Mesenchymal stem cells in the treatment of pediatric diseases

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Background: **In recent years, the incredible interests in mesenchymal stem cells have boosted the expectations of both patients and physicians. Unlike embryonic stem cells, neither their procurement nor their use is deemed controversial. Moreover, their immunomodulatory capacity coupled with low immunogenicity has opened up their allogenic use, consequently broadening the possibilities for their application. In May 2012, Canadian health regulators approved Prochymal, the first mesenchymal stem cells-based drug, for acute graft-versus-host diseases in children who have failed to respond to steroid treatment. The aim of this article is to review the recent advances in mesenchymal stem cells for pediatric diseases.**

Data sources: **A literature review was performed on PubMed from 1966 to 2013 using the MeSH terms "mesenchymal stem cells", "clinical trials" and "children". Additional articles were identified by a hand search of the references list in the initial search.**

Results: **The following categories are described: general properties, mechanisms of action, graft-versus-host diseases, cardiovascular diseases, liver diseases, inflammatory bowel diseases, osteoarticular diseases, autoimmune diseases, type 1 diabetes, and lung diseases.**

Conclusions: **Mesenchymal stem cells, owing to their availability, immunomodulatory properties, low immunogenicity, and therapeutic potential, have become one of the most attractive options for the treatment of a wide range of diseases. It is expected to see more and more clinical trials and applications of mesenchymal stem cells for pediatric diseases in the near future.**

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Review article

Introduction

The concept of stem cells originated at the end of the 19th century as a theoretical term to account for the ability of certain tissues (blood, skin, etc.) to self-renew for the lifetime of an organism even though they are composed of short-lived cells. The term mesenchymal stem cells (MSCs), a synonym for stromal stem cells, was first coined in 1991 by Caplan.[1] The literature can be traced to classical experiments demonstrating that transplantation of bone marrow (BM) to heterotopic anatomical sites resulted in *de novo* generation of ectopic bone and marrow.[2] The revolutionary work came from Friedenstein et al,[3] when they first reported the development of fibroblast colonies in monolayer cultures of guinea-pig BM and spleen cells. They later discovered a minor subpopulation of BM cells called stromal stem cells were responsible for transferring the microenvironment of hematopoietic tissues.[4] The concept of MSCs in BM did not receive worldwide attention until additional similar work was published in 1999 by Pittenger et al.[5] During the last 15 years, the MSCs field has experienced a major boost. Their capacities for differentiation and immunoregulatory functions have made them an outstanding candidate for the treatment of many clinical diseases such as graft-versus-host diseases (GVHD), myocardial infarction, autoimmune diseases, osteoarticular diseases, etc. In the following paragraphs, the properties of MSCs, mechanisms of action, as well as preclinical and clinical investigations, especially in disorders of children, will be discussed in detail.

MSCs: general properties

MSCs are a heterogeneous group of progenitor cells. This wide variety of origins, methodologies, and

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acronyms prompted standardization in 2005 by the International Society for Cellular Therapy, which set minimum requirements for MSCs definition. MSCs are defined as being plastic adherent, bearing certain stromal surface markers (CD73, CD90, and CD105), and lacking hematopoietic cell markers such as CD11a, CD14, CD19, CD34, CD45, and major histocompatibility complex (MHC) class II. In addition, they should have adipogenic, osteogenic, and chondrogenic differentiation potential.[6] Apart from meeting the minimum requirements, MSCs also have the ability to differentiate into cells not only in the mesenchymal lineage but also cells in ectodermal (epithelial cells and neuroglial-like cells) and endodermal (muscle cells, lung cells, gut epithelial cells, and hepatocyte-like cells) lineages.[5,7] However, the differentiation capacity of MSCs remains relatively limited under physiological environment.[8] An important subject in regenerative medicine is to promote the differentiation potential of MSCs through priming. Priming human MSCs with integrin α5 was shown to stimulate osteoblast differentiation and osteogenesis.[9] Tsai et al[10] reported MSCs primed with valproate and lithium robustly migrate to infarcted regions and facilitate recovery in a stroke model. Priming of MSCs with oxytocin enhances the cardiac repair in a rat model of ischemia/reperfusion injury.[11]

The BM has been historically the prime source of MSCs. Although this is not entirely understood, BM-MSCs are thought to act as supporters and nurturers of other cells within the marrow.[12,13] Despite the fact that there is a relatively small population (0.0001% -0.01% of nucleated cells in human BM), MSCs can be easily purified by plastic adherence and expanded after BM extraction.[5] However, the harvest of BM is a highly invasive procedure and the number, differentiation potential, and maximal life span of MSCs from BM decline with increasing age.[14,15] Because of its ease of procurement and the ability to bank cells from the prospective patient, the adipose tissue has garnered significant attention in the field over the past few years.[16] Another MSCs-rich, easily bankable tissue that could potentially be used is the umbilical cord blood.[17] No significant differences concerning the morphology and immune phenotype of the MSCs derived from these three sources are obvious. The colony frequency was the lowest in umbilical cord blood, whereas it was the highest in adipose tissue. The umbilical cord blood MSCs could be cultured in the longest period and showed the highest proliferation capacity, whereas BM-MSCs possessed the shortest culture period and the lowest proliferation capacity.[18]

MSCs treatment has been proven to be safe so far. A meta-analysis of 8 studies with randomized control trials did not detect an association of MSCs administration with acute infusional toxicity, organ system complications, infection, death or malignancy. Nevertheless, there was a significant association between MSCs and transient fever.[19] However, the safety of MSCs in clinical applications is in controversy. Several reports in animal models have raised concerns about tumor formation by using MSCs. This was observed after *in vitro* growth of murine MSCs derived from the BM.[20,21] However, malignant transformation of transplanted human MSCs has not yet been noted so far.[22] Increased risk of thrombosis is another concern. Moll et al[23] reported short-term expanded MSCs triggered only weak blood responses *in vitro*, while extended culture and coculture with activated lymphocytes increased their prothrombotic properties. After systemic infusion to patients, increased formation of blood activation markers was detected with a dose of $1\text{-}3\times10^6$ cells per kilogram of body weight.[23] There is also ongoing discussion on whether to use allogeneic or human leukocyte antigen (HLA)-matched MSCs for a number of therapies. A remarkable unique feature of MSCs is that they are considered to be immune-privileged as they express low levels of cell-surface HLA class I molecules, whereas HLA class II, CD40, CD80, and CD86 are not detectable on the cell surface.[24-26] Stimulation with interferon (IFN)-γ has been shown to increase both class I and class II molecules. However, MSCs do not express co-stimulatory molecules CD80 (B7-1), CD86 (B7-2) or CD40, even after IFN-γ stimulation.[24] These features allow MSCs to escape from the immune surveillance.[27] Therefore, therapeutic potency, safety, and efficacy of the treatment with MSCs might reside to a large extent in their immunologically privileged phenotype and in their immunosuppressive capacity. Although some claim a proposed immunoprivilege of MSCs primarily through the inhibition of effector functions which would prevent their rejection in an allotransplantation setting,[28] others report that MSCs lose their immune privilege upon differentiation[29] or are rejected right away.[30] Thus, it has been suggested that effects of MSCs on the recipient's immune system may be affected not only by cell-to-cell interactions but also by environmental factors not yet fully understood.[28]

With the application of MSCs in the clinical setting, issues have been raised regarding how to expand these cells with good-manufacturing practice (GMP).[31] Most existing expansion protocols use DMEM supplemented with fetal bovine serum (FBS). However, FBS is an undesirable source of xenogeneic antigens and bears the risk of transmitting animal viral, prion, and zoonose contaminations.[32] In some cases, immunological reactions and anti-FBS antibodies have been observed

and considered as having possibly affected the therapeutic outcomes.[33] As an alternative for FCS, platelet lysate, both autologous and allogeneic human serum, and serum free medium have been applied for MSCs expansion. The use of allogeneic human serum has been reported to result in MSCs growth arrest and death in some studies.[34] The application of autologous serum allows a faster proliferation compared to FBS at least during the first few passages, avoids the exposure to allogeneic antigens, and minimizes the risk of infection. Nevertheless, the amount of autologous serum required for a sufficient expansion exceeds the amount a donor could provide.[35] Plate lysate is derived by mechanical disruption of platelet concentrates through repeated freezing and thawing or chemical lysis of the membrane. Subsequent centrifugation steps separate the platelet debris from the supernatant, including all bioactive platelet factors presenting within the platelets.[36] Platelet lysate is capable of shortening human MSCs culture duration and then minimizes the risk of entering senescence and transformation.[37] However, human serum or platelet lysate still contains ill-defined factors, which vary from donor to donor and can exert different biological effects on MSCs proliferation and differentiation. In terms of standardization in GMP, only a chemically defined medium can be regarded as ideal. One of such xeno-free and serum-free culture systems has recently been reported.[38]

MSCs: mechanisms of action

MSCs act via multifaceted pathways that are not completely identified. The potential mechanisms through which MSCs display their reparative/regenerative effects after tissue damage include the capacity to home to sites of injury, the ability to release anti-inflammatory soluble factors, and the capacity to modulate immune responses.[39-41] Because MSCs have ability to differentiate into various cell types, it was initially thought that engraftment and differentiation into injured tissues were the mechanisms involved in their regenerative properties. In fact, engrafted MSCs have been identified at sites of injury along with improvements in regeneration and function.[42,43] However, it is difficult to correlate the extent of limited engraftment with dramatic functional improvement. The secretory or paracrine function of MSCs has been proposed to be the other mechanisms of MSCs effects.

It has been demonstrated that MSCs release several soluble molecules and chemokines, either constitutively or following cross-talk with other cells. These include indoleamine 2,3-dioxygenase, prostaglandin-E2 (PGE2), transforming growth factor-β (TGF-β), tumor necrosis factor (TNF)-stimulated gene 6 (TSG-6), and nitric oxide (NO).[39,44] Release of IFN-γ by damaged target cells is able to induce the release of indoleamine 2,3-dioxygenase by human MSCs, which, through the depletion of tryptophan, results in an antiproliferative effect.[45,46] MSCs constitutively produce PGE-2 that is enhanced by stimulation with IFN-γ and TNF-α[47] as well as by toll-like receptor 3 (TLR3) but not TLR4 ligands.[48] A large body of data supports the role of MSCs-derived PGE-2 in the suppression of T-cell activation and proliferation both *in vitro* and *in vivo*.[46,49] In addition to T lymphocyte-specific effects, PGE-2 produced by MSCs has an important role in MSCs reprogramming of macrophages[50,51] and dendritic cells.[52] More recently, MSCs have been shown to inhibit mast cell function through a COX-2-dependent mechanism.[53] Several studies[27,54] have identified TGF-β1 as one of the key factors of immunomodulation by MSCs. By using neutralizing monoclonal antibody, TGF-β1 was identified as the mediator of MSCs in a cell culture system.[27] TGF-β1 also suppresses production of proinflammatory cytokines by impact on macrophages and lymphocytes.[55] TSG-6 is an interleukin-1 (IL-1)/TNF-α inducible protein with anti-inflammatory properties. MSCs-derived TSG-6 was shown to mediate protective effects in murine models of myocardial infarction,[56] corneal injury,[57] allogeneic corneal transplant,[58] and zymosan-induced peritonitis.[59] In all models, TSG-6 inhibited the early inflammatory response including neutrophil infiltration and pro-inflammatory cytokines. NO is produced as a result of the enzymatic reaction of inducible NO synthase and has the capacity to inhibit T-cell proliferation and induce T-cell apoptosis. Treatment of MSCs with IFN-γ, TNF-α, or IL-1 induced expression of inducible NO synthase in mouse MSCs.[60] MSCs-derived NO induced apoptosis of alloreactive T cells through suppression of signal transducer activation of transcription-5 phosphorylation.[61] MSCs production of NO enhanced cardiac allograft survival,[62] attenuated delayed-type hypersensitivity responses through induction of T-cell apoptosis,[63] and prevented GVHD.[60]

The anti-inflammatory/antiproliferative effect of MSCs might also be due to their ability to stimulate the generation/differentiation of regulatory T cells (T_{reg}). MSCs favor the generation of T_{reg} and this corresponds with a decrease in Th1, Th2 and Th17 lymphocytes.[64,65] The regulation of MSCs on T_{reg} requires cell contact as well as PGE-2 and TGF-β1, and these purified T_{reg} were shown to functionally suppress alloreactive T lymphocyte proliferation.[47] In an experimental murine model of Crohn's disease, the infusion of MSCs was associated with the induction of $FoxP3^+$ T_{reg} cells, which was efficacious in both preventing and curing colitis.[41]

Moreover, it has been recently demonstrated that heme oxygenase-1 produced by human MSCs is able to promote the formation of Tr1 and Th3 T_{reg} cells *in vitro*, and this process is influenced by the environment in which MSCs and target cells interacts.[66]

Graft-versus-host diseases

Hematopoietic stem cell transplantation (HSCT) is an effective therapeutic modality for a variety of hematological disorders such as leukemia. However, GVHD after allogeneic HSCT is associated with a high mortality, especially in the case of steroid-resistant GVHD.[67] Because of the immunosuppressive properties, MSCs therapy is ideal for treating GVHD. Several studies have evaluated the effect of MSCs together with HSCT on engraftment, safety, and GVHD in pediatric patients (Table). Ball et al[70] reported a Phase I/II trial in which 14 children received 1-3.3 million donor MSCs/kg body weight 4 hours before peripheral blood HSCT of HLA-disparate relative donors. While they observed a graft failure rate of 15% in 47 historic controls, all patients given MSCs showed sustained hematopoietic engraftment without any adverse reaction. Approximately 14% of treatment group patients developed acute GVHD versus 30% in historical controls. In a study reported by Bernardo et al,[68] a total of 13 pediatric patients (median age 2 years, range 0.8-14) with hematological disorders were enrolled and received co-transplantation of umbilical cord blood (UCB) cells and parental-derived MSCs. The results of these 13 patients were compared with those obtained in a group of 39 historical controls (median age 4 years, range 0.8-17). They observed no differences in hematological recovery or rejection rates compared with 39 matched historical controls, most of whom received granulocyte-colony stimulating factor (G-CSF) after UCB transplantation. However, the rate of grade III and IV acute GVHD was significantly decreased in the study cohort when compared with controls, thus resulting in reduced early treatment related mortality (TRM). Although these data do not support the use of MSCs in UCB transplantation, they suggest that MSCs, possibly because of their immunosuppressive effect, may abrogate life-threatening acute GVHD and reduce early TRM.[68] Macmillan et al[69] conducted a Phase I/II clinical trial in 15 pediatric patients receiving parental MSCs co-transplanted with unrelated donor UCB. Patients received a dose of 0.9-5 million MSCs/kg body weight MCSs, 4 hours before transplantation of UCB and three of them were given a second dose at day 21. The cumulative incidence of grade II-IV acute GVHD by day 100 was 38%. All three patients receiving the second dose had grade II acute GVHD and were treated successfully with methylprednisolone. No patient developed chronic GVHD. Although there was a trend toward improved 3-year survival after transplant in the MSCs group compared with the historical controls, it was not statistically significant.[69]

Other studies have evaluated the effectiveness of

Table. MSCs for treatment of pediatric graft-versus-host diseases (GVHD)

Study	Patient number	MSCs sources	HSCT sources	Dose	Response
Bernardo et al[68] 2011	13	Paternal BM MSCs	Umbilical cord blood	1-3.9 million/kg, single dose at day of HSCT	Reduced grade III-IV GVHD compared to historic controls
Macmillan et al[69] 2009	15	Parental BM MSCs	Umbilical cord blood	0.9-5 million/kg at day of HSCT, three patients received second dose at day 21	Three patients developed acute grade II GVHD, and no patient developed chronic GVHD
Ball et al[70] 2007	14	Haploidentical relative BM MSCs	Haploidentical relative	1-3.3 million/kg, single dose at day of HSCT	14% in MSCs group developed acute GVHD versus 30% in historical controls
Prasad et al[71] 2011	12	Prochymal™ (HLA mismatched unrelated BM MSCs)	HLA matched or mismatched	8 million/kg/dose in 2 patients and 2 million/kg/dose in the rest patients twice a week for 4 wks started at a median of 98 days post-transplant	Of the 12 stage III or IV acute GVHD patients, 7 (58%) patients had complete response, 2 (17%) partial response, and 3 (25%) mixed response
Lucchini et al[72] 2010	11	HLA mismatched unrelated BM MSCs	HLA matched or mismatched	0.7-3.7 million/kg/dose, two doses	Overall response in 71.4% of GVHD patients, with complete response of 23.8%
Fang et al[73] 2007	2	Haploidentical adipose MSCs	HLA matched	1 million/kg/dose, single dose approximately 110 days post transplant	Complete response in both patients
Wu et al[74] 2011	2	HLA mismatched unrelated umbilical cord MSCs	Peripheral blood HLA matched in one Umbilical cord blood HLA mismatched in the other	One patient 3.3-8.0 million/kg/dose for 3 doses, the second patient 4.1 million/kg/dose for 1 dose	Acute GVHD improved dramatically

MSCs: mesenchymal stem cells; BM: bone marrow; HLA: human leukocyte antigen; HSCT: hematopoietic stem cell transplantation.

MSCs in treating existing GVHD (Table). Fang et al[73] reported the use of human adipose-derived MSCs as salvage therapy for the treatment of severe GVHD. Their study included 2 pediatric patients who developed severe GVHD after HLA matched unrelated HSCT. The GVHD was successfully treated with adipose-derived MSCs from HLA-mismatched unrelated donors. Lucchini et al[72] reported a multicenter study of 11 pediatric patients diagnosed with acute or chronic GVHD and treated for compassionate use with unrelated HLA-disparate MSCs. Eleven patients received intravenous MSCs for acute GVHD or chronic GVHD, which was resistant to multiple lines of immunosuppression. The median MSCs dose was 1.2 million/kg (range: 0.7-3.7 million/kg). No acute or chronic side effects were reported at a median follow-up of 8 months (range: 4-18 months). Overall response was obtained in 71.4% of patients, with complete response in 23.8%. None of the patients presented GVHD progression upon MSCs administration, but 4 patients had GVHD recurrence 2 to 5 months after infusion. Two patients developed chronic limited GVHD. MSCs efficacy seems to be greater in acute GVHD than in chronic GVHD even after failure of multiple lines of immunosuppression. Recently, Wu et al[74] infused *in vivo* expanded umbilical cord-derived MSCs into 2 patients with severe, steroid resistant acute GVHD. They reported that umbilical cord blood-derived MSCs have higher proliferative potential and more immunosuppressive effect compared with BM-MSCs. The acute GVHD improved dramatically in both patients after MSCs treatment.

Another study used Prochymal™ (Osiris Therapeutics, Inc., Baltimore, Maryland) in 12 children with acute GVHD. All patients had stage III or IV gut symptoms and half had additional liver and/or skin involvement. The GVHD was refractory to steroids in all patients and additionally to a median of 3 other immunosuppressive therapies. The MSCs were started at a median of 98 days (range: 45-237 days) post-transplant. The MSCs (8×10^6 cells/kg/dose in 2 patients and 2×10^6 cells/kg/dose in the rest) were infused intravenously twice a week for 4 weeks. Partial and mixed responders received subsequent weekly therapy for 4 weeks. Overall, complete response was seen in 7 (58%) patients, partial response in 2 (17%), and mixed response in 3 (25%). Complete resolution of gastrointestinal symptoms occurred in 9 (75%) patients. Two patients relapsed after initial response and showed partial response to retreatment. The cumulative incidence of survival at 100 days from the initiation of Prochymal™ therapy was 58%. Five (42%) of the 12 patients were alive after a median follow-up of 611 days (range: 427-1111 days). No infusion or other identifiable acute toxicity was seen in any patient.[71] The favorable observation in patients with an otherwise grave prognosis indicated that MSCs hold potential for the treatment of acute GVHD. Results from this study paved the way for the approval of Prochymal™ in children with acute GVHD by health authorities of Canada and New Zealand.

Cardiovascular diseases

MSCs transplantation has been shown to significantly improve cardiac function in several animal models, as measured by end systolic and diastolic volumes, and left ventricular ejection fraction.[75,76] Related to improve cardiac function, MSCs-treated animals have a reduced mortality rate.[75] The mechanisms behind these beneficial effects are not entirely clear, but appear to be a combination of increased myocardial perfusion, reduced scar formation, regeneration of cardiomyocytes, and recruitment and activation of endogenous progenitor populations.[77,78]

On the basis of rigorous preclinical testing in animal models, clinical trials have been initiated for acute myocardial infarction (MI), ischemic cardiomyopathy, and heart failure. Phase I/II clinical data have been reported with intravenous therapy,[79] intracoronary infusion,[80] and intramyocardial injection.[81] Intravenous MSCs therapy was exemplified by a randomized, double-blind, placebo-controlled study of 53 patients, in whom the allogeneic MSCs product, Prochymal™, was evaluated after acute MI. MSCs were delivered via peripheral intravenous route within 10 days of percutaneous intervention for MI. The allogeneic product was safely tolerated despite preclinical data highlighting the risk of pulmonary entrapment after systemic administration.[82] Six-month benefit was reported for left ventricular ejection fraction and reverse remodeling in patients with anterior infarcts and, moreover, there was evidence for a reduction in arrhythmic events.[79] Chen and colleagues[80] investigated the effects of intracoronary infusion of autologous BM-MSCs ($8\text{-}10\times10^9$, n=34) or saline (n=35) in patients with subacute MI. Positron emission tomographic imaging showed improvement in perfusion defects at 3 months after BM-MSCs therapy, and left ventriculography demonstrated improved ejection fraction (EF) and left ventricular (LV) chamber sizes in MSCs-treated patients compared with placebo. Importantly, this study showed that intracoronary MSCs infusion in MI patients was safe, with no deaths reported during follow-up, and electrocardiographic monitoring showed no arrhythmias. In a trial of intramyocardial delivery of MSCs, eight patients with ischemic cardiomyopathy received transendocardial

injection of autologous BM-MSCs in LV scar and border zone. All patients tolerated the procedure with no serious adverse events. Cardiac MRI at one year demonstrated a decrease in end diastolic volume, a trend toward decreased end systolic volume, decreased infarct size, and improved regional LV function by peak in the treated infarct zone. Improvements in regional function were evident at 3 months, whereas the changes in chamber dimensions were not significant until 6 months. Improved regional function in the infarct zone strongly correlated with reduction of end diastolic volume and end systolic volume.[81] Recently, a prospective, multicenter, randomized MSCs trial was conducted in patients with heart failure of ischemic origin who received intramyocardially standard-of-care or standard-of-care plus autologous lineage-specified BM-MSCs. In the cell therapy arm, MSCs were exposed to a cardiogenic cocktail. LVEF was improved by cell therapy versus standard-of-care alone, and was associated with a reduction in left ventricular end-systolic volume. Cell therapy also improved the 6-minute walk distance, and provided a superior composite clinical score encompassing cardiac parameters in tandem with New York Heart Association (NYHA) class, quality of life, physical performance, hospitalization and event-free survival.[83]

Rupp et al[84] reported for the first time intracoronary administration of autologous BM-derived progenitor cells in a 2-year-old child with severe dilated cardiomyopathy [NYHA III, B-type natriuretic peptide (BNP)=1150 pg/mL]. 270 million autologous total BM-derived progenitor cells were administered to the patient by an intracoronary bolus injection with the stop-flow technique on the same day of BM harvest. After low-pressure inflation (<1 atm) of a 2×20 mm coronary balloon dilation catheter placed in the left anterior descending artery (LAD), cells were infused over three minutes twice without any complications. The left ventricular injection fraction augmented from 24% one month after stem cell therapy up to 41% three months later and 45% six months after stem cell therapy. During this observation period, BNP values decreased from 787 to 191 pg/mL corresponding to an improvement of the functional class to NYHA II three months after stem cell therapy and NYHA I six months after stem cell therapy.[84]

Another report showed the beneficial effect of MSCs in an 11-year-old boy with severe dilated cardiomyopathy (NYHA IV).[85] About 6 mL of the prepared material containing 4.8×10^6/mL autologous MSCs were injected into the proximal left main coronary artery through a five-Fr left Judkins catheter. It was delivered in five portions with 3-minute intervals between injections. The clinical condition of the patient improved gradually after the procedure with cardiac functional class changed from initial IV to III and II. The need for hospitalization was reduced substantially. Pathologic review of the myocardial specimen after stem cell administration showed a small number of newly generated myofibers.[85]

Liver diseases

Orthotopic liver transplantation is the only proven effective treatment for fulminant hepatic failure (FHF), but its use is limited because of organ donor shortage, high costs, and the requirement for lifelong immunosuppression. MSCs therapy may have the potential to become a new avenue for the treatment of FHF. Kuo et al[86] injected MSCs-derived hepatocytes and undifferentiated MSCs intrasplenically or intravenously into immunodeficient mice with carbon tetrachloride–induced liver failure. The administration of both types of cells rescued the animals from hepatic failure, which was accompanied by an attenuated oxidative stress and an accelerated repopulation of hepatocytes. In a D-galactosamine-induced rat model of acute liver injury, systemic infusion of MSCs-conditioned medium provided a significant survival benefit by preventing the release of liver injury biomarkers. Furthermore, the therapy resulted in a 90% reduction of apoptotic hepatocellular death and a three-fold increment in the number of proliferating hepatocytes, suggesting that soluble factors released by MSCs might be capable of promoting regeneration of hepatocytes.[87] In a small-for-size liver graft model in rats, implantation of MSCs overexpressing hepatocyte growth factor through the portal vein prevented liver failure and thus reduced animal mortality.[88] Khuu et al[89] demonstrated that transplanted adult liver MSCs were able to differentiate in the non pre-conditioned SCID mouse liver mainly in the peri-portal area. In response to partial hepatectomy, integrated adult liver MSCs proliferated and participated in liver regeneration of recipient mouse.

In a phase I trial, four patients with decompensated liver cirrhosis were treated with autologous MSCs infusion through a peripheral vein. Both end-stage liver disease scores and the quality of life of the patients improved twelve months after treatment.[90] Another study was conducted in patients with end-stage liver disease caused by hepatitis B, hepatitis C, alcoholic liver disease, and cryptogenic fibrosis. There was a modest but significant improvement in liver function without severe adverse effects, suggesting that MSCs might be useful for the treatment of end-stage liver disease with satisfactory tolerability.[91] Recently, Zhang et al[92]

reported human umbilical cord MSCs improved liver function and ascites in patients with decompensated liver cirrhosis. Another study[93] showed short-term efficacy of MSCs in liver failure patients was favorable, but long-term outcomes were not markedly improved.

Inflammatory bowel diseases

The immunomodulatory effects of MSCs on dendritic cells, B and T lymphocytes provide a therapeutic option for patients with chronic inflammatory bowel diseases who are refractory to all established therapies. One phase I trial was conducted to treat Crohn's fistulas with autologous adipose-derived MSCs. Eight fistulas in four patients were inoculated with MSCs. Six fistulas were considered healed as the external openings were covered with epithelium at the end of week 8. In the other two fistulas, there was only incomplete closure of the external openings with a decrease in output flow. No abscess or other complication occurred within the follow-up of 24 months.[94] A more recent publication by the same group confirmed the therapeutic success of MSCs therapy in a phase II trial. Patients with complex perianal fistulas were randomly assigned to intralesional treatment with either fibrin glue or fibrin glue plus adipose-derived MSCs. Among the 24 patients treated with MSCs, the authors[95] observed a significant superiority on fistula occlusion of 71% versus 16% in fibrin glue alone.

In addition to adipose-derived MSCs, BM-MSCs have also been tested to treat Crohn's disease. In one study, BM-MSCs were isolated and expanded in nine patients with refractory Crohn's disease. MSCs isolated from patients with Crohn's disease showed similar morphology, phenotype and growth potential compared with MSCs from healthy donors. Importantly, immunomodulatory capacity was intact, as MSCs from patients significantly reduced peripheral blood mononuclear cell proliferation *in vitro*. Patients received two doses of 1-2×10^6 cells/kg body weight intravenously 7 days apart. Baseline median Crohn's disease activity index (CDAI) was 326 (224-378). Three patients showed clinical responses with CDAI decrease ≥70 from baseline to 6 weeks post-treatment; conversely 3 patients required surgery because of disease worsening.[96] Ciccocioppo et al[97] documented the efficacy of intrafistular injections of autologous BM-MSCs every 4 weeks in 10 Crohn's disease patients. Sustained complete closure was observed in 7 patients and an incomplete closure in 3 patients with a parallel reduction of CDAI and perianal disease activity indexes. The percentage of mucosal and circulating regulatory T cells significantly increased during the treatment and remained stable until the end of follow-up.[97] MSCs have also been shown to reduce gut GVHD. Prasad et al[71] reported 12 children having grade III and IV acute GVHD with gut symptoms were treated with Prochymal™. The MSCs were infused intravenously over 1 hour twice a week for 4 weeks. Gut involvement responded to MSCs and achieved a complete response in 9 patients. The remaining 3 patients responded with a 1 to 2 stage improvement in their gut symptoms.

Osteoarticular diseases

Results in mouse model bearing segmental fractures indicated that mobilization of BM-MSCs with AMD3100 provided significant augmentation of bone growth as determined by micro-CT and histomorphometry.[98] Obermeyer et al[99] reported pre-injury alcohol exposure resulted in a significant impairment in biomechanical strength and a decrease in callus volume. MSCs transplants restored both fracture callus volume and biomechanical strength in animals with alcohol-impaired healing. Cell imaging demonstrated a time-dependent MSCs migration to the fracture site. Diekman et al[100] showed intra-articular delivery of MSCs prevented the development of post-traumatic arthritis after 8 weeks. Cytokine levels in serum and synovial fluid were affected by treatment with MSCs, including elevated systemic IL-10 at several time points. In a report from Dufrane's group, a critical size femoral defect and a four-level spinal fusion in pigs were used to assess the ability of MSCs to achieve bone formation. An osteoblastic three-dimensional autologous graft made of adipose-derived MSCs was developed to solve this issue. The autograft was obtained by supplementing the osteoblastic differentiation medium with demineralized bone matrix. New bone formation was demonstrated in both animal models as determined by micro-CT scan and histology/histomorphometry.[101]

One patient was reported to have novel maxillary reconstruction with ectopic bone formation by adipose-derived MSCs.[102] The patient underwent a hemimaxillectomy due to a large keratocyst. The defect was reconstructed with a microvascular flap using autologous adipose-derived MSCs, beta-tricalcium phosphate and bone morphogenetic protein-2. The flap had developed mature bone structures and vasculature in the defect area. In 2011, first donor-less trachea transplant was achieved in a 36-year-old man who had tracheal cancer. First, a glass scaffold that was an exact replica of the original windpipe was made using results from a CT scan. The scaffold was incubated with autologous BM-MSCs. A brand new trachea was created in just a few days. The new trachea has since been transplanted into the patient.[103]

In 1999, Horwitz el al[104] reported the initial results of allogeneic BM transplantation in three children with osteogenesis imperfecta, a genetic disorder in which osteoblasts produce defective type I collagen, leading to osteopenia, multiple fractures, severe bony deformities and considerably shortened stature. Three months after osteoblast engraftment (1.5%-2.0% donor cells), representative specimens of trabecular bone showed histologic changes with new dense bone formation. All patients had increases in total body bone mineral content ranging from 21 to 29 grams, compared with predicted values of 0 to 4 grams for healthy children with similar changes in weight. These improvements were associated with increases in growth velocity and reduced frequencies of bone fracture.[104] Another study from the same group demonstrated the feasibility of MSCs therapy. They used neomycin-marked, BM-derived MSCs to treat six children who had undergone standard BM transplantation for severe osteogenesis imperfecta. Each child received two infusions of the allogeneic MSCs. Five of six patients showed engraftment in one or more sites, including bone, skin, and marrow stroma. Patients had an acceleration of growth velocity during the first 6 months post-infusion. This improvement ranged from 60% to 94% of the predicted median values for age- and sex-matched unaffected children, compared with 0% to 40% over the 6 months immediately preceding the infusions. There was no clinically significant toxicity except for an urticarial rash in one patient just after the second infusion. Failure to detect engraftment of cells expressing the neomycin phosphotransferase marker gene suggested the potential for immune attack against therapeutic cells expressing a foreign protein.[105] Le Blanc et al[106] reported a female fetus with multiple intrauterine fractures, diagnosed as severe osteogenesis imperfecta, underwent transplantation with allogeneic HLA-mismatched male fetal MSCs in the 32nd week of gestation. At 9 months of age, bone biopsy revealed 0.3% of XY-positive cells in a specimen. Whole Y genome fluorescent in situ hybridization staining showed a median of 7.4% Y-positive cells. Bone histology showed regularly arranged and configurated bone trabeculae. Patient lymphocyte proliferation against donor MSCs was not observed in co-culture experiments performed *in vitro* after MSCs injection. Complementary bisphosphonate treatment began at 4 months. During the first 2 years of life, three fractures were noted. At 2 years of corrected age, psychomotor development was normal.[106] Another study was conducted to evaluate the enhancing effect of recombinant platelet derived growth factor on MSCs in secondary alveoloplasty in children. Three children with 4 alveolar defects were selected for this study. MSCs were mounted on biphasic scaffolds and combined with platelet derived growth factor in the operating room to make a triad of the scaffold, growth factor, and cells. The triads were placed in anterior maxillary cleft defects and closed with lateral advancement gingival flaps. The postoperative cleft bone volume was measured with cone beam CT scans. A mean of 51.3% fill of the bone defect was calculated 3 months post-operation.[107]

Autoimmune diseases

The immunomodulatory properties of MSCs make them an ideal tool for treating autoimmune diseases. In systemic lupus erythematosus (SLE) patients, there are conflicting results on the therapeutic use of MSCs. One publication concerned two young lupus patients (19 and 25 years old) who were administered intravenously autologous BM-MSCs at a dose of 1 million/kg.[108] No adverse effects or changes in SLE disease activity indices (SLEDAIs) and British Isles Lupus Activity Group were noted during 14 weeks of follow-up, although circulating T_{reg} cells increased markedly. Four months after the infusion, one patient with previous kidney involvement had a renal flare requiring methylprednisolone pulses and cyclophosphamide.[108] Liang et al[109] reported 15 cyclophosphamide failing patients including three children with persistently active SLE who received a single intravenous infusion of 1 million/kg BM-derived MSCs. All patients improved clinically following MSCs treatment with a marked decrease in the SLEDAI score and 24 hours proteinuria. At 12-month follow-up, SLEDAI scores decreased from 12.2 to 3.2 and proteinuria decreased from 2505 to 858 mg/24 hours. At 1-year follow-up, 2 out of 13 patients had a relapse of proteinuria, whereas the other 11 continued to have decreased disease activity on minimal treatment. Anti-dsDNA levels decreased and nonrenal-related manifestations also improved significantly. No serious adverse events were reported. The percentage of T_{reg} significantly increased at 1 week, 3 months, and 6 months after MSCs administration.[109] The same group also studied whether double MSCs transplantation is superior to single transplantation. Fifty-eight refractory SLE patients including several children were enrolled in this study, in which 30 were randomly given a single dose of MSCs, and the other 28 were given two doses of MSCs. Patients were followed up for survival rates, disease remission, and relapse, as well as transplantation-related adverse events. SLEDAI and serologic features were evaluated. The results showed no remarkable differences between the single and double doses of MSCs infusion in terms of disease remission, relapse, amelioration of disease activity, and serum indexes with more than one year follow-up. This study demonstrated that single

MSCs transplantation at the dose of one million MSCs per kilogram of body weight was sufficient to induce disease remission for refractory SLE patients.[110]

In patients with rheumatoid arthritis, the number of mesenchymal progenitor cells in synovial fluid is reduced.[111] This might be explained by an impaired recruitment of MSCs to the joint[112] or a suppressed proliferation potential of MSCs[113] associated with decreased telomere length.[114] Calkoen et al[115] performed a comparative analysis of BM-derived MSCs from children with juvenile idiopathic arthritis (JIA) and healthy pediatric controls and reported some unexpected findings. MSCs were successfully expanded from 11 patients with JIA and 10 controls. MSCs from patients with JIA and controls showed no differences in their immunosuppressive effect using control peripheral blood mononuclear cells. The immunosuppressive effect of both MSCs groups was diminished in the presence of indomethacin. MSCs from patients with JIA and controls suppressed interleukin-2-induced natural killer cell activation to a similar extent. In addition, MSCs of patients with JIA and controls inhibited the differentiation of monocytes to dendritic cells. The comparable immunosuppressive characteristics of MSCs derived from patients with JIA to age-matched controls support the potential use of patient-derived MSCs in the treatment of JIA.[115]

Type 1 diabetes (T1D)

The World Health Organization estimates 347 million people worldwide have diabetes. As many as 3 million Americans may have T1D, and each year, more than 15 000-40 000 children are diagnosed with T1D in the United States. Historically, approaches aiming to cure T1D have made a negligible number of patients insulin-independent. A successful approach should preserve the remaining β-cells, restore β-cell function, and protect the replaced insulin producing cells from autoimmune destruction.[116] The use of MSCs holds great promise for the cure of T1D due to their ability to differentiate into insulin producing cells and immunological characteristics.[117] Hisanaga et al[118] reported that addition of activin A and betacellulin accelerated MSCs differentiation, and immunoreactive insulin was detected 14 days after the treatment. Insulin-containing secretory granules were observed in differentiated cells by electron microscopy. MSCs treated with conophylline and betacellulin responded to a high concentration of glucose and secreted mature insulin. When these cells were transplanted into streptozotocin-treated mice, they markedly reduced the plasma glucose concentration, and the effect continued for at least 4 weeks. Another study[119] showed that MSCs, when cultured under defined conditions, were induced to trans-differentiate into insulin-producing cells. Furthermore, these insulin-producing cells formed aggregates that, upon transplantation into mice, acquired architecture similar to islets of Langerhans. These aggregates showed endocrine gene expression for insulin (I and II), glucagon, somatostatin, and pancreatic polypeptide. Immunohistochemistry also confirmed that these aggregates were positive for insulin, somatostatin, pancreatic polypeptide and C-peptide.[119] In the nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mouse model, ELISA assays demonstrated that blood levels of mouse insulin were higher in the human MSCs-treated as compared with untreated diabetic mice, but human insulin was not detected. There was also an increase in pancreatic islets and β cells producing mouse insulin. Most of the β cells in the islets were mouse cells that expressed mouse insulin. In the kidneys of human MSCs-treated diabetic mice, human cells were found in glomeruli.[120]

Few clinical trials using MSCs for T1D are ongoing. The first is a US-based trial on the use of allogeneic MSCs (Prochymal™) to determine safety and efficacy in patients including children affected by T1D *(www.ClinicalTrials.gov; identifier, NCT00690066*). Patients from 12 to 35 years old with newly diagnosed T1D will receive either Prochymal or placebo. University of Sao Paulo is also conducting a trial studying safety and efficacy of MSCs in newly-diagnosed T1D patients including children (*www.ClinicalTrials.gov; identifier, NCT01322789*). Patients aged from 12 to 35 years with recently diagnosed (less than 6 weeks) T1D, proven by anti-pancreatic beta cell antibodies, will be included in this study. First, BM-derived adult MSCs will be collected from a first degree relative and cultured. After that, the patients will receive 4 intravenous infusions of MSCs with 1 week apart followed by 4 infusions with 4 months apart. In China, a clinical trial is recruiting participants to evaluate the efficacy of autologous transplantation of MSCs in patients aged from 10 to 40 years with T1D (*www.ClinicalTrials.gov; identifier, NCT01157403*).

Lung diseases

Acute lung injury and acute respiratory distress syndrome (ARDS) are life-threatening conditions which affect both adults and children. In a mouse bleomycin-induced lung injury model, Ortiz et al[121] reported that exogenous infused MSCs could be found in the injured lungs and these cells appeared to adopt characteristics of epithelial cells. MSCs administration immediately after exposure to bleomycin also significantly reduced the degree of bleomycin-induced inflammation and collagen

deposition within lung tissue. Our group administered bleomycin to mice with or without busulfan-induced myelosuppression. We found that myelosuppression increased mice susceptibility to bleomycin injury and that MSCs transfer was protective. Protection was associated with the differentiation of engrafted MSCs into specific and distinct lung cell phenotypes, with an increase in circulating levels of G-CSF and GM-CSF (known for their ability to promote the mobilization of endogenous stem cells) and a decrease in inflammatory cytokines.[122] In a lipopolysaccharide (LPS)-induced mouse acute lung injury model, we demonstrated that infused MSCs administration suppressed the LPS-induced increase in circulating proinflammatory cytokines without decreasing circulating levels of anti-inflammatory mediators. Histologic analysis revealed that MSCs, but not fibroblasts, significantly reduced lung neutrophils at 6, 24, and 48 hours after LPS treatment.[40] In the same model, Gupta and colleagues[123] reported that intratracheally MSCs administration had improved survival relative to PBS-treated mice: 80% versus 42% at 48 hours and 64% versus 18% at 72 hours. MSCs reduced the severity of lung injury as measured by excess lung water, wet/dry ratio, and bronchoalveolar lavage (BAL) protein concentration. Histologic analysis at 48 hours revealed less hemorrhage and edema. Mei and colleagues[124] found that albumin, total protein, and immunoglobulin M in BAL were increased 3 days after intratracheal LPS and the phenomena were attenuated by MSCs infusion. MSCs transfected with angiopoietin-1 resulted in further improvement in both alveolar inflammation and permeability. Krasnodembskaya et al[125] reported the antibacterial effects of MSCs in a mouse model of pneumonia. They reported that mice given live E coli intratracheally had increased BAL lavage protein 18 hours later, and BAL protein was significantly decreased by intratracheal delivery of MSCs (but not fibroblasts) 4 hours after the injury. We are currently conducting a clinical trial to treat adult ARDS with human MSCs.

Cystic fibrosis (CF) affects more than 30 000 kids and young adults in the United States. CF is caused by mutations of CF transmembrane conductance regulator (CFTR) that particularly affects the lungs and digestive system and makes patients more vulnerable to repeated lung infections. Restoration of the abnormal CFTR function to CF airway epithelium is considered the most direct way to treat the disease. Several reports studied the potential of MSCs as a therapy for CF. Wang et al[126] found that MSCs possess the capacity of differentiating into airway epithelia. MSCs from CF patients are amenable to CFTR gene correction, and expression of CFTR does not influence the pluripotency of MSCs. Moreover, the CFTR-corrected MSCs from CF patients are able to contribute to apical chloride secretion in response to cAMP agonist stimulation, suggesting the possibility of developing cell-based therapy for CF. Another study[127] found that MSCs from cord blood differentially expressed mRNA for Clara cell secretory protein, CFTR, surfactant protein C, and thyroid transcription factor-1 when cultured in specialized airway growth media or with specific growth factors. Furthermore, systemically administrated cord blood-MSCs can localize to the airway and alveolar epithelium of immunotolerant (NOD/SCID) mice and acquire CFTR expression.

Future perspectives

The field of MSCs therapy is evolving rapidly, but some fundamental information potentially impacting the therapeutic effect is still missing. First, a better understanding the mechanisms of MSCs homing and immunomodulation is necessary to optimize their therapeutic effects. Also, signaling pathways mediating the expression and secretion of relevant MSCs trophic factors and the mechanisms of how they synergistically impact tissue repair need to be further investigated. At present, there is still no standard treatment protocol for MSCs. The routes of administration, cell numbers, and duration of therapy are some of the factors that remain challenges and need to be sought out to generate effective treatment. In addition, the majority of current MSCs-based clinical trials focus on the potential benefits of the immunomodulatory and trophic properties of MSCs rather than their potential to generate new tissues directly. The regeneration ability of MSCs needs to be further explored. Finally, understanding why some MSCs donors have more immunomodulatory potential and why some patients are more responsive to MSCs therapy would allow an optimized selection for donors and patients in trials and could highly impact the clinical outcome of MSCs treatments.

Conclusions

MSCs have become promising therapeutic agents for many diseases due to their low immunogenicity, immunomodulatory ability, self-renewal, and differentiation capacity. Data from clinical trials support the use of MSCs in the treatment of diseases of both adults and children. In Canada and New Zealand, Prochymal™, the first MSCs-based drug, has been approved for acute GVHD in children who have failed to respond to steroid treatment. With an ever increasing

number of clinical trials using MSCs, the exponential growth of knowledge will lead to new approvals of MSCs in other diseases. However, care should be taken as the potential benefits of cell therapy are transferred from adults to pediatric patients, since children are not mini-adults. In addition, other potential risks such as tumor formation have been observed in animal models. On the other hand, limitations to the use of MSCs by regulating agencies in different countries should not be considered as major setbacks, but rather as hurdles that need to be overcome through gathering more knowledge with regard to cell therapy. As more research is funded and more clinical trials are granted, MSCs hold a great future for the treatment of more diseases in children.

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