1083-8791/05/1105-0001$30.00/0
doi:10.1016/j.bbmt.2005.01.005

Immunobiology of Human Mesenchymal Stem Cells and Future Use in Hematopoietic Stem Cell Transplantation

Katarina Le Blanc, Olle Ringdén

Center for Allogeneic Stem Cell Transplantation and Division of Clinical Immunology, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm, Sweden

Correspondence and reprint requests: Olle Ringdén, MD, PhD, Division of Clinical Immunology, F79, Karolinska University Hospital, Huddinge, SE-141 86 Stockholm, Sweden (e-mail: olle.ringden@labmed.ki.se).

Received July 16, 2004; accepted January 6, 2005

ABSTRACT

Mesenchymal stem cells (MSCs) may be derived from adult bone marrow, fat, and several fetal tissues. In vitro, MSCs can be expanded and have the capacity to differentiate into several mesenchymal tissues, such as bone, cartilage, and fat. They escape the immune system in vitro, and this may make them candidates for cellular therapy in an allogeneic setting. They also have immunomodulatory effects, inhibit T-cell proliferation in mixed lymphocyte cultures, prolong skin allograft survival, and may decrease graft-versus-host disease (GVHD) when cotransplanted with hematopoietic stem cells. MSCs induce their immunosuppressive effect via a soluble factor. Some candidates have been suggested, and various mechanisms have also been suggested, although contradictory data exist; this may be due to differences in the cells and systems tested. A major problem has been that it has been difficult to identify and isolate MSCs after transplantation in vivo. However, MSCs seem to enhance hematopoietic engraftment in recipients of autologous and allogeneic grafts. Recently, they were found to reverse grade IV acute GVHD of the gut and liver. No tolerance was induced, however. Controlled studies are warranted. Thus, in allogeneic stem cell transplantation, MSCs may be used for hematopoiesis enhancement, as GVHD prophylaxis, and for the treatment of severe acute GVHD. They are also of potential use in the treatment of organ transplant rejection and in autoimmune inflammatory bowel disorders where immunomodulation and tissue repair are needed.

KEY WORDS

Mesenchymal stem cells • Allogeneic hematopoietic stem cell transplantation • Immune response • Graft-versus-host disease

INTRODUCTION

Stem cells are characterized by a capacity for self-renewal and an ability to differentiate into at least 1 mature cell type. In addition to hematopoietic stem cells (HSCs), bone marrow also contains mesenchymal stem cells (MSCs). Although MSCs do not fulfill all criteria for a true stem cell, they have been so called for convenience. MSCs were first recognized by Friedenstein and associates, who identified an adherent, fibroblast-like population that could regenerate rudiments of normal bone in vivo [1-4]. Apart from postnatal marrow, MSCs have also been isolated from adipose tissue and fetal liver, blood, bone marrow, lung, and cord blood [5-8]. They have the capacity to differentiate in vitro and in vivo into several mesenchymal tissues, including bone, cartilage, tendon, muscle, adipose tissue, and, possibly, bone marrow stroma [9-11].

As progenitors of well-differentiated tissues, MSCs have enticed researchers to explore their role in regenerative medicine. Their use to create new bone in segmental bone defects has been demonstrated in the athymic rat implanted with human MSCs and in a canine model using autologous MSCs [12-14]. Furthermore, culture-expanded MSCs have been demonstrated to regenerate articular cartilage defects and repair Achilles tendon ruptures in rabbit models [15-

17]. Human synovial membrane–derived MSCs engraft and differentiate into muscle cells in a mouse model of muscular dystrophy [18]. Under certain conditions, MSCs may acquire a cardiomyogenic phenotype [19]. Several attempts have been made to make MSCs engraft in the myocardium, but it has been controversial whether MSCs undergo in situ differentiation [20-22]. However, there are no data showing functional integration of MSCs into cardial muscle. It therefore remains to be proven whether MSCs will play a role in cellular cardiomyoplasty. Adipocytes derived from MSCs are functional and may be of value in adipocyte research as a renewable source of adipocytes [23]. MSCs have been suggested to be precursor cells for the bone marrow stroma that provides a 3-dimensional scaffold and enhances proliferation of HSCs. They may therefore also enhance engraftment after autologous or allogeneic stem cell transplantation (ASCT).

MSCs possess immunomodulatory properties and inhibit T-cell proliferation in vitro [24-29]. An immunosuppressive effect of MSCs in vivo has been shown in a baboon model, in which infusion of ex vivo–expanded matched donor or third-party MSCs delayed the time to rejection of histoincompatible skin grafts [25]. An immunosuppressive function of MSC grafts in human beings, as a corollary to the immunosuppressive effect of MSCs in vitro and in preclinical animal models, suggests that MSCs may be used for the prevention and treatment of graft-versus-host disease (GVHD) in ASCT, in organ transplantation to prevent rejection, and in autoimmune disorders. Severe acute GVHD is associated with high mortality and is a major threat to successful ASCT [30-32]. Currently, no effective therapy exists for severe steroid-refractory acute GVHD [33-42]. A variety of drugs and antibodies have been used, mostly with discouraging results. Recently, MSCs were used to successfully treat a 9-year-old boy with severe treatment-resistant acute GVHD; this suggests that they have a powerful immunosuppressive effect in humans [43]. This review will deal with MSC expansion, which is necessary for clinical use. Furthermore, we will briefly present surface markers, cytokine production, and interaction with hematopoietic cells, to understand the role of MSCs in hematopoiesis. A major focus is on immune escape and immunosuppression of MSCs, which so far are dominated by in vitro studies. Several mechanisms behind the immunomodulatory effect have been suggested. We have, among other things, tried to explain the divergent findings. Finally, we have reviewed the limited clinical experience with using MSC in HSC transplantation, focusing on their potential role for prophylaxis and treatment of acute GVHD.

MSC EXPANSION

Expansion of MSCs is a necessity for clinical use. Most information regarding the phenotypic and functional properties of the cells we refer to as MSCs comes from studies performed on cells expanded ex vivo. Plating studies indicate that MSCs are rare in the human body [10,44-47]. Relatively little is known about the characteristics of the primary precursor cells in vivo. One problem is the inability to prospectively isolate MSCs because of their rarity and the lack of markers to facilitate their isolation and enrichment.

MSCs are rare in the human body but can be expanded in vitro to hundreds of millions of cells from a 10- to 20-mL bone marrow aspirate [9,44-47]. Isolated from other cells in the bone marrow by adherence to plastic and consecutive passaging, MSCs proliferate to spindle-shaped cells in confluent cultures. Although homogeneous by light microscopy, even single cell–derived colonies form a molecularly heterogeneous population of cells that vary to some extent in their differentiative capacity [10,48,49]. Even if MSCs rapidly expand >1 billion-fold, individual cells in a culture exhibit a highly variable expansion potential [46,50-54]. Furthermore, the cell yield after expansion varies with the age and condition of the donor and with the harvesting techniques [46,50,54-57]. Naturally, differences in isolation techniques, culture conditions, media additives, and subculturing techniques greatly affect cell yield and possibly also the phenotype of the expanded cell product [58].

Is the MSC a true stem cell? The gene expression/proteomics of MSCs that have been culture expanded depend on the culture conditions, passage, species, and other factors or may or may not reflect in vivo events. One may therefore question whether MSCs are real stem cells, because there seems to be an expansion limit. MSCs have not been demonstrated in vivo at a single-cell level to be capable of regenerating or maintaining a tissue compartment. One problem concerning MSCs is the inability to prospectively isolate MSCs from tissue, characterize them, and observe their biologic properties. According to a less distinct definition, MSCs have the capacity for self-renewing and giving rise to 1 or more types of differentiated progeny. In vitro, MSCs have vast proliferative potential, can clonally regenerate, and can give rise to differentiated progeny. So far, the designation of MSCs as stem cells is based on extrapolation of in vitro data. More in vivo data showing the therapeutic potential and biology of MSCs is required before they can be claimed to be true stem cells. Moderate subcultivation will not change the karyotype or telomerase activity of MSCs, but if the cells are cultured beyond the Hayflick limit of approximately 50 population doublings, signs of senescence and apoptosis appear [46,50,51].

Stromal precursors can be found in the peripheral blood of mice, but whether MSCs circulate in the bloodstream of humans to repair injured tissues remains an open question [59]. MSCs have been detected in the peripheral blood of granulocyte colony-stimulating factor–mobilized breast cancer patients, but not in peripheral blood collected from healthy donors [60-63].

ADHESION MOLECULES, CYTOKINE PRODUCTION, AND INTERACTIONS WITH HEMATOPOIETIC CELLS

There is a need for a quantitative assay to assess MSCs in a given population, because there is no specific marker or combination of markers that specifically identifies MSCs. Therefore, MSCs have been defined by using a combination of phenotypic markers and functional properties. Controversy still exists over the in vivo phenotype of MSC; however, ex vivo–expanded MSCs do not express the hematopoietic markers CD34, CD45, or CD14 [10,64]. In addition to their stem cell characteristics of self-renewal, high proliferative capacity, and multipotentiality, they can be identified as cells that stain positive for CD73, CD105, CD166, CD90, and CD29 by flow cytometry [10,11,65-67].

It has been speculated that MSCs participate in the marrow microenvironment, because MSCs produce a vast array of matrix molecules, including fibronectin, laminin, collagen, and proteoglycans [10,51,68-70]. They also express various integrin α- and β-subunits and their noncovalent associations that constitute receptors for extracellular matrix components, including collagen (α1β1 and α2β1), laminin (α6β1 and α6β4), fibronectin (α3β1 and α5β1), and vitronectin (αvβ1 and αvβ3) [10,51,65,71]. It is likely that MSCs play a role in the organization of the extracellular matrix. Characterization of surface molecules by flow cytometry has determined that MSCs also express ligands for surface molecules present on cells of the hematopoietic lineage, including intercellular adhesion molecule (ICAM)–1, ICAM-2, vascular cell adhesion molecule 1, lymphocyte function–associated antigen 3, CD72, and activated leukocyte cellular adhesion molecule [10,51,65,72,73]. In coculture experiments, MSCs form cell clusters with HSCs, including megakaryocytes and osteoclast progenitors [74,75]. At the same time as they provide physical support for HSCs, they constitutively secrete cytokines important for HSC differentiation [65,72,74-77]. When cocultured with hematopoietic progenitors in vitro, MSCs have the capacity to maintain and expand lineage-specific colony-forming units from $CD34^+$ marrow cells in long-term bone marrow culture [75,78].

Stromal cells are also essential for lymphopoiesis. Early B cells adhere to stromal cells, and differentiation does not occur when lymphocytes and stromal cells are separated in a diffusion chamber system [79,80]. Immature T cells preferentially adhere to mesenchymal bone marrow stroma, which, at least in culture, supplies the appropriate stimuli for proliferation of thymus precursor cells [81,82]. Moreover, donor-derived stromal cells in bone marrow migrate into the thymus and participate in the positive selection of T cells after bone marrow transplantation plus bone grafts [83].

Both $CD4^+$ and $CD8^+$ lymphocytes bind to MSCs, and the affinity is increased for activated T cells [71]. Several adhesion molecules expressed by MSCs are essential for the interaction with T cells. Vascular cell adhesion molecule 1, ICAM-2, and lymphocyte function–associated antigen 3 are present on unstimulated MSCs, whereas the expression of ICAM-1 is inducible [10,27,65,71].

MSCs ESCAPE THE IMMUNE SYSTEM IN VITRO

MSCs seem to escape the immune system, and this makes them potentially useful for various transplantation purposes. Adult MSCs express intermediate levels of HLA major histocompatibility complex (MHC) class I molecules. The expression on fetal MSCs is lower [10,27,65,84,85]. Adult MSCs do not express HLA class II antigens of the cell surface. However, HLA class II is readily detectable by Western blot on whole-cell lysates of unstimulated adult MSCs, thus suggesting that MSCs contain intracellular deposits of HLA class II alloantigens [84]. Cell-surface expression can be induced by treatment of the cells with interferon (IFN)–γ for 1 or 2 days. Unlike adult MSCs, the fetal liver–derived cells have no HLA class II intracellularly or on the cell surface [85]. The presence of IFN-γ in the growth medium for 2 days initiated the intracellular synthesis of HLA class II in fetal MSCs, although 7 days of exposure was required for cell surface expression. After differentiation of MSCs into bone, cartilage, or adipose tissue, both adult and fetal MSCs continued to express HLA class I, but not class II [84,85].

In vitro, undifferentiated MSCs fail to elicit a proliferative response from allogeneic lymphocytes, thus suggesting that the cells are not inherently immunogenic [24,26,27,86]. When precultured with IFN-γ for full HLA class II expression, MSCs still escape recognition by alloreactive T cells [84,85]. In coculture experiments, MSCs differentiated into adipocytes, osteoblasts, and chondrocytes, like undifferentiated MSC, are nonimmunogenic [84,85]. However, in vivo, limited data demonstrate the persistence

of allogeneic MSCs after transplantation into immunocompetent hosts. Therefore, the question of whether MSCs are recognized by an intact allogeneic immune system in vivo remains open, although the in vitro data support the theory that MSCs escape the immune system.

MSCs do not express FAS ligand or costimulatory molecules, such as B7-1, B7-2, CD40, or CD40L [27,87]. When costimulation is inadequate, T-cell proliferation can be induced by the addition of exogenous costimulation. However, MSCs differ from other cell types, and no T-cell proliferation can be observed when they are cultured with HLA-mismatched lymphocytes in the presence of a CD28-stimulating antibody [27]. One potential avenue for the initiation of graft rejection is bystander activation. Given that the proportion of T cells with allospecificity is estimated to be 1% to 5% in the course of an infection, T cells that are capable of recognizing MSCs or their progeny could be activated by chance. However, in agreement with the in vitro data, infusion or implantation of allogeneic, MHC-mismatched MSCs into baboons has been well tolerated in most animals [88-90]. Unique immunologic properties of MSCs were also suggested by the fact that engraftment of human MSCs occurred after intrauterine transplantation into sheep, even when the transplantation was performed after the fetuses became immunocompetent [91].

MSC neither fail to activate T cells nor are targets for $CD8^+$ cytotoxic lymphocytes [92]. Phytohemagglutinin (PHA) blasts generated to react against a specific donor will lyse chromium-labeled mononuclear cells from that individual, but not MSCs derived from the same donor. Furthermore, killer cell inhibitory receptor (KIR ligand)–mismatched natural killer cells do not lyse MSCs [92]. Thus, MSCs, although incompatible at the MHC, escape the immune system. These in vitro data may suggest that allogeneic fetal or adult MSCs can be transplanted without being rejected. However, although MSCs are transplantable across allogeneic barriers, a delayed type hypersensitivity reaction leading to transplant rejection occurred in a xenogenic model when human MSCs were rejected in immunocompetent rats [93]. In this study, human MSCs were identified in the heart muscle of severe compromised immune deficiency rats, in contrast to that of immunocompetent rats. In the latter group, peripheral blood lymphocytes proliferated after restimulation with human MSCs in vitro, thus suggesting cellular immunization. Such a proliferative response in vitro has not been detected in humans treated with intravenous (IV) infusion of allogeneic MSCs (Le Blanc and Ringdén, unpublished data, 2004).

IMMUNOMODULATION BY MSCs

The immunomodulatory effects of MSCs may make them useful for immunotherapy, although the exact mechanism of action is unknown. As mentioned previously, MSCs are immunosuppressive and inhibit T-cell alloreactivity induced in mixed lymphocyte cultures (MLCs) or by nonspecific mitogens [24-29, 87,94]. Whether they suppress lymphocyte responses induced by recall antigens is controversial [29,71,94]. MSC-induced T-cell suppression seems to include both naive and memory T cells. It has no immunologic restriction; it is significant whether the MSCs are autologous with the stimulatory or the responder lymphocytes or are derived from a third party [25-28]. This suggests that MSCs used in ASCT may not need to be derived from the HSC donor. The degree of MSC suppression is dose dependent [26,29]. High doses of MSC are inhibitory, whereas low doses enhance lymphocyte proliferation in MLCs. The T cells do not become apoptotic or anergic, because they can be restimulated if MSCs are removed [25,29].

MSCs decreased the expression of $CD4^+$ activation markers, CD25, CD38, and CD69 on PHA-stimulated lymphocytes [95]. Recent data suggest that MSCs increase the number of regulatory T cells [96]. Data from our own laboratory suggest that suppression may be mediated by different mechanisms, depending on the T-cell stimulus [97]. For instance, MSCs increased the transcription and translation of interleukin (IL)–2 and soluble IL-2 receptors in MLCs, whereas the levels decreased if MSCs were present among the PHA-stimulated lymphocytes. T-cell inhibition may occur before IL-2 is secreted. MSCs inhibit lymphocyte proliferation induced by concanavalin A. However, when IL-2 was added to concanavalin A–stimulated lymphocytes, the inhibition by MSCs was partly abrogated [25]. Furthermore, coculture of MSCs with purified activated dendritic cells led to decreased tumor necrosis factor α secretion and increased IL-10 secretion [96]. MSCs cocultured with effector T cells or purified natural killer cells led to a decrease in IFN-γ secretion or an increase in IL-4 secretion, respectively. Dependent on the kinetics, MSCs can enhance or depress IL-10 levels in MLCs [97]. These various effects may in part explain the immunosuppressive effects by MSCs in vitro.

The way in which MSCs suppress T-cell activation and modulate the immune response has not been completely resolved. However, several mechanisms have been proposed, and MSCs have been shown to have various significant effects. Suppression seems to be mediated by a soluble factor or factors produced by human MSCs, because suppression still occurs if MSCs and lymphocytes are separated in a transwell system [24,27,92]. It is unlikely that the factor(s) are

Table 1. *Clinical Experience of Mesenchymal Stem Cells in Hematopoietic Stem Cell Transplantation*

Disease	No. Patients	Source of MSCs/SCT Setting	Outcome	Study
Hematologic malignancies	15	Autologous IV infusion	No adverse events of 1, 10, and 50 × 10^6 cells	Lazarus et al. [101]
Breast cancer	28	Autologous in autologous SCT	IV infusion was safe; autologous SCT recovery was rapid	Koç et al. [102]
Inborn errors of metabolism	11	HLA-identical from SCT donor	No immune response against donor; improved nerve-conduction velocity in metachromatic leukodystrophy	Koç et al. [125]
Osteogenesis imperfecta	5	HLA-identical from SCT donor	Gene-marked MSCs engrafted; new dense bone formation; few fractures	Horwitz et al. [104]
Acute myeloid leukemia	1	HLA-haploidentical from SCT donor	SCT engraftment with no GVHD	Lee et al. [133]
Leukemia	31	HLA-identical from SCT donors	Rapid platelet engraftment; low incidence of acute GVHD	Frassoni et al. [134]
Severe aplastic anemia	1	Allogeneic MSC	Engraftment; improved stroma	Fouillard et al. [139]
Severe acute GVHD	1	Haploidentical MSC; unrelated donor SCT	Clearance of grade IV acute GVHD, twice	Le Blanc et al. [43]

constitutively secreted by MSC, because cell-free MSC culture supernatants fail to suppress alloreactivity, whereas supernatants from MSC/lymphocyte cocultures are suppressive [29,95,98]. In contrast, in mice, inhibition by MSCs was reported to require cell contact [28]. The soluble factors mediating the suppressive effect were suggested to be composed of the hepatocyte growth factor and transforming growth factor (TGF)–α [24]. Experiments showed that the addition of anti–hepatocyte growth factor and anti–TGF-α restored T-cell proliferation in the presence of MSCs. However, we were unable to reproduce these experiments [95]. Aggarwal and Pittenger [96] suggested that MSC-produced prostaglandin E_2 accounted for reduced lymphocyte proliferation. Another study suggests that indoleamine 2,3-dioxygenase–mediated tryptophan depletion by MSCs can act as a T cell–inhibitory effector mechanism [99]. Indoleamine 2,3-dioxygenase, which is induced by IFN-γ, catalyzes the conversion from tryptophan to kynurenine and inhibits T-cell responses [100]. However, in the hands of Tse et al., neither MSC production of IL-10, TGF-β1, and prostaglandin E_2 nor tryptophan depletion in the culture medium was responsible for the immunosuppressive effect [27]. In addition, MSCs produce bone morphogenetic protein 2, which may mediate immunosuppression via the generation of $CD8^+$ regulatory cells [98]. The controversial data may be due to the use of MSCs generated by different techniques; the use of different stimuli, culture conditions, doses, and kinetics; and different lymphocyte populations tested. Such differences may in turn affect cytokine and chemokine secretion, with seemingly contradictory results. Furthermore, apparent species-specific differences, particularly between murine and human MSCs, add to the confusion [28]. A major problem is that MSCs cannot be identified by a specific marker or a combination of markers. One likely conclusion from the available data is that several mechanisms are involved in the MSC-mediated immunosuppressive effect.

First-trimester fetal liver–derived MSCs have different immunomodulatory properties [85,86]. Fetal MSCs suppress mitogenic responses, but not alloreactivity, in MLCs. However, when precultured with IFN-γ for full HLA class II expression, fetal MSCs inhibit lymphocyte proliferation at a magnitude similar to that seen with adult MSCs. Thus, despite the upregulation of class II alloantigens, other concurrent events induced by IFN-γ seem to enhance the antiproliferative effect that fetal MSCs exert on lymphocyte proliferation.

CLINICAL EXPERIENCE OF MSC INFUSION

There is an urgent need for better treatment and prevention of GVHD after ASCT. Therefore, the clinical experience with and safety of MSCs are of the utmost interest. The published clinical experience of MSCs in the hematopoietic stem cell transplantation setting is limited but is summarized in Table 1. A phase I trial was performed to determine the feasibility of collection, expansion, and IV infusion of human MSCs in the autologous setting [101]. Fifteen patients participated; 5 patients in each group received 1, 10, and 50 × 10^6 MSCs, respectively. No adverse reactions were observed.

After IV infusion, MSCs circulate in the human body for a short period. Koç et al. [102]. detected circulating clonogenic MSCs in some, but not all, patients within the first hour of infusion, but not thereafter. Human MSCs engraft in multiple tissues and demonstrate site-specific differentiation after intrauterine transplantation into sheep [91,103]. Potential in vivo engraftment in bone has also been shown after IV MSC infusion in children with osteogenesis imperfecta (OI) [104] and in 1 patient with severe aplastic anemia [105]. However, in the vast majority of reports, long-term engraftment of transplanted MSCs has not been demonstrated.

MSCs MAY ENHANCE ENGRAFTMENT

Because the bone marrow stromata that support hematopoiesis may derive from MSCs, it is of interest to study whether MSCs enhance hematopoiesis. After ASCT, the immune and hematopoietic systems are, in most cases, entirely donor in origin [106-108]. After high-dose chemoradiotherapy before autologous or allogeneic stem cell transplantation, the marrow stroma is damaged and slow to reconstitute [56,109-114]. Because damage to the stroma may affect hematopoietic engraftment after stem cell transplantation, reconstitution of stromal cells by infusion of MSCs may enhance hematopoiesis after transplantation. Because less than 1 in 10 000 cells in the bone marrow is an MSC, a conventional bone marrow graft should contain at the most approximately 10 000 MSCs per kilogram of recipient weight [10,47]. However, stromal progenitors are predominantly recipient in origin, and this suggests that MSCs have a limited capacity for reconstituting the marrow microenvironment [57,115].

Nevertheless, both fetal and adult human MSCs promote engraftment of unrelated and umbilical cord–derived HSCs in nonobese-diabetic/severe compromised immune deficiency mice and fetal sheep [29,116-119]. Whether this is due to MSCs or to specific cytokines needs to be determined. The enhancing effect is most prominent when the dose of hematopoietic cells is limiting. In the study by Maitra et al., 2 of 10 mice engrafted when transplanted with a low number of MSCs, whereas 8 of 10 mice cotransplanted with umbilical cord blood cells and MSCs showed persistent engraftment [29]. MSC support for reconstitution is not lineage restricted but involves cells of myeloid, lymphoid, and megakaryocytic lineages [118-120]. The mechanism of enhancement is not understood and may or may not require homing of MSCs to the marrow.

In an initial study to explore whether MSCs enhance engraftment in autologous stem cell transplantation in humans, MSCs were isolated and expanded in breast cancer patients receiving peripheral blood stem cell infusions [102]. Twenty-eight patients were infused IV with 1 to 2 $\times$ 10^6/kg MSCs. There were no cases of toxicity. The median time to achieve an absolute neutrophil count of $>0.5 \times 10^9$/L was 8 days (range, 6-11 days). A platelet count of $>20 \times 10^9$/L was reached in a median of 9 days (range, 4-19 days). The observed rapid hematopoietic recovery suggests that MSC infusion may have a positive effect on hematopoiesis in vivo. Controlled clinical trials are now needed to clarify whether MSCs have a role in accelerating hematopoietic engraftment after transplantation.

MSCs FOR INBORN ERRORS OF METABOLISM

ASCT is indicated for several inborn errors of metabolism [121-124]. MSCs express high levels of arylsulfatase A and α-L-iduronidase [57]. Arylsulfatase A deficiency is the cause of metachromatic leukodystrophy (MLD), and α-L-iduronidase deficiency is the cause of Hurler disease. To obtain further beneficial effects by providing enzyme replacement to the tissue that MSCs distribute to, donor-derived MSCs were expanded and given IV to patients with MLD and Hurler disease who had previously undergone ASCT. Eleven patients were enrolled and infused with donor MSCs; they had no significant toxicity within 15 to 31 months of follow-up [125]. No immune response against donor MSCs was detected with enzyme-linked immunospot assay using recipient lymphocyte and donor MSCs. Although there was no major improvement in the overall health of the patients, in 4 of 5 patients with MLD, there was clear evidence of improvement in nerve-conduction velocity.

OSTEOGENESIS IMPERFECTA

MSCs can differentiate into bone in vitro. Therefore, MSCs may be useful in the treatment of bone disorders. OI is caused by a mutation of 1 of the 2 genes that encode type I collagen. This genetic disorder leads to generalized osteopenia, bony deformities, excessive fragility with fracturing, and short stature. There is no cure for OI, nor is there any effective therapy [126].

In a mouse model of OI, MSCs expressing normal type 1 collagen were infused [127]. A small number of donor MSCs and osteoblasts engrafted, and normal collagen was detected in the bone of the OI mice. Studies in humans indicate a possible therapeutic effect of MSCs in OI [128]. In 5 children with OI, representative specimens of trabecular bone showed histologic changes indicative of new dense bone for-

mation after successful ASCT. All patients had an increase in total body bone mineral content compared with predicted values for healthy children. These improvements were associated with increased growth velocity and fewer bone fractures. The data suggest that ASCT can lead to engraftment of functional MSCs, thus indicating the feasibility of this strategy in the treatment of OI and maybe other MSC disorders. Furthermore, gene-marked MSCs were demonstrated to engraft in children with OI [104]. These data encouraged us to perform fetal MSC transplantation in utero in a fetus with OI. A female fetus with bilateral intrauterine femur fractures, diagnosed with severe OI, underwent transplantation with HLA-mismatched male fetal MSCs in the 32nd week of gestation. At 9 months of age, a bone marrow biopsy showed 7.4% Y-positive cells by fluorescence in situ hybridization (FISH). The bone was regularly arranged, with configured bone trabeculae lined by a columnar layer of normal osteoblasts. During the first year of life, this girl had only 2 suspected fractures: a clavicular fracture at 6 weeks of age and a costal fracture at 9 months. The data showed that allogeneic HLA-mismatched fetal MSCs engraft and differentiate into bone in an immunocompetent fetus.

MSCs IN ALLOGENEIC HSC TRANSPLANTATION

In ASCT, MSCs may be used to enhance engraftment of white blood cells and platelets. Furthermore, MSCs may be used to modulate the immune system, as prophylaxis to prevent GVHD, and as treatment for established GVHD. Several studies have compared bone marrow and peripheral blood as a source of stem cells in patients receiving allografts [129-131]. The higher CD34 cell dose often obtained by using peripheral stem cell harvest is associated with faster neutrophil and platelet engraftment. However, when 881 adult patients with acute myeloid leukemia in first complete remission were retrospectively compared for transplant-related mortality, leukemia-free survival, and overall survival, the outcome was significantly better for patients who received a high ($>2.7 \times 10^8$/kg) bone marrow dose than for high-dose peripheral blood stem cell transplantation [132]. Because whole bone marrow contains many cell types, it is possible that accessory cells in the graft contribute to the improved outcome when stem cell doses are similar. Thus, a graft engineered to include both a large stem cell dose and an increased number of stromal precursor cells may further improve outcome in ASCT.

A 20-year-old woman with acute myeloid leukemia received peripheral blood stem cells combined with MSCs from her HLA-haploidentical father [133]. The patient engrafted rapidly, with no acute or chronic GVHD, and was doing well 31 months after the ASCT. This is a most remarkable case. After haploidentical transplantations with conventional immunosuppression, the risk of rejection or life-threatening acute GVHD is substantial. This finding, of course, needs to be confirmed.

In a multicenter clinical trial, HSCs and MSCs derived from HLA-identical sibling donors were infused to promote hematopoietic engraftment and limit GVHD [134]. Thirty-one patients received myeloablative conditioning and HLA-identical sibling bone marrow or peripheral blood stem cells. Escalating doses of MSC from 1 to 5×10^6/kg were given. There were no incidences of MSC infusion–related toxicity. The incidence of grade II to IV acute GVHD was 15% in the cotransplanted group, compared with 40% in a matched control group ($P = .01$). The most significant difference was the higher platelet count on day +50 after transplantation in the cotransplanted group ($P = .0001$). Delayed platelet engraftment is a known risk factor for chronic GVHD [135]. In agreement with an improved platelet reconstitution, the incidence of chronic GVHD was lower in the group receiving MSCs (12% versus 67% in the control group; $P = .002$). Survival at 6 months in the patients receiving MSCs was 88%, compared with 68% in the control group. This preliminary study suggests that coinfusion of MSCs in ASCT may enhance engraftment, decrease GVHD, and improve survival. These data have to be interpreted with caution, because the study is not published in a refereed journal. Furthermore, a new analysis has been performed with a matched control group from the Centre of International Blood and Marrow Transplant Research. This new analysis has not yet been presented or published. Prospective dose-finding and randomized studies are under way in the United States and in Europe.

MSCS FOR TREATMENT OF ACUTE GVHD

There is no successful therapy for steroid-refractory acute GVHD. The possible role of MSCs in this context is therefore of potential interest. Recently, we reported a case of grade IV acute GVHD of the gut and liver in a patient who had undergone ASCT with cells from an unrelated female donor [43]. The male patient was unresponsive to all types of immunosuppression, including prednisolone 2 mg/kg daily, repeated IV infusions of methylprednisolone, extracorporeal treatment with psoralen and UV-A light 1 to 4 times per week for 6 weeks, infliximab and daclizumab for 4 weeks, mycophenolate mofetil, and methotrexate. He was treated for repeated bacterial, viral, and invasive fungal infections. After an infusion of 2×10^6 MSCs per kilogram from his HLA-haploidentical

mother, his GVHD responded miraculously with a decline in bilirubin and normalization of stools. The patient had high-risk acute lymphoblastic leukemia in third remission at the time of transplantation. After the MSC infusion, DNA analysis of his bone marrow showed the presence of minimal residual disease [136]. Cyclosporine treatment was discontinued to allow a maximum graft-versus-leukemia effect. When immunosuppression was discontinued, the patient again developed severe acute GVHD, with diarrhea and an increase in bilirubin to 360 mmol/L within a few weeks.

Colonoscopy showed a normal colon with mild GVHD and 4% female epithelium detected by FISH. He received a repeat infusion of MSCs from his mother (1×10^6/kg). After 1 week, his stools were normal, and he started to eat again. Bilirubin subsequently normalized. One year after transplantation, he was home and well, with no minimal residual disease in the blood or bone marrow. However, 1.5 years after the transplantation, an attempt was made to discontinue immunosuppression, and the GVHD recurred. Immunosuppression was again initiated. Unfortunately, in the meantime, he developed repeated pneumonias requiring ventilation and died in his home hospital 19 months after ASCT. Several lessons can be learned from this case. In particular, MSCs exert therapeutic effects on severe GVHD, probably through immunosuppression and healing of the gut and liver. Although a profound immunomodulatory effect was seen, it is clear from this case that tolerance was not induced by MSCs. Immunosuppression with cyclosporine and steroids was needed because severe acute GVHD recurred after the withdrawal of cyclosporine. However, the same MSCs could be used again and had the same dramatic effect on GVHD in the gut and liver. In accordance with previous in vitro reports, allogeneic HLA-incompatible MSCs did not induce an immune response in vivo [24,26,27,84]. Furthermore, a dose of 1×10^6 MSCs per kilogram seems sufficient for a prompt response. It could not be proven that the female epithelial cells in the colon detected by FISH were from the MSC donor, because the HSC donor was also female. However, it is most likely that these cells were from the MSC donor, because a study in baboons showed that MHC-mismatched MSCs became engrafted in gastrointestinal tissue after IV infusion [90,137].

Two additional patients with grade II to IV acute GVHD of the gut responded to MSC therapy from their respective HLA-identical ASCT donors at our unit. Both patients were alive and well 32 and 6 months after ASCT (Le Blanc and Ringdén, unpublished data, 2005). A promising effect of MSCs on acute GVHD has also been confirmed in 1 patient who recovered from steroid-resistant acute GVHD after infusion of MSCs from an HLA-matched donor by the team in Genua (Frassoni, personal communication, 2004). So far, our experience of using MSCs to treat acute GVHD is limited to a few cases. Additional data from prospective controlled studies are needed, and such studies are under way.

FUTURE PERSPECTIVES FOR MSCs IN ASCT

Many questions regarding MSCs cannot be answered today. Most of what is known about MSCs is derived from in vitro experiments. When administered in vivo, MSCs have been difficult or almost impossible to detect. There is very limited experience with MSCs administered to humans. For instance, gene-marked MSCs were found to engraft in children with OI [104]. Using FISH, we were able to find Y chromosome–positive cells in a bone marrow biopsy sample when male fetal MSCs were injected in utero to a female recipient. In this patient, genomic HLA typing by polymerase chain reaction (PCR) could not detect any donor cells. Furthermore, we have not been able to identify donor HLA by PCR in any tissue after IV infusion of allogeneic MSCs. We can only speculate about the difficulties in identifying MSCs in vivo. Many cells seem to lodge in the pulmonary vascular bed, and in other tissues MSCs may appear with low frequency, if at all [138]. Clinical effects of MSCs have clearly been observed; however, it is possible that the effect of the MSCs has been due to local production of growth factors rather than to direct participation of MSCs in the healing process. After fulfilling this function, MSCs may have died. It cannot be excluded that MSCs may have been rejected in an allogeneic setting, although an immune response in vitro, determined via lymphocyte proliferation, has not been detected. Much more work, especially in vivo, is required to increase our knowledge of how MSCs act and their fate. However, we need not wait for such additional data, because significant effects, albeit anecdotal, have already been noted in the clinic.

One reason that it has been difficult to detect donor MSCs in bone marrow aspirates may be that MSCs are located in the endosteum [139]. In a 68-year-old woman with end-stage severe aplastic anemia, MSCs from her son (10^7 MSCs per kilogram on 1 occasion and 6×10^6 MSCs per kilogram on another occasion) were injected. The bone marrow showed donor chimerism by PCR that was not detected in bone marrow aspirates. Such a discrepancy between bone marrow biopsies and aspirations has already been observed in baboons [88].

In HSC transplantation, MSCs may be important for several indications. Overall, in ASCT, MSCs may enhance engraftment of hematopoietic cells. This may

be particularly important in cord blood transplantation, in which the limited cell dose delays engraftment of the absolute neutrophil count and platelets and in which there is an increased risk of graft failure [140,141]. Whether MSCs in this setting have immunomodulatory effects and prevent rejection remains to be proven. Furthermore, with nonmyeloablative conditioning, the risk of graft failure is increased compared with myeloablative conditioning [142]. Whether MSCs enhance donor cell engraftment and prevent rejection may be worthwhile to explore. Because MSCs produce arylsulfatase A and α-L-iduronidase, cotransplantation with HSCs may also be important in patients with various types of inborn errors of metabolism [57]. The beneficial role of MSCs in this context may be more difficult to assess, because these are rare disorders. MSCs may also be used as GVHD prophylaxis in ASCT, as indicated in the pilot trial reported by Frassoni et al. [134].

Two prospective randomized studies are under way in Europe to address this issue. One is being performed in HLA-identical siblings by using MSCs from the HSC donor, and the other is being performed in recipients of unrelated ASCT, in which MSCs are expanded from the recipients' haploidentical sibling or parent. Because MSCs have immunomodulatory effects, it is also important to evaluate their effect on the graft-versus-leukemia effect. Acute and especially chronic GVHD decrease the risk of leukemic relapse [143-145]. Assessment is therefore needed of whether cotransplantation with MSCs, while decreasing acute and chronic GVHD, can increase the risk of leukemic relapse with an unchanged leukemia-free survival.

Exogenously administered MSCs tend to survive and proliferate in the presence of malignant cells in animal models [146] .They seem to potentiate tumor growth in some solid tumors and exert an inhibitory effect in others [98,147]. Little is known about what effect MSCs have on leukemia. It is also important to assess whether MSCs depress immune responses against infections caused by bacteria, fungi, and viruses, which often compromise already-immunocompromised ASCT patients. Because of these concerns, it is possible that MSCs may have their most important application in the treatment of steroid-resistant acute GVHD. Such patients have a high mortality despite treatment with a wide range of new immunosuppressive drugs [30-42].

It has also been suggested that MSCs may be used to treat rejections of organ allografts. Indeed, 1 rat cardiac allograft study showed that MSCs home to the site of allograft rejection [148]. Furthermore, MSCs may have applications in autoimmune inflammatory bowel disease because of their immunomodulatory effect and their capacity for healing damaged gut epithelium [43].

ACKNOWLEDGMENTS

We thank Inger Hammarberg for typing the manuscript. This study was supported by grants from the Swedish Cancer Society (0070-B02-16XAC and 4562-B02-02XBB), the Children's Cancer Foundation (2000/067 and 01/039), the Swedish Research Council (K2003-32X-05971-23A and K2003-32XD-14716-01A), the Cancer Society in Stockholm, and the Karolinska Institute.

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