan	Adult periodontitis	Ex vivo cultured mesenchymal stem cells	I and II	Completed
NCT01076920	University Hospital, Toulouse	Chronic myocardial ischemia and left ventricular dysfunction	Autologous mesenchymal stem cells	I and II	Recruiting
NCT00891501	Cairo University	Degenerative arthritis and chondral defects	Autologous mesenchymal stem cells	II and III	Recruiting
NCT00790764	TCA Cellular Therapy	Ischemic heart disease	Bone marrow-derived mononuclear and mesenchymal stem cells	II	Ongoing
NCT01223664	Sun Yat-Sen University	Liver cirrhosis	Allogeneic bone marrow stem cell transplantation	II	Ongoing
NCT01221454	Sun Yat-Sen University	Liver failure	Allogeneic bone marrow stem cell transplantation	II	Ongoing

Note: *ClinicalTrials.gov.

which restores functional pancreatic tissue appears to be the best alternative for patients with diabetes.

Bone marrow transplantation has been shown to contribute to the prevention of islet destruction,[101] and clinical trials have demonstrated the ability of allogeneic islet transplants to impact positively on glycemic control in patients with type 1 diabetes. However, the need for lifelong immunosuppression currently limits the indication of islet transplantation.[100,101] Another potential application of MSCs could be enhancement of allogeneic islet cell engraftment and survival. This property has already been demonstrated in a nonhuman primate model.[102] Additional infusions of donor-specific or third party MSCs resulted in reversal of rejection episodes in animals. In sublethally irradiated mice with type 1 diabetes induced by streptozotocin, serum blood glucose and insulin returned to normal levels in parallel with efficient tissue regeneration after a single injection of bone marrow cells and MSCs. The cell therapy was only effective when both types of cells were combined. This was the result of a reparative and not regenerative process, since no donor-derived cells were found in the pancreas of treated animals. Beta cell-specific T lymphocytes disappeared in the pancreas as a result of MSC injection, demonstrating a dual effect of cell repair and immunomodulation.[103]

In addition to the abovementioned immunomodulatory effect, there is evidence that MSCs are able to participate in the islet regenerative process on the basis of their capacity to generate insulin-producing cells.[104,105] These insulin-producing cells express multiple genes related to the development or function of pancreatic beta cells, including high expression of pancreatic and duodenal homeobox 1, insulin, and glucagon, and could release insulin in a glucose-dependent manner that led to amelioration of diabetes in streptozotocin-treated nude mice.[104,105] It is interesting that in vivo hyperglycemia appears to be an important factor in bone marrow-derived MSC differentiation into insulin-producing cells capable of normalizing hyperglycemia in a diabetic animal model.[106–108]

The well studied effects of MSCs on angiogenesis and myogenesis also have importance in the treatment of the cardiovascular complications of diabetes. MSCs induce myogenesis and angiogenesis by releasing angiogenic, mitogenic, and antiapoptotic factors, including vascular endothelial growth factor, insulin-like growth factor-1, adrenomedullin, and hepatocyte growth factor.[109] Transplanted MSCs were shown to differentiate into cardiomyocytes and improve myogenesis and angiogenesis, with improvement in cardiac disorders.[109] This effect has also been attributed to the release of MSC-derived paracrine factors capable of cardioprotection. Autologous MSCs have been successfully used in the treatment of severe diabetic limb ischemia.[110] Bradycardia, decreased left ventricular pressure, decreased contractility index, and increased arterial pressure occur in diabetic animals because of cardiac sympathetic nerve impairment.[111] It is accepted that insulin can improve cardiac function by its inotropic effect of reducing blood glucose levels to prevent further myocardial remodeling.[112] Treatment of diabetic rats with allogeneic MSCs results in a significant increase in heart rate, left ventricular pressure, and contractility index, as well as a notable reduction of systolic blood pressure. These results appear to be associated with lowering of serum glucose and increased serum insulin levels, with homing of implanted cells detected in the pancreas and heart.[113] In mice with streptozotocin-induced type 1 diabetes, injection of MSCs reduced albuminuria, and the glomeruli were histologically normal. In the corresponding control group, untreated diabetic mice showed glomerular

hyalinosis and mesangial expansion, in association with pancreatic islet degeneration.[114] Data from studies using NOD/SCID mice transplanted with human MSCs and C57Bl/6 mice transplanted with murine MSCs indicate that injected MSCs engraft in damaged kidneys, differentiate into renal cells, and regulate the immune response, resulting in efficient treatment of diabetic nephropathy because MSCs are able to reconstitute the necrotic segments of diabetic kidneys.[114–116] Due to their intense paracrine activity, with release of angiogenic and neurotrophic factors, as well as their already mentioned potential capacity to convert in bone marrow mononuclear cells, MSCs have been studied for their capacity to improve diabetic neuropathy and associated wound healing impairment.[117,118] A list of clinical trials using autologous and allogeneic MSCs for a wide range of conditions is showed in Table 2.

Conclusion

In vitro, in vivo animal, and human clinical data show a wide range of potential applications for MSCs. There is overwhelming evidence of their usefulness in regenerative medicine, tissue engineering, and immune therapy. Although adult MSC transformation can be observed in vitro and in animals, no malignant transformation of implanted cells has been reported in patients. Infusion of allogeneic MSCs appears to be free of major hazardous events and does not raise any ethical issues, such as those related to use of human embryonic stem cells. MSCs migrate to sites of tissue injury in response to local signals, with critical phenotypic changes and intense paracrine activity that contributes to the reparative process. Among the MSCs, allogeneic MSCs have unique immunomodulatory properties. Their immunosuppressive capabilities without evidence of immunosuppressive toxicity at a global level define their application in the treatment of diseases in which the pathogenesis involves uncontrolled activity of the immune system. Infusion of allogeneic MSCs or their coinfusion with autologous MSCs has shown promising results in GVHD, diabetes, lung injury, Crohn's disease, and multiple sclerosis. It is important to mention the data related to the regenerative capacity of allogeneic MSCs in cardiac and neural disease, as well as in diabetes-related loss of functional mass in the kidney. In addition, the capacity of allogeneic MSCs to facilitate engraftment of other cells and their potential association with smart scaffolds used to seed other stem cells with regenerative purposes should be explored further.

The limited availability of autologous MSCs has been a major obstacle to their application. This has been resolved by extended culture methods. However, this generalized procedure is not free of potential risks. The variability in culture methods conspires to make the establishment of standard treatment procedures and generation of definitive data for clinical application more difficult. Minimal changes in the culture procedure will produce heterogeneous cell populations. The longer the culture period, the greater the risk of cytogenetic changes being observed in vitro. The use of supplements to reduce culture time may have undesirable and unpredictable effects on the immunogenicity and biological activity of the cells implanted. The use of premanufactured allogeneic MSCs from controlled donors under optimal conditions and their application in highly standardized clinical trials would lead to a better understanding of their clinical applications and reduce the time to clinical translation.

Disclosure

The authors report no conflicts of interest in this work.

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