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Chimerism of bone marrow mesenchymal stem/stromal cells in allogeneic hematopoietic cell transplantation

Is it clinically relevant?

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Multipotent mesenchymal stem/stromal cells (MSCs) have been extensively used as a transplantable cell source for regenerative medicine and immunomodulatory therapy. Specifically in allogeneic hematopoietic stem cell transplantation (HSCT), co-transplantation or post-transplant infusion of MSCs derived from bone marrow (BM) of non-self donors has been implicated in accelerating hematopoietic recovery, ameliorating graft- vs.-host disease, and promoting tissue regeneration. However, irrespective of the use of MSC co-administration, post-transplant chimerism of BM-derived MSCs after allogeneic HSCT has been reported to remain of host origin, suggesting that the infused donor MSCs are immunologically rejected or not capable of long-term engraftment in the host microenvironment. Also, hematopoietic cell allografts currently used for HSCT do not seem to contain sufficient amount of MSCs or their precursors to reconstitute host BM microenvironment. Since the toxic conditioning employed in allo-HSCT may impair the function of host MSCs to maintain hematopoietic/regenerative stem cell niches and to provide a local immunomodulatory milieu, we propose that new directions for enhancing immunohematopoietic reconstitution and tissue repair after allogeneic HSCT include the development of strategies to support functional replenishment of residual host MSCs or to support more efficient engraftment of infused donor MSCs. Future areas of research should include in vivo tracking of infused MSCs and detection of their microchimeric presence in extra-marrow sites as well as in BM.

Introduction

Multipotent mesenchymal stem/stromal cells (MSCs) are originally isolated from human bone marrow (BM) as adherent, fibroblast-like shaped cells with an ability to differentiate into the mesenchymal lineage cells of mesoderm such as osteoblasts, adipocytes, and chondrocytes.[1,2] In addition to such multi-differentiation capacity, MSCs are shown to have protean immunomodulatory properties to ameliorate immune dysfunctions caused by pathologic effector cells. In the last decade, substantial efforts have been made to develop regenerative or anti-inflammatory cellular therapy using culture-expanded autologous or allogeneic MSCs.[3,4] Specifically in the field of allogeneic hematopoietic stem cell transplantation (HSCT), various clinical trials have been performed with the aim of accelerating engraftment or ameliorating graft- vs. -host disease (GVHD) by infusion of MSCs obtained from the hematopoietic cell donors or third-party donors. However, in the allogeneic setting, most of infused MSCs are considered short-lived in the host and the mechanisms by which MSC-based therapy can work in clinics remain mostly unclarified. In this short review, we critically look back on the biological basis of cellular therapy using culture-expanded allogeneic MSCs in the context of allogeneic HSCT. We also propose that a new area of translational research should include functional replenishment of host-type MSCs by pharmacologic agents or chimerism enhancement of donor-type MSCs by their in situ infusions.

Biological Characteristics of Human Mesenchymal Stem/Stromal Cells (MSCs)

In a current understanding of the biology of human MSCs, they are characterized by the following in vitro features:[3,5] (1) their ability to adhere to plastic plate and to form colony forming unit-fibroblastic (CFU-F); (2) their ability to differentiate into osteoblasts, adipocytes and chondrocytes; (3) their positive surface expression of CD105 (endoglin), CD73, and CD90 (Thy-1) in the absence of pan-leukocyte (CD45), endothelial/primitive hematopoietic (CD34), and hematopoietic lineage markers as well as in the absence of surface human leukocyte antigen (HLA)-class II molecules. MSCs or MSC-like cells appear to be widely distributed in the human body, because the cells with similar biological characteristics to BM-derived MSCs have been demonstrated to be isolated from a variety of adult organs or tissues

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including adipose tissues, cartilages, fetal liver, and fetal lungs, although their mutual identities remain elusive.[3]

Whether umbilical cord blood (CB) and peripheral blood (PB) contain MSCs or not has been a great interest since, as well as BM, CB and cytokine-mobilized PB have been successfully used as stem cell sources for allogeneic HSCT. Recent studies have clearly demonstrated that CB contains MSCs, but its frequency is estimated to be extremely low around one in 1×10^8 mononuclear cells compared with that in BM around one in 1×10^6 to 1×10^4 mononuclear cells. However, CB-derived MSCs show tremendous proliferation to generate much more progeny than BM-derived MSCs and can be expanded to the order of 10^8 cells, which are deemed sufficient for clinical studies.[6,7] CB-derived MSCs differ from BM-derived MSCs in their variable expression levels of CD146, a marker for multipotency and differentiation potential: CB-derived MSCs show robust potential for chondrogenic differentiation, while their potential for adipogenic differentiation is not obvious. Therefore, the use of an appropriate origin or unit of MSCs is important for achieving satisfactory effects according to the planned clinical application.

On the other hand, the presence of MSCs in steady-state PB is a matter of controversy, although several investigators have reported that MSCs or MSC-like cells can be isolated in postnatal PB or in PB samples obtained from individuals receiving granulocyte-colony stimulating factor,[8,9] patients with breast cancer,[10] and patients with osteoporosis.[11] These findings might reflect one of the potential features of MSCs that they might be occasionally supplied into the blood circulation from the MSC niche in response to systemic or local hormones, cytokines, and growth factors to migrate toward organs or tissues in need.[12,13] However, further studies are necessary to confirm if MSC-like multipotent cells can be reproducibly obtained from the steady-state circulating blood.[14]

Immunomodulatory Properties of MSCs and Treatment of Immunologic Diseases by Culture-Expanded MSCs

Lines of evidence demonstrate that MSCs have immunomodulatory properties with anti-inflammatory, anti-proliferative and immunosuppressive capacities. The possible mechanisms underlying such immunomodulatory effects of MSCs are thought to be mediated by a combination of soluble molecules in the microenvironment where MSCs can directly interact with immune cells. MSCs are initially demonstrated to exhibit anti-proliferative effects on T cells,[15] but these effects are observed on other immune cells including B cells, NK cells, and dendritic cells.[16,17] Mature mesenchymal cells such as chondrocytes can also show anti-proliferative effects on immune cells.[18] However, they do not exert the immunomodulatory effects mediated by MSCs,[19] implying the unique property of MSCs in the immune system. Effective immunosuppression by MSCs is supposed to be achieved in local microenvironment where MSCs reside. In mouse experiments, MSCs become activated by proinflammatory cytokines interferon-γ, tumor necrosis factor-α, interleukin (IL)-1-α or IL-1-β and express T-cell-specific chemokines CXCL-9 and CXCL-10 to attract T cells into close proximity with MSCs.[20,21] Conversely, MSCs express chemokine receptors that are involved in migration of MSCs to the sites where they participate in immune responses.[22-24] In such a milieu, MSCs exert their inhibitory effects on T cell proliferation through the secretion of soluble factors such as nitric oxide,[25] indoleamine-2,3-dioxygenase,[26-28] heme oxygenase-1,[29] prostaglandin E_2,[30,31] transforming growth factor-β1,[22] and leukemia inhibitory factor.[32] Some of these soluble molecules are also demonstrated to be involved in the suppression of proliferation and cytotoxicity of NK cells or maturation and function of dendritic cells.[28,33-35] However, MSC-mediated immunosuppression is thought to be a "combined" effect that cannot be fully explained by any one of those molecules released from MSCs. Furthermore, direct cell-to-cell interactions might also be associated with the MSC-mediated immunosuppression. Although activating NK cells are capable of killing MSCs with low levels of HLA class I molecules expressed on their surface, upon simulation of MSCs with interferon-γ, the expression of HLA class I molecules is upregulated,[36] resulting in inhibition of NK cell-mediated lysis of MSCs.[37]

Given such protean immunomodulatory properties, infusions of allogeneic MSCs have been suggested to be beneficial for the treatment of diseases in which the immune system is dysregulated such as multiple sclerosis, type 1 diabetes mellitus, Crohn's disease, systemic lupus erythematosus, and rheumatoid arthritis/Sjögren syndrome.[3] Le Blanc et al. was the first to report successful treatment of steroid-resistant severe acute GVHD of the gut and liver by intravenous infusion of culture-expanded BM-derived MSCs from a haploidentical mother in a boy who had previously received unrelated HLA-matched HSCT for his acute lymphoblastic leukemia.[38] In this report, they demonstrated the partial female donor epithelium chimerism (4%) in the biopsy specimens of colon by X and Y chromosomes fluorescence in situ hybridization analysis, which might imply the contribution of donor MSCs to the improvement of gut GVHD. A large study from the same group showed that infusion of MSCs from multiple donor sources conferred overall response rate of 69% including complete response for the treatment of steroid-resistant acute GVHD.[39] These studies support the experimental and preclinical observations that the infused MSCs can directly or indirectly exert immunomodulatory effects possibly through the production of soluble factors and/or direct cell-to-cell interactions. However, intriguingly, many of these immunomodulatory effects of infused MSCs can be exerted without their long-term persistence in the host.[40,41]

Effects of Ex Vivo Expanded MSCs on Hematopoietic Reconstitution after Hematopoietic Cell Transplantation

Clinical application of MSCs for tissue/organ regeneration has also been extensively considered based on their capability of secreting trophic factors, homing to sites of damage, and multidifferentiation. One of the important roles of BM-derived MSCs is to provide the microenvironment of hematopoietic stem cell (HSC) niche that supports durable and effective hematopoiesis.

In animal models, nestin-positive cells are shown to be MSCs that comprise the HSC niche in BM.[42] Osteoblasts, CD146-expressing sub-endothelial stromal cells, and adipo-osteogenic progenitors expressing CXCL12 also are demonstrated to be cellular constituents of HSC niche;[43,44] these cells are derived from MSCs or have similar characteristics to MSCs. Conversely, aberrant MSC-derived osteoprogenitor cells have been demonstrated be associated with disease pathophysiology in animal models.[45-48] Given the results of studies demonstrating the importance of MSCs or MSC-derived stromal cells for the maintenance of hematopoiesis, the Lazarus group intravenously administered autologous culture-expanded MSCs in patients with hematologic malignancies without any infusion-related adverse reactions.[49] Their group also reported the ability of MSCs to enhance hematopoietic recovery by co-infusion of autologous PB progenitor cells and culture-expanded MSCs in patients with breast cancer receiving high-dose chemotherapy.[50]

Upon allogenic HSCT especially when using HLA-mismatched grafts, primary graft failure is one of the early and very serious complications. Co-administration of MSCs with HSCs could be a promising strategy to overcome this shortcoming. In myeloablative allogeneic HSCT using BM or PB progenitor cells from HLA-identical siblings, co-transplantation of culture-expanded BM-derived MSCs was safe without immediate or late toxicities but showed no apparent acceleration of engraftment.[51] Importantly, at 6 and 18 mo after transplantation, microsatellite-based chimerism analysis of MSCs in BM aspirates revealed that loss or very rare presence of infused donor-type MSCs.[51] In a situation of graft failure or graft rejection after the first allogenic HSCT, a pilot study showed the utility of co-infusion of MSCs in the subsequent salvage HSCT to improve engraftment of the second graft.[52] In haploidentical allogenic HSCT using cytokine-mobilized CD34 positive progenitor cells, co-transplantation of haploidentical MSCs did not confer acceleration of either neutrophil or platelet engraftment but prevented graft rejection.[53] In this study, donor-type chimerism of MSCs evaluated by PCR analysis using donor/recipient polymorphisms was only transiently detected at very low levels in 3 out of 14 patients at 3 mo after transplantation. In unrelated HLA-mismatched CB transplantation for children with high risk hematological malignancies, co-transplantation of haploidentical parental MSCs conferred relatively rapid hematopoietic recovery.[54] More recently, de Lima et al. reported that ex vivo coculture of CB cells with MSCs derived from BM of haploidentical family members or third-party donors is feasible and effectively expanded CD34+ cells to accelerate hematopoietic recovery when infused with the second unexpanded CB unit.[55] Although these pilot studies suggest the utility of MSCs on hematopoietic recovery after allogeneic HSCT, further studies are needed to elucidate the appropriate dose of MSCs and timing of their infusions for achieving optimal outcomes.

Chimerism of Donor-Derived MSCs after Allogeneic Hematopoietic Cell Transplantation

Although there is experimental evidence suggesting the presence of a common mesoderm cell as origin of both hematopoietic and mesenchymal progenitor cells in an animal model,[56] it is still controversial if durable engraftment of native donor-derived MSCs without ex vivo treatment can occur in the recipient of allogeneic HSCT.[57-63] In this context, clinical studies of culture-expanded high-dose MSCs transplantation for children with severe osteogenesis imperfecta (OI) are very informative.[64,65] Horwitz et al. reported the engraftment of donor-type MSCs in children who underwent allogeneic marrow transplantation and subsequent infusion of culture-expanded booster MSCs as treatment for severe OI; the infused MSCs were derived from the BM of the original donors and gene-marked for tracking.[66] Despite high doses of infused MSCs, the fraction of donor cells at any samples was less than 1% when PCR-based chimerism analyses of osteoblasts, fibroblasts, and marrow stromal cells of these children were performed between 18 and 34 mo after the MSC infusions.

Similarly to gene-marking, recent development of noninvasive imaging techniques has enabled us to have a better insight into in vivo cell tracking of transplanted MSCs in a real-time manner.[66] These experimental imagings have revealed that intravenously transplanted MSCs are mainly distributed in the lungs and liver, while their distribution to the other tissues such as BM is barely detectable.[67] Also, in conventional BM transplantation where MSCs are being transferred in their native form without an ex vivo expansion phase, it is believed that only a small proportion of MSCs can reach to the marrow because most of them are trapped in the microvasculatures of lungs, particularly when MSCs are intravenously administered. By contrast, in a rat model of myocardial infarction, Barbash et al. reported that direct left ventricular infusion of MSCs could enhance their migration and colonization to the area of ischemic myocardium as compared with systemic intravenous infusion.[68] Similarly, there is accumulating evidence that intra-BM transplantation of hematopoietic cell graft relatively rich in MSCs may be a promising procedure to facilitate the sustained engraftment of donor MSCs in a series of experiments using large animal models.[69] To get a better understanding of the fate of donor-derived MSCs after allogeneic HSCT or other MSC-based therapy, development of reliable imaging methods to track infused MSCs is highly required. Furthermore, it is warranted to develop a technique for detecting microchimeric presence of infused MSCs in extra-marrow sites as well as in BM.

Future Prospects

One caveat in allogeneic HSCT using current protocols might be very low chimerism of donor-type MSCs at least in the hematopoietic microenvironment. Although it is well known that recipients of allogeneic HSCT successfully recover normal hematopoietic function despite lack of engraftment of donor-derived MSCs in the majority of cases, host-type MSCs that survived after sublethal conditioning for HSCT can harbor genetic abnormalities that may compromise crucial functions of MSCs to support effective hematopoiesis and tissue regeneration.

Although little information exists regarding the function of MSCs residing in the recipient of allogeneic HSCT, BM-derived

MSCs from a long-term survivor after myeloablative transplant can have complex chromosome abnormalities (TI, unpublished observation). Since various cytokines including erythropoietin and parathyroid hormone are reported to have activities to enhance MSC-mediated bone formation, hematopoiesis, and tissue regeneration,[70-72] we propose that future direction of MSC-based therapy should include pharmacologic upregulation of their functions. Another promising approach will be to improve the engraftment of donor-type MSCs by their in situ administration into the BM cavity of the recipient. Attempts to reconstitute abnormal microenvironment with allogeneic normal MSCs and to achieve appropriate donor MSC chimerism could be a novel therapeutic approach for the treatment of intractable hematologic and MSC-associated disorders.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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