Experimental & Molecular Medicine (2013) 45, e2; doi:10.1038/emm.2013.2

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REVIEW

The potential use of mesenchymal stem cells in hematopoietic stem cell transplantation

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In the last 10 years, mesenchymal stem cells (MSCs) have emerged as a therapeutic approach to regenerative medicine, cancer, autoimmune diseases, and many more due to their potential to differentiate into various tissues, to repair damaged tissues and organs, and also for their immunomodulatory properties. Findings *in vitro* and *in vivo* have demonstrated immune regulatory function of MSCs and have facilitated their application in clinical trials, such as those of autoimmune diseases and chronic inflammatory diseases. There has been an increasing interest in the role of MSCs in allogeneic hematopoietic stem cell transplantation (HSCT), including hematopoietic stem cell engraftment and the prevention and treatment of graft-versus-host disease (GVHD), and their therapeutic potential has been reported in numerous clinical trials. Although the safety of clinical application of MSCs is established, further modifications to improve their efficacy are required. In this review, we summarize advances in the potential use of MSCs in HSCT. In addition, we discuss their use in clinical trials of the treatment of GVHD following HSCT, the immunomodulatory capacity of MSCs, and their regenerative and therapeutic potential in the field of HSCT.
Experimental & Molecular Medicine (2013) **45,** e2; doi:10.1038/emm.2013.2; published online 10 January 2013

Keywords: clinical trials; graft-versus-host disease; hematopoietic stem cell transplantation; mesenchymal stem cells; therapeutic use; transplantation immunology

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) has been used to treat patients with various malignant and non-malignant diseases during the last 40 years. In 1957, Thomas *et al.*[1] first reported the infusion of bone marrow (BM) into patients who received radiation and chemotherapy. These early experiences led to the use of allogeneic HSCT to promote recovery of hematopoietic function after myeloablative therapy. In 1968, the first successful human BM transplantion (BMT) was performed by Park *et al.*[2] By the late 1970s, Thomas *et al.*[3] used allogenic BM from human leukocyte antigen (HLA)-identical siblings, following total body irradiation (TBI) and administration of cyclophosphamide. *In vivo* transplantation of BM elements suggested that mesenchymal stem cells (MSCs) were precursors to BM connective tissue cells. In the 1990s, non-myeloablative stem cell transplant was used for hematologic diseases and solid tumors.[4,5] Kessinger *et al.*[6,7] introduced the use of peripheral blood stem cells for allogeneic transplant.

The history of stem cells began with the discovery in the mid-1800s that some cells had the ability to generate other cells.[8] Stem cell studies were performed by Maximow and Friedenstein.[9,10] Maximow discovered stem cells in the blood that could differentiate into various blood cells, and observed the relationship between hematopoiesis and the mesoderm during development.[9] Friedenstein first isolated adult non-hematopoietic stem cells from the BM and demonstrated ectopic BM formation by transplanting marrow stromal cells.[11] In the 1970s, Friedenstein *et al.*[10] first isolated adherent stromal cells from whole BM *in vitro* culture. These adherent stromal cells were fibroblast-like, clonogenic cells with multilineage potential to differentiate into different mesenchymal tissues and hematopoietic-supporting stroma when a single colony-forming unit-fibroblast (CFU-F) was retransplanted *in vivo*.[10] In the 1980s, Caplan and Owen further refined isolation methods and identified MSC markers.[12,13] In 1998, Thomson *et al.*[14] isolated cells from the inner cell mass of early embryos and developed the first

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Received 3 July 2012; accepted 6 October 2012

human embryonic stem cell lines.[14] Gearhart derived germ cells from fetal gonad tissue.[15]

Following the discovery of isolation and culture methods for MSCs, MSC-based therapies for stem cell research and clinical application began to be developed. MSCs have gained attention due to their potential for cell therapy and regenerative medicine. Recently, studies of the immune-suppressive capacity and regenerative potential of MSCs have generated clinical interest in the field of HSCT in terms of preventing graft rejection and controlling graft-versus-host disease (GVHD), as well as facilitating tissue engineering.

DEFINITION OF MSCS

MSCs, also known as mesenchymal stromal/stem cells, are non-hematopoietic. They were originally defined as self-renewing, multipotent progenitor cells with multilineage potential to differentiate into other types of cells of mesoderm origin, such as adipocytes, osteocytes, chondrocytes, tenocytes and skeletal myocytes, as well as cells of non-mesodermal origin, such as hepatocytes, neural cells and epithelial cells.[16–19] MSCs were initially identified in the BM and are commonly isolated by gradient centrifugation to separate nucleated cells, followed by *in vitro* culture and serial passage. However, many publications suggested the lack of 'stemness' of MSCs.[20] To improve the definition of MSCs, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) designated the name 'multipotent mesenchymal stromal cells' for the plastic-adherent cells found under standard culture conditions.[20] The surface phenotype of culture-expanded MSCs, by the defined ISCT standards, is negative for surface CD14 or CD11b, CD45, CD34, CD79 or CD19, and HLA-DR. MSCs are generally, but not homogeneously, positive for a number of cell-surface markers, including CD73, CD90 and CD105. Also, MSCs must differentiate into bone, fat and cartilage by addition of exogenous growth factors, and must be plastic-adherent *in vitro*.[21] Additionally, MSCs uniformly express CD49b, CD49e, CD54, CD166, CD50, CD62L and CD106. MSCs lack expression of the co-stimulatory molecules CD80, CD86, CD40 and CD40L.[22,23] Expression of chemokine receptors, including CCR1, CCR7, CXCR4, CXCR5 and the chemokine CX_3CL1, by late-expanded MSCs is downregulated compared with that of early-passage expanded MSCs.[24] The expression of surface antigens, including GD2, CD271 and frizzled-9, on MSCs has led to the isolation of MSCs using antibody-based immunomagnetic beads and/or fluorescence-activated cell sorting.[25–28] The phenotypic markers of MSCs are summarized in Table 1; however, no single marker that definitively distinguishes MSCs from all other cell types has been identified.

Although MSCs can differentiate into various mature cells, their intrinsic capacity to secrete cytokines and growth factors at sites of tissue injury and inflammation contributes significantly to their therapeutic capacity. The production of these tropic mediators is defined by their *in vivo* location, niche and severity of injury. MSCs are reservoirs for the production of cytokines, chemokines and extracellular matrix components, which have the ability to support stem cell survival and proliferation.[27,29,30] MSCs possess differentiation potential and may regenerate damaged or diseased tissues *in vivo*, as well as have a potential role in immunomodulation, providing a basis for a variety of clinical applications.

SOURCE OF MSCS

MSCs are most commonly isolated from BM,[17] but stromal cells with identical properties of BM-derived MSCs have been identified in many other tissues. These cells have been obtained from adipose tissue,[31–33] placenta,[34] amniotic fluid,[35–38] umbilical cord blood (UCB),[39,40] connective tissues of skeletal muscle and dermis,[41] dental tissue,[42] and fetal tissues such as lung and blood.[35] Mobilized peripheral blood cells have also been reported as a source of MSCs.[43] Although reports of UCB as a source of MSCs were initially controversial, there is general agreement that MSCs do reside

Table 1 Characterization of MSCs

	Phenotypic markers		*Other surface molecules used for isolation*		
Source	*Negative*	*Positive*	*Co-stimulatory (low expression)*	*Chemokines/receptors (low expression)*	*Others*
Bone marrow					
Adipose tissue					
Placenta					
Amniotic fluid	CD14 or CD11b		CD80	CCR1	
Umbilical cord blood	CD45	CD73	CD86	CCR7	GD2
Connective tissues of skeletal muscle and dermis	CD34	CD90	CD40	CXCR4	CD271
Dental tissue	CD79α or CD19	CD105	CD40L	CXCR5	Frizzled-9
Fetal tissue	HLA-DR			CX_3CL1	
Peripheral blood cells					

CCR, CC chemokine receptor; CD, cluster of differentiation; CXCR, CXC chemokine receptor; CX_3CL, CX_3C chemokine; GD2, neural gaglioside; HLA-DR, human leukocyte antigen-DR; MSC, mesenchymal stem cell.

within cord blood and that the volume and storage time of the cord blood are critical for successful isolation of MSCs.[39]

The developmental relationship between these different MSCs has not yet been determined, and there is no consensus as to whether BM-derived MSCs are the same as those isolated from other tissues. Gene expression studies have demonstrated that MSC populations are highly heterogeneous, including those from the same tissue source.[44] Thus, MSC gene expression profiles, including expression levels of MSC markers and some of their functional properties, differ according to the tissue source.[45] With regard to their ability to renew and differentiate, all cells derived from the majority of tissues behave in a similar manner. Additionally, the major functional properties, such as regulation of immunological tolerance, wound healing, inflammation and fibrosis, are common to all MSCs.[46] The heterogeneity of the MSC population suggests that different tissue sources may generate MSCs particularly suited to specific clinical applications.

IMMUNOMODULATORY PROPERTIES OF MSCS

MSCs not only provide stromal support for hematopoietic stem cells in the BM but also have potent immunosuppressive and anti-inflammatory effects. MSCs suppress T-cell proliferation induced by alloantigens or mitogens via increasing the number of regulatory T cells.[47,48] The interactions between T cells and MSCs have significant clinical implications in HSCT. MSCs have been shown to lessen complications of GVHD after HSCT[49] and immune-mediated disease.[50–52] In addition, MSCs inhibit function of B cells,[53] natural killer cells[54] and dendritic cells.[55] The main immunosuppressive function of MSCs is to induce soluble factors, including transforming growth factor-β,[56] hepatocyte growth factor,[57] nitric oxide,[58] HLA-G[59] and indoleamine 2,3-dioxygenase[60] (Table 2); however, these cells can also exert immunosuppressive effects by direct cell-to-cell interaction.[61] The immunosuppressive capacity of MSCs is enhanced under inflammatory conditions in the presence of the proinflammatory cytokines interferon (IFN)-γ, tumor necrosis factor-α and interleukin (IL)-6. MSCs constitutively produce large amounts of IL-6 and IL-8, and the chemokine CCL-2. When MSCs were treated with IFN-γ, there was secretion of ICAM-1, CXCL-10 and CCL-8, whereas IL-8 production was decreased.[62] This phenomenon suggests that MSCs target neutrophils and monocytes under non-inflammatory conditions, but attract monocytes, dendritic cells, T cells and natural killer cells under inflammatory conditions.[62] A number of studies reported transforming growth factor-β as a key mediator of immunomodulation by MSCs. There is some evidence that the transforming growth factor-β-transduced MSCs used in our study showed enhanced immunomodulatory effects on T-cell-mediated immunity.[56] Under immunologically quiescent conditions, MSCs promote T-cell survival and can induce the activation and proliferation of CD4+ T cells.[63,64] The dual immunomodulatory properties of MSCs suggest that environmental factors may have a crucial role in induction of MSC-mediated immunomodulation.

Recent studies reported that MSCs must be 'licensed' to exert their immunomodulatory effects. Marigo and Dazzi[65] showed that MSCs are not constitutively inhibitory, but require a licensing step to produce acute inflammatory helper T-lymphocyte (Th1)-type cytokines. If MSCs are transplanted during acute inflammation, the microenvironment containing polarized M1 macrophages 'licenses' MSCs to inhibit effector T, B, natural killer and dendritic cells. In contrast, if MSCs are licensed after the polarization of M2 macophages by Th2-type cytokines during chronic inflammation, the microenvironment provides alternative licensing and recruits MSCs to the fibrosis process.[65] In conclusion, MSC therapy in an inflammatory microenvironment that is licensing-dependent. Under conditions of mild or chronic inflammation, the lack of licensing fails to provide therapeutic effects, leading to weakened MSC immunosuppressive activity.

Table 2 Immunomodulatory molecules produced by MSCs

Molecule	*Function*
Transforming growth factor-β	Suppress T-lymphocyte proliferation
Hepatocyte growth factor	Suppress T-lymphocyte proliferation
Nitric oxide	Suppress T-cell function and responsiveness
Human leukocyte antigen-G	Suppress naive T-cell proliferation
Indoleamine 2,3-dioxygenase (IDO)	IDO-mediated T-cell inhibition by converting tryptophan tokynurenin, a T-cell-inhibitory effector pathway in APCs
Chemokines: CCL-2, ICAM-1, CXCL-10, CCL-8	Drive T-cell migration toward MSCs

APC, antigen-presenting cell; CCL, CC chemokine; CXCL, CXC chemokine; ICAM-1, intercellular adhesion molecule 1; MSC, mesenchymal stem cell.

THERAPEUTIC POTENTIAL OF MSCS FOR TREATMENT OF ACUTE AND CHRONIC GVHD

GVHD is a severe inflammatory condition, which results from immune-mediated attack of recipient tissue by donor T cells contained in the allogeneic graft. The immunomodulatory properties of MSCs have led to clinical trials of MSC-based therapy to prevent acute GVHD (*a*GVHD) and chronic GVHD (*c*GVHD), major complications that occur after allogeneic HSCT.[66] For a more detailed review of GVHD, see Schroeder and DiPersio.[67] Without intervention before HSCT, almost all allotransplant recipients develop significant GVHD. These data suggest that enhancing the immunomodulatory capacity of MSCs has potential for treatment of GVHD following HSCT.[68] Multiple drugs and strategies are used to deplete T cells to prevent donor anti-host immunological complications of allotransplantation. Recently, the immunomodulatory and tissue-repair properties of MSCs have led to many studies and clinical trials of their use as a treatment for GVHD. The rationale for studies of MSCs was based on their immunomodulatory properties identified by numerous *in vitro* assays and *in vivo* models, as explained above.

*a*GVHD involves direct cytotoxic effects of donor T cells on recipient tissues, activation of antigen-presenting cells and an inflammatory cascade that produces cytokines, including IL-1, IL-6, IL-12, IFN-γ and tumor necrosis factor-α.[69] At first, there was a scarcity of preclinical models demonstrating the efficacy of MSCs in ameliorating *a*GVHD before clinical studies started. In fact, most preclinical models were developed after the demonstration of clinical efficacy. The clinical efficacy of MSCs in *a*GVHD was initially observed in a 9-year-old boy suffering from steroid-resistant grade IV *a*GVHD who received haploidentical third-party MSCs.[70] In the next phase II clinical trial, which involved 55 patients with the same condition, the administration of MSCs significantly improved the overall survival rate.[71] However, in another phase I/II clinical trial, a single infusion of MSCs given at the time of the transplant did not prevent the development of *a*GVHD.[72] This discrepency was also seen in animal models and was later explained by the findings of preclinical studies. Initial studies of MSCs in a murine *a*GVHD model demonstrated that a single infusion of MSCs at the time of HSCT did not prevent *a*GVHD,[73] but this could be mitigated by multiple doses given at weekly intervals subsequent to HSCT.[74] Polchert *et al.*[75] reported that MSCs can be used to treat *a*GVHD when administered at an appropriate time in the presence of IFN-γ. That study showed that the survival rate of mice increased when MSCs were administered at day +2 or +20 of HSCT, when IFN-γ levels were at their peak. This study demonstrated that timing was critical because an appropriate inflammatory environment was needed to 'license' the MSCs. The role of IFN-γ and of the inflammatory environment in activation of MSCs to exhibit inhibitory activty has been described *in vitro*.[76]

It has been suggested that inflammatory cytokines, including IFN-γ, can recruit MSCs to the site of inflammation and tissue injury.[77] In addition to their immunomodulatory effects, MSCs might enhance the healing of wounded tissue by providing soluble factors, transdifferentiation and cell fusion. In a previous clinical trial, patients were treated with MSCs to ameliorate tissue toxicty following HSCT.[78] Tissue injuries, such as hemorrhagic cystisis and pneumomediastium, were cleared in several patients after treatment with MSCs. In one patient with *a*GVHD, symptoms of perforated diverticulitis and peritonitis were reversed by MSC therapy.[78] Furthermore, a series of experiment used bioimaging to track the biodistribution of MSCs in a murine model of *a*GVHD.[79] In these experiments, donor C57BL/6 splenocytes, which expressed enhanced green fluorescent protein, were used to induce *a*GVHD. Then, MSCs were generated from C57BL/6 donor mice expressing red fluorescent protein (RFP). RFP-MSC were injected, and both fluorescent signals were detected consistently. Enhanced green fluorescent protein was first detected in the lungs, but spread to the gastrointestinal (GI) tract, liver, skin, and lymph nodes, all of which are known clinical targets of *a*GVHD. After injection, RFP signals co-localized with EGFP signals at the *a*GVHD target sites, proving that MSCs can home to sites of *a*GVHD and potentially exert direct cell-cell contact-mediated effects as well as paracrine effects for tissue repair. Murine MSCs that were engineered to express the anti-inflammatory cytokine IL-10 significantly reduced the severity of *a*GVHD compared to unmodified MSCs.[80] It is likely that safely engineered MSCs may provide more targeted and effective cell therapy for *a*GVHD.

*c*GVHD occurs after the first 100 days of HSCT and is characterized by autoimmune-like dysregulation. While *a*GVHD involves mainly the skin, liver, and GI tract, *c*GVHD affects almost any organ and reduces the quality of life, organ function, and overall survival.[81] In contrast to *a*GVHD, the pathophysiology of *c*GVHD is poorly understood. A clinical trial of the use of MSCs to treat *c*GVHD has been reported recently.[72] The first report involved co-transplantation of HLA-identical sibling culture-expanded MSCs with HLA-identical sibling HSCT in patients with hematologic malignancies. Clinical improvement was identified in 22 of 36 (61%) patients who survived at least 90 days.[72] Another report suggested that BM-derived MSCs may be used successfully to treat adult patients with sclerodermatous *c*GVHD,[82] because IFN-γ, IL-2, IL-10, and IL-4 producing cells were detected both before and after MSC infusion. Before MSC infusion, Th2-type cells were markedly increased compared to Th1 type cells, whereas after MSC infusion, the proportion of Th1 type cells increased.[82] In another study, infusion of culture-expanded MSCs was investigated as a therapeutic approach for patients with steroid-resistant *c*GVHD.[68] Although 14 of 19 patients (73.7%) responded to MSC administration, only four patients showed complete remission.[68] The majority of patients showed only partial or mixed responses, suggesting that MSC may not be a potent immunomodulator in a *c*GVHD environment. The number of studies of use of MSCs in *c*GVHD is insufficient; it is apparent that unlike *a*GVHD, the therapeutic effect of MSCs on *c*GVHD is limited.

Despite *in vitro* and *in vivo* evidence that MSCs can ameliorate GVHD, clinical trials of the treatment of GVHD remain incomplete. Our recent data showed that transforming growth factor-β-transduced MSCs were able to successfully treat autoimmune arthritis by inducing Foxp3 levels and inhibiting IL-17 production; however, MSCs themselves did not suppress IL-17 production.[56] These findings suggest that while MSCs exert immunomodulatory properties via an IFN-γ (thast is, Th1)-dominant response, MSCs may not effectively inhibit Th17 responses. While the role of Th17 in *a*GVHD pathogenesis is still not clearly defined, recent studies have revealed that GVHD involves a combination of both Th1 and Th17 responses;[83] thus, the monitoring of both Th1 and Th17 responses, rather than Th1 or Th17 responses alone, could be an accurate indicator of GVHD severity after HSCT.[84] Furthermore, Yi *et al.*[85] suggested that blocking Th1 or Th17 cells alone was ineffective for treatment of GVHD. Blocking Th1 cells led to the exacerbation of Th17 responses, and *vice versa*. Thus, new studies are proposing that simultaneous inhibition of Th1 and Th17 differentiation could be a new strategy to treat GVHD following HSCT.[86] Finally, a more standardized study design is needed for clinical trials, in order

Table 3 *In vivo* immunosuppressive effects of MSCs

Animal, model	*MSCs*	*Outcome*	*Reference*
Mouse, CIA	TGF-β-transduced MSCs	Suppressed development of autoimmune arthritis and joint inflammation	Park *et al.*[56]
Mouse, *a*GVHD	Single infusion of MSCs	No effect on prevention of GVHD	Sudres *et al.*[73]
Mouse, *a*GVHD	Multiple infusions of MSCs after HSCT, once GVHD has been fully established	Increased survival rate and amelioration of disease	Tisato *et al.*;[74] Polchert *et al.*;[75] Joo *et al.*[79]
Mouse, *a*GVHD	IL-10-transduced MSCs	Reduced severity of *a*GVHD	Min *et al.*[80]
Mouse, graft rejection	Co-transplantation of fetal and adult human MSCs	Long-term engraftment	Almeida-Porada *et al.*[87]
Sheep, graft rejection	Co-transplantation of fetal and adult human MSCs	Long-term engraftment	Noort *et al.*[88]
Primate, graft rejection	Autologous intra-BM transplantation of MSC	Improved engraftment	Masuda *et al.*[90]
Mouse, graft rejection	Allogeneic MSCs	Increased rejection	Nauta *et al.*[91]
Mouse, non-obese diabetic	Allogeneic MSCs	Induction of mixed chimerism and prevention of insulitis	Asari *et al.*;[98] Itakura *et al.*[99]
Rat, hindlimb transplant	Co-infusion of allogeneic MSCs and bone marrow cells	Induction of stable high-level chimerism	Pan *et al.*[100]
Mouse, chimerism	Intra-bone marrow-bone marrow transplantation with allogeneic MSCs	Induction of mixed chimerism	Wang *et al.*[101]

*a*GVHD, acute graft-versus-host diasease; BM, bone marrow; CIA, collagen-induced arthritis; GVHD, graft-versus-host diasease; HSCT, hematopoietic stem cell transplantation; IL-10, interleukin 10; MSC, mesenchymal stem cell; TGF-β, transforming growth factor-β.

Table 4 Clinical trials of MSC therapy for hematopoietic stem cell transplantation

Target	*Source of MSCs*	*Type of trial*	*Observations*	*Reference*
Treatment of *a*GVHD	BM	I	CR (100%)	Le Blanc *et al.*[70]
Treatment of *a*GVHD	BM	II	30 CR (54%) 9 PR (16%)	Le Blanc *et al.*[71]
Prevention of *a*GVHD, *c*GVHD	BM, PBSC	I	22/46 (50%) still developed *a*GVHD 22/36 (61%) still developed *c*GVHD	Lazarus *et al.*[72]
Prevention of *a*GVHD, *c*GVHD	BM	I	Incidence of *a*GVHD in MSC-treated patients was 31%, whereas control was 41%. None of the MSC-treated patients developed *c*GVHD.	Bernardo *et al.*[95]
Treatment of *c*GVHD	BM	I	Decreased signs and symptoms in all patients	Zhou *et al.*[82]
Treatment of *c*GVHD	BM	I	4 CR (21%) 10 PR (52%)	Weng *et al.*[68]
Facilitation of engraftment	BM	I–II	Engraftment prompt in all patients without toxicity of MSC	Koc *et al.*,[92]
Facilitation of engraftment	BM	I–II	All patients given MSC showed sustained hematopoietic engraftment without adverse reaction	Ball *et al.*[93]

*a*GVHD, acute GVHD; BM, bone marrow; cGVHD, chronic GVHD; CR, complete response; MSC, mesenchymal stem cell; PBSC, peripheral blood stem cell; PR, partial response.

to accurately evaluate the effects of MSCs on GVHD. Data from preclinical murine GVHD models suggest that the timing of MSC administration is critical to its effectiveness, but the optimal treatment schedule has not yet been defined. To-date a variety of dosing schedules has been used; however, the optimal treatment method should be determined. Furthermore, as mentioned above, a consensus must be formed regarding the optimal culture and manufacturing conditions to generate uniform MSCs. Therefore, more definitive studies and longer follow-ups during clinical trials are necessary to assess the long-term efficacy and toxicity associated with MSC use. In future studies, the use of genetically modified MSCs holds considerable promise. In conclusion, both preclinical (Table 3) and clinical (Table 4) trials using MSCs as a potential therapeutic agent for the treatment of GVHD are encouraging, yet the data remain incomplete.

FACILITATING ENGRAFTMENT BY MSCS

MSCs have been suggested to be the progenitor cells that modulate alloreactivity and promote hematopoietic reconstitution. Therefore, MSCs have been suggested to enhance

engraftment and to prevent rejection after HSCT. Co-transplantation of fetal and adult human MSCs promoted long-term engraftment in immunodeficient (non-obese diabetic/SCID) mice and fetal sheep.[87,88] Infusion of allogeneic BM-derived MSCs inhibited lymphocyte proliferation and prolonged skin allograft survival.[89] Co-transplantation with MSCs improved HSC engraftment after autologous intra-BM (IBM) transplantation in non-human primates.[90] In murine models, the infusion of host MSCs enhanced engraftment of allogeneic hematopoietic cells; however, donor MSCs increased rejection of an allogeneic stem cell graft.[91] During the first clinical trial, rapid hematopoietic recovery was accelerated by the use of culture-expanded marrow MSCs and autologous blood transplantation.[92] In a phase I/II clinical trial, co-transplantation of donor MSCs in 14 children given T-cell-depleted HLA-disparate CD34+ cells accelerated leukocyte recovery and prevented graft rejection.[93] In a pediatric phase I/II trial, infusion of *ex vivo* culture-expanded third-party haploidentical MSCs into unrelated pediatric UCB transplantation prompted hematopoietic recovery.[94] Co-infusion of parental MSCs in pediatric patients given allogeneic UCB graft prevented *a*GVHD, but did not affect engraftment or hematopoietic recovery.[94,95] In an adult phase I/II clinical trial, patients receiving UCB transplantation with co-infusion of third-party donor, mobilized hematopoietic stem cells did not affect the kinetics of engraftment or *a*GVHD.[96] These findings indicate that co-infusion of hematopoietic cells and MSCs is safe *in vivo* and clinically, while the engraftment capacity of MSCs in terms of efficacy remains uncertain.

INDUCTION OF MIXED CHIMERISM USING MSCS

Induction of mixed chimerism and achieving immunological tolerance is an important goal in the efforts to reduce the morbidity, mortality and shortage of organ transplants as well as to combat hematological malignancies. Mixed chimerism entails coexistence of recipient and donor hematopoietic cells following transplantation of donor BM into conditioned recipients. These protocols involve T-cell depletion, co-stimulation blockade and therapeutic use of regulatory T cells.[97] Recent studies of MSC-mediated anti-GVHD effects, their supportive role in hematopoietic engraftment and their immunomodulatory properties have led to increasing use of MSCs in mixed chimerism protocols.

Most mixed chimerism protocols utilizing MSCs use recipient conditioning regimens to enhance the engraftment of donor BM followed by the co-administration of BM cells and MSCs. In non-obese diabetic mouse models, known to be highly resistant to chimerism induction, recipient mice were treated with a preconditioning regimen consisting of 3 Gy TBI and anti-CD3 monoclonal antibody injection.[98] The co-injection of allogeneic BM cells and MSCs facilitated engraftment, induced mixed chimerism with a success rate greater than 78%, and prevented insulitis and the onset of diabetes. Furthermore, no GVHD developed with this treatment regimen. Similar results were demonstrated in streptozotocin-diabetic rats.[99] The recipient rats received a conditioning regimen consisting of anti-lymphocyte serum and 5 Gy TBI, followed by co-infusion of allogeneic MSCs, BM cells and islets. Although all recipients rejected the islets initially, half developed stable mixed chimerism and donor-specific immune tolerance, shown by donor skin engraftment and a second round of islet transplants. In another experiment, recipient Lewis rats received a similar conditioning regimen consisting of anti-lymphocyte serum, rapamycin immunosuppressive therapy (from days 0 to 130) and 3 Gy TBI, followed by co-infusion of allogeneic MSCs and BM cells.[100] Additionally, a hindlimb allotransplant was performed 30 days after the BMT. The immunosuppressive therapy was stopped 100 days after hindlimb transplantations. Fourteen of fifteen recipients developed stable and high-level chimerism, and the survival time of hindlimb allografts was prolonged even after the withdrawal of rapamycin in the group with co-administration of MSCs. In contrast, a protocol that induced mixed chimerism with no cytotoxic conditioning was evaluated.[101] Wang *et al.*[101] established mixed chimerism by IBM–BMT combined with BM-derived allogeneic MSC treatment in mice. IBM-BMT is a method that administers donor BM directly into the recipient BM. Because of the low morbidity of GVHD and rapid recovery of hematopoietic function, IBM-BMT is considered one of the best strategies, and intraosseous infusion is an established method for patients receiving critical care in the clinical setting. The donor-derived MSCs were transplanted intravenously daily for 4 days before the actual transplantation, and IBM-BMT was performed immediately after the fourth injection. The majority of the mice developed 20–25% chimerism levels among myeloid lineage cells, whereas no chimerism was detected in either control group (IBM-BMT or BM-MSC treatment alone).

MSC IMMUNOGENICITY

MSCs are considered to be immunoprivileged because of their absent or low expression of major histocompatibility complex class II and other co-stimulatory molecules. MSCs have also been found to have an immunosuppressive role. Thus, MSCs have been assumed to be a powerful therapeutic tool that could be used regardless of the major histocompatibility complex identity between donor and recipient. However, recent research has revealed that MSCs can stimulate immune responses under certain conditions. Depending on the IFN-γ level, MSCs can exhibit antigen-presenting properties.[102] At low IFN-γ levels, MSCs can upregulate the expression of major histocompatibility complex II and gain the ability to act as an antigen-presenting cell.[102] IFN-γ-treated MSCs have also been demonstrated to induce ovalbumin-specific immune responses.[103] The IFN-γ-treated syngeneic MSCs could process the ovalbumin antigen peptide, present it on major histocompatibility complex II molecules, and activate ovalbumin-specific T cells.[103] MSC immunogenicity has also been demonstrated *in vivo* models. The presence of allogeneic MSCs in a non-myeloablative transplantation setting resulted in a significantly increased graft rejection.[91,104,105]

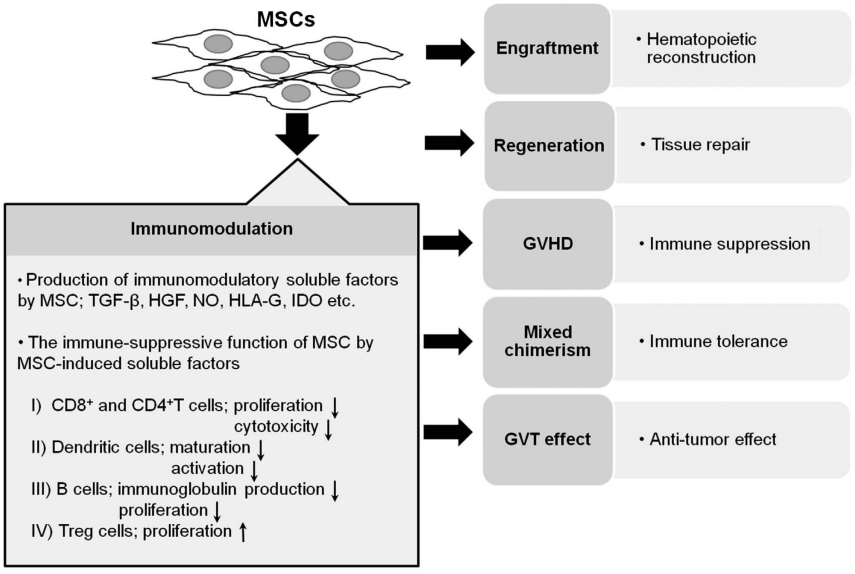

Figure 1 MSC-mediated therapies targeting for hematopoietic stem cell transplantation. The potential uses of MSCs include treatment of GVHD, facilitation of hematopcietic engraftment, induction of mixed chimerism and induction of the GVT effect. MSCs possess unique properties of immune modulation and tissue regeneration. ↓ : suppression; ↑: promotion. GVHD, graft-versus-host disease; GVT, graft-versus-tumor; HLA, human leukocyte antigen; IDO, indoleamine 2,3-dioxygenase; MSCs, mesenchymal stem cells; TGF-β, transforming growth factor-β.

Furthermore, the administration of allogeneic MSCs induced T-cell responses in naive immunocompetent host mice. It appears that MSCs can engraft immunocompromised hosts, but have limited capacity to elicit an immune response in an immunocompetent host. Many aspects of the immunogenic properties of MSCs remain to be elucidated; therefore, further studies should validate the efficacy and clinical consequences of the use of MSCs.

GRAFT-VERSUS-TUMOR EFFECT FOLLOWING MSC THERAPY

Allogeneic donor lymphocytes produce a strong graft-versus-tumor (GVT) effect. However, its clinical efficacy is limited by conditioning-related toxicity, GVHD, and engraftment failure. Barnes and Loutit[106] first reported GVT alloreactivity of allogeneic HSCT in a murine model. The GVT response is mediated largely by minor histocompatibility antigens,[107–109] natural killer cells[110–113] and donor lymphocyte infusion.[114–116] A major focus of allogeneic HSCT is augmentation of GVT effects without GVHD. Recent investigations have focused on infusion of cells, novel pharmacologic agents and biological agents that may specifically prevent GVHD without affecting GVT reactions. More specifically relevant to GVT effects, 20 patients with hematologic malignancies received MSCs from HLA-mismatched donors after conditioning with TBI and fludarabine.[117] HLA-mismatched non-myeloablative HSCT with MSC co-infusion exhibited a therapeutic effect on the hematologic malignancies. Furthermore, the results suggested that MSC co-infusion prevented GVHD while preserving GVT effects.[117] Although the induction of tumor inhibition and enhancement are polar opposites, MSCs have numerous beneficial properties in terms of promoting GVT effects due to both their immunomodulatory properties after HSCT that may suppress tumors, as well as their tropism toward the tumor microenvironment.

CONCLUSION

HSCT was initiated in 1957 by Thomas *et al.*[1] by the infusion of human BM into leukemia patients. Currently, a variety of stem cell sources other than the BM, such as peripheral blood, amniotic fluid and UCB, are being used in transplantation settings, and new treatment methods are continually being discovered. However, the limitations of HSCT, including infection, relapse of disease, engraftment and complications of GVHD, have not been resolved. At present, MSCs are an excellent candidate with clinical therapeutic potential for HSCT. Preclinical and clinical studies of MSC therapy are revealing new methods of overcoming these limitations. The potential uses of MSCs include treatment of GVHD, facilitation of hematopoietic engraftment, induction of mixed chimerism and induction of the GVT effect. In addition, MSCs possess unique properties of immune modulation and tissue regeneration that highlight their potential as a potent therapeutic tool (Figure 1). Based on the results of clinical studies, to improve the safety and efficiency of MSC therapy, studies of specific markers that identify MSCs, cell dose and the timing and route of administration are crucial and must continue. Furthermore, enhancement of the therapeutic activity of MSC transplants is required to increase our knowledge of how MSCs regulate the host immune response, and therefore, further studies may be needed to identify the immunoregulatory mechanisms and *in vivo* biological activities of MSCs after administration.

ACKNOWLEDGEMENTS

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry for Health & Welfare, Republic of Korea (A092258).

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