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Mesenchymal Stem Cells: Will They Have a Role In the Clinic?

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Abstract In addition to hematopoietic stem cells (HSC), human post natal bone marrow contains another stem cell capable of giving rise to multiple mesenchymal cell lineages. Termed mesenchymal stem cells (MSCs) based on their capacity for multi-lineage differentiation, these cells can easily be obtained following a simple bone marrow aspiration procedure and subsequently expanded in culture through as many as 50 population doublings. This extensive capacity for expansion in vitro at clinical scale has recently facilitated the development of clinical trials designed to assess the safety, feasibility, and efficacy of transplanting MSC for a variety of pathological conditions. This review focuses on the background and rationale for performing clinical studies of MSC transplantation and will discuss the potential role that MSC may play in the correction or modification of human diseases. J. Cell. Biochem. Suppl. 38: 73–79, 2002.
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Based on their pioneering studies initiated more than thirty years ago, Friedenstein et al. [1968] were the first to propose the concept that human post natal bone marrow contained a precursor cell for multiple mesenchymal cell lineages [Owen, 1988]. Over the ensuing decades, marrow stromal cells have been characterized, based largely upon their properties in vitro or following transplantation in various animal model systems [Bianco and Gehron Robey, 2000; Deans and Moseley, 2000]. The term colony-forming units fibroblastic (CFU-F) was coined by Friedenstein to describe cells isolated from the bone marrow stroma of a variety of post natal organisms that are adherent, nonphagocytic, fibroblastic, and clonogenic in nature [Friedenstein et al., 1974]. Under well-defined in vitro and in vivo conditions, a proportion of CFU-F can give rise to multiple mesenchymal tissues including bone, adipose, cartilage, myelosupportive stroma, smooth muscle, cardiomyocytes, and tendon. The term mesenchymal stem cells (MSCs) is based on the demonstration that there exist clonogenic populations of adherent human bone marrow derived cells which possess the capacity to differentiate into at least three well-defined mesenchymal cell lineages (osteocyte, adipocyte, and chondrocyte) when placed in the appropriate differentiative conditions [Pittenger et al., 1999]. Recently, methodologies describing the purification and expansion of human MSC have generated a new wave of enthusiasm for their study [Pittenger et al., 1999]. The capacity to expand MSC to clinical scale numbers has paved the way for the current trials evaluating the effects of transplanting MSC. Nevertheless, numerous controversies abound regarding the appropriate phenotypic and molecular description of MSC, the optimal conditions for their purification and expansion in vitro, and the proper model systems to best define the functional properties of MSC following transplantation. Very little is known currently regarding the behavior and fate of MSC following either systemic infusion or local implantation. Further, while it is envisioned, it is as of yet unproven that MSC can serve as useful tools for genetic modification in skeletal

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gene therapy applications. These and many other issues will require substantial clarification before the therapeutic potential of MSC can be fully realized.

How Does One Define a Mesenchymal Stem Cell?

Presently, there are no well-defined criteria with which to characterize MSC. To date, the definitions have been rather loose and therefore, significant controversy exists as to what we mean when we label a cell (or more likely a group of cells) as an MSC. As with any putative stem cell, MSC can be defined by their extensive in vitro self-renewal capacity and multi-lineage potentiality. Efforts to define a distinct phenotype characteristic of MSC have been confounded by the fact that these cells can express a range of cell-lineage specific antigens which may vary depending on the culture preparation (e.g., serum-containing vs. serum-deprived), culture duration, or plating density. Therefore, no single set of phenotypic markers appears capable of identifying a cell as an MSC. In one set of studies, human MSC were shown to express a homogeneous (> 98% purity) non-hematopoietic (lacking expression of CD14, CD34, and CD45) phenotype identifiable by the markers SH-2 (now known to recognize CD105), SH-3, and SH-4 [Pittenger et al., 1999]. However, cultures of human MSC may become morphologically and phenotypically homogeneous only after they are passaged several times in culture at high density, which may lead to loss of multi-lineage potential [Bianco and Gehron Robey, 2000]. The antibody STRO-1 has been used by some groups to enrich, for cells having multi-lineage differentiation capacity, while others have not found this antibody useful to identify MSC [Colter et al., 2001; Gronthos et al., 1994]. Another group has recently shown that human MSC are morphologically, phenotypically, and functionally heterogeneous early in culture, particularly if plated at low density [Colter et al., 2001]. Many of the discrepancies among groups can at least in part be accounted for by differences in the methods used to either isolate or culture MSC.

Given these inconsistencies, any interpretation of in vitro or in vivo studies involving putative MSC must be made with these limitations in mind. Table I presents a listing of many of the surface markers that have been used to characteristize MSC.

TABLE I. Surface Phenotype of Human MSC

Surface marker category	Expression
Growth factor receptor	
IL-1R (CD121)	+
IL-2R (CD25)	−
IL-3R (CD123)	+
Transferrin receptor (CD71)	+
SCF-R (CD117)	±
G-CSF-R (CD114)	−
PDGF-R	±
EGF-R	±
Hematopoietic markers	
CD1a	−
CD11b	−
CD14	−
CD34	−
CD45	−
CD133	−
Adhesion molecules	
ALCAM (CD166)	+
ICAM-1 (CD54)	+
ICAM-2 (CD102)	+
ICAM-3 (CD50)	±
L-Selectin (CD62L)	+
E-Selectin (CD62E)	−
PECAM (CD31)	±
VCAM (CD106)	+
Hyaluronate receptor (CD44)	+
Integrins	
VLA-α1 (CD49a)	+
VLA-α2 (CD49b)	+
VLA-α3 (CD49c)	+
VLA-α4 (CD49d)	−
VLA-α5 (CD49e)	+
VLA-β (CD29)	+
β4 integrin (CD104)	+
Other markers	
Thy-1 (CD90)	±
Endoglin (CD105)	+
SH-3	+
SH-4	+
B7-1 (CD80)	−
B7-2 (CD86)	−

+ indicates routinely positive in all studies; ± indicates variably expressed; − indicates lack of expression.

Regardless of how one might define a cell in vitro as an MSC, it is clear that the ultimate test of a "stem cell" rests on a demonstration of its functional capacity following transplantation in appropriate animal models. This issue is particularly germane when one considers that several clinical trials evaluating the effects of transplanting culture expanded MSC have recently been initiated, accelerating concerns over whether MSC are capable of being administered safely and of performing useful, clinically meaningful functions following transplantation. With this issue in mind, the remainder of this review will focus on recent in vivo models of MSC transplantation and will discuss the current clinical trials of MSC transplantation. Finally, future avenues for research will be defined.

Can Marrow Stromal Cells be Transplanted Systemically?

Given the inherent simplicity of the approach, initial clinical studies of MSC transplantation have evaluated the effects of delivering culture expanded MSC by the intravenous route [Lazarus et al., 1995; Koc et al., 2000]. This raises the question as to what actually occurs following the systemic administration of MSC and whether they are transplantable by this route. Do the cells "home" to particular organ sites such as the bone marrow or do they simply disperse widely and implant randomly? Currently, it is unproven whether marrow stromal cells infused via the systemic circulation are capable of any (not to say durable) engraftment [Bianco and Gehron Robey, 2000]. Furthermore, whether "engrafted" cells are capable of functioning within the site of transplantation awaits formal demonstration. This issue is certainly far from trivial when one considers the potential uses of MSC for the repair of diffuse bone, muscle, or cartilagenous disorders, or for the repair or regeneration of myelo-supportive marrow stroma following HSC transplantation. At present, there has been no definitive demonstration of a circulating stromal cell progenitor, although a circulating skeletal muscle precursor cell has recently been described [Kuznetsov et al., 2001]. Furthermore, studies analyzing stromal cell chimerism following HSC transplant clearly demonstrate that the stromal microenvironment remains of host origin following a conventional allogeneic transplant [Devine and Hoffman, 2000]. The inability to identify stromal cells of donor origin following conventional allogeneic blood or marrow transplantation has generated a number of hypotheses to explain this result. One plausible yet rather simplistic argument suggests that the reason is quantitative. That is, a conventional bone marrow transplant does not supply sufficient stromal cells to result in significant levels of donor stromal chimerism. Furthermore, since circulating stromal cells or MSC have not been conclusively identified in either peripheral blood or umbilical cord blood grafts, transplantation of these stem cell sources would not be expected to result in significant donor stromal cell engraftment [Lazarus et al., 1997; Koc et al., 1999]. Another argument suggests that stromal cell competition may play a role. Since resident marrow stromal cells are relatively radiation resistant and mitotically quiescent, they would not likely be depleted in the host to any significant degree prior to infusion of the donor graft and therefore, there would be few if any niches available to replace endogenous stroma [Bianco and Gehron Robey, 2000]. Finally, since marrow stromal cells reside in the extravascular compartment, it is unclear whether they possess the cellular machinery (adhesion molecules, chemokine receptors) necessary to negotiate the marrow sinusoidal wall in a manner similar to HSC. Therefore, it may be too simplistic to apply the HSC transplant paradigm to intravenously infused MSC. Although ongoing clinical trials are attempting to address this issue, recent animal models provide some clues. Pereira et al. [1995] demonstrated long term engraftment of marrow stromal cells in bone marrow, bone, spleen, and lung of irradiated mice. Nilsson et al. [1999] detected donor-derived osteocytes in cortical bone of mice receiving marrow grafts. Interestingly, this occurred in the absence of any pretransplant conditioning. Hou et al. [1999] demonstrated bony engraftment of cells transduced with a reporter gene driven by the osteocalcin promoter. These data provide some evidence of the engraftment of marrow stromal cells following systemic infusion in mice, although very little is known regarding the functional capacity of these cells after engraftment.

More recently, the fetal sheep model has been used to demonstrate the capacity of human MSC to migrate to and engraft numerous organs when transplanted via the intraperitoneal cavity in very high quantities [Liechty et al., 2000]. Importantly, human MSC were capable of site specific differentiation into chondrocytes, adipocytes, myocytes and cardiomyocytes, bone marrow stromal cells, and thymic stroma. No functional data were presented in these studies. Finally, using a more clinically relevant immunocompetent non-human primate model (*Papio anubis*), our group has demonstrated the capacity for long term engraftment (up to 21 months) of intravenously infused autologous and allogeneic MSC into myeloablated and non-myeloablated baboons [Devine et al., 2001b]. Baboon MSC transduced with an enhanced green fluorescent (eGFP) reporter gene were detected in bone marrow biopsies at virtually all time points up to 21 months following intravenous infusion. In a follow-up to this study, three baboons which had received eGFP marked MSC

underwent necropsy at between 9 and 21 months following the initial MSC transplant and then had multiple non-hematopoietic tissues randomly sampled and analyzed for the presence of the reporter gene by a real time PCR methodology [Devine et al., 2001a]. Surprisingly, multiple non-hematopoietic tissues including the small and large intestine, lung, liver, kidney, pancreas, and thymus gland had eGFP signal detected at relatively high quantities (from 0.1% to 2% of total input DNA). Unfortunately, we have not yet been able to identify by immunohistochemical means any of the transplanted MSC or their progeny in these tissues, possibly due to gene silencing events, rendering these data difficult to interpret. Nevertheless, the data gained from this model suggest that MSC are widely distributed following systemic administration. It has yet to be determined if MSC migrate early into a variety of tissues followed by local proliferation or if they are recruited later from a reservoir site. Further, whether MSC remain undifferentiated or undergo site specific differentiation in non-human primates will require in situ assays presently under development within our group. All the same, the detection of gene marked cells in immunocompetent large animal models such as the baboon and post-immune fetal sheep suggest that MSC may not be recognized to any significant degree by the recipient immune system or may possess unique immunologic characteristics conveying upon them an "immune privilege" within various tissue microenvironments. Given the theoretical advantage of infusing an always available or "universal" allogeneic MSC product, studies aimed at dissecting the immunological properties of MSC have been undertaken recently.

Do MSC Possess Immunomodulatory Properties?

Studies unveiling immunologic properties of MSC are relevant when considering the potential widespread clinical applicability of allogeneic MSC. Human MSC constitutively express a number of molecules required for antigen specific interactions with T cells. Human MSC express MHC Class I but not Class II molecules, unless induced by interferon gamma [McIntosh and Bartholomew, 2000]. They also express numerous adhesion molecules (VCAM-1, ICAM-1, L-selectin, CD72, and LFA-3) [Pittenger et al., 1999]. Human MSC typically do not express the co-stimulatory molecules B7-1 or B7-2, although mRNA for B7-1 can be detected by reverse transcriptase PCR [McIntosh and Bartholomew, 2000].

Human MSC are not well-recognized by alloreactive T cells. Culture of human MSC with resting allogeneic T cells fail to elicit T cell proliferation or the expression of T cell activation molecules such as CD25 and CD134 [McIntosh and Bartholomew, 2000]. This does not appear to be due to lack of co-stimulatory molecule expression, since retroviral transduction of MSC with either B7-1 or B7-2 does not result in a significant response. Although the ability to present antigen in the absence of co-stimulatory molecules might result in the induction of T cell tolerance, MSC appear not to be tolerogenic, since T cells cultured with allogeneic MSCs or donor-matched irradiated peripheral blood mononuclear cells (PBMC) for seven days and rested for three days were able to be re-stimulated with irradiated PBMC with secondary kinetics [McIntosh and Bartholomew, 2000]. This result suggests priming of T cells by MSC without inducing proliferation. Furthermore, in vitro studies indicate that human MSC may suppress activated T cells, since the addition of MSC to alloreactive T cells in a mixed lymphocyte reaction (MLR) culture results in near complete suppression of T cell proliferation, whether the MSC or added to the MLR at the initiation culture or midway through a seven day culture [Klyushnenkova et al., 1999]. Suppression is not dependent on treatment with interferon gamma or fas-ligand and was not MHC restricted, since MSC from third party donors were similarly suppressive as MSCs matched to responder or stimulator cells in the MLR. Moreover, this immunosuppressive effect appears to be mediated at least in part by a soluble factor based on the results of cultures of MLR cultures undertaken in transwells [McIntosh and Bartholomew, 2000]. Taken together, these in vitro data suggest that human MSC possess immunosuppressive effects which may render them either "immune privileged" or perhaps immunosuppressive in vivo. These findings may explain the lack of rejection of xenogeneic, allogeneic, or gene transduced MSC following their transplantation in large animal models.

To further investigate whether MSC possess immunosuppressive properties in vivo, our group used a stringent baboon skin transplant

model to test this effect [Bartholomew et al., 2001b]. We detected a statistically significant prolongation of allogeneic skin grafts when MSC were co-transplanted on the day of skin grafting. In concordance with the in vitro data, the effect on skin allograft prolongation was not MHC restricted, as third party MSC were equally suppressive and capable of prolonging skin graft survival as MSC from either the skin graft donor or recipient. These intriguing findings suggest that allogeneic MSC may be clinically useful for the repair or regeneration of mesenchymal tissue disorders, for enhancing allograft or xenograft acceptance, and for down-regulation of deleterious graft-versus-host disease (GVHD) responses. Interestingly, preliminary data from an ongoing phase I/II trial of MSC in the HLA-identical allogeneic HSC transplantation setting suggest a decrease in the expected occurrence of both acute and chronic GVHD [Lazarus et al., 2000]. The immunomodulatory effects of MSC appear unrestricted by species and clearly require further study.

Potential Clinical Applications of MSC Transplantation

Given their capacity to give rise to multiple mesenchymal tissues, the most obvious clinical application of MSC would in repairing or regenerating damaged or mutated tissues of mesenchymal origin (Table III). As proof of principle, a recent clinical study suggests that transplantation of marrow-derived osteoblasts may be useful for the correction of osteogenesis imperfecta, as was suggested by a preclinical model [Pereira et al., 1998; Horwitz et al., 1999]. Additional animal models suggest that allogeneic MSC may be effective for repairing bone defects or fractures and for repairing cartilaginous defects (e.g., damaged knee joints) [Devine et al., in press].

Recently, it has been suggested that adult bone marrow contains cells capable of migrating to and repairing damaged skeletal and cardiac muscle [Ferrari et al., 1998; Orlic et al., 2001]. Whether the cells responsible for the tissue repair are of hematopoietic, mesenchymal, or endothelial origin remains unresolved. Preliminary studies suggest that culture expanded MSC may possess the capacity to repair ischemic myocardial damage when directly injected into the infarct or the peri-infarct zone or, intriguingly, when given by systemic infusion soon after induction of myocardial ischemia [Devine et al., in press]. Although further refinements in these models are required, such results suggest a potential clinical application for MSC following myocardial infarction and studies are being planned.

Preclinical models also suggest that the co-transplantation of stromal cells simultaneously with hematopoietic cells may enhance the engraftment potential of the hematopoietic cells [Anklesaria et al., 1989; Hashimoto et al., 1997; El-Badri et al., 1998]. In addition, MSC express a number of cytokines that are crucial for the support of hematopoiesis (Table II) [Deans and Moseley, 2000]. Based on these data, clinical trials attempting to exploit the potential of MSC in this setting are ongoing. A phase I trial demonstrated the safety and feasibility of infusing autologous MSC into patients receiving high dose chemotherapy for breast cancer [Koc et al., 2000]. Recently, a phase I/II clinical trial evaluating combining allogeneic blood or marrow grafts with allogeneic MSC was initiated and preliminary data suggest this approach is both feasible and may be efficacious, although further study in a phase III setting will be required [Lazarus et al., 2000]. A trial combining unrelated donor umbilical cord blood with allogeneic, MHC-mismatched related MSC has also been initiated. Furthermore, given the in vitro immunosuppressive effects of MSC, trials evaluating the down-regulation of GVHD in these settings are planned.

TABLE II. Cytokine Expression by Human MSC

Expressed in long term bone marrow culture
FLT-3 Ligand
SCF
LIF
IL-6
IL-7
IL-8
IL-11
IL-12
IL-14
IL-15
Expression induced by IL-1
G-CSF
GM-CSF
IL-1
IL-6
IL-8
IL-11
Not expressed in culture or after IL-1
IL-2
IL-3
IL-4
IL-10
IL-13

TABLE III. Potential Clinical Applications of MSC

Modification or correction of disorders of mesenchymal tissues
Osteogenesis imperfecta
Skeletal defects/fractures
Cartilage repair
Ischemic myocardial damage
Enhancement of hematopoietic engraftment
Unrelated umbilical cord blood
Volunteer unrelated donor
Haploidentical related donor
Non-myeloablative transplants
Delivery of corrective genes
Skeletal disorders
Hemophilia
Lysosomal storage diseases (e.g., Fabry disease)
Immune regulation
Enhancement of solid organ allograft acceptance
Down regulation/prophylaxis of GVHD

Several properties of MSC suggest they may be suitable targets for genetic modification. In vitro, MSC proliferate rapidly and are relatively easily transduced using retroviral vectors. In vivo, stromal cells turn over slowly and may be expected to be long lived. Moreover, gene marked MSC may not be recognized to any significant degree by the host immune system. We and others have demonstrated the feasibility of infusing retrovirally transduced marrow stromal cells in in vivo models [Allay et al., 1997; Hurwitz et al., 1997; Bartholomew et al., 2001a]. We have recently demonstrated that baboon MSC, transduced to express the human erythropoietin (hEPO) gene, can be implanted into allogeneic or autologous recipients, and are capable of secreting hEPO for up to 137 days following implantation [Bartholomew et al., 2001a]. Such data suggest the need for further studies evaluating the feasibility and efficacy of genetically-modified MSC in a variety of clinical settings.

FUTURE DIRECTIONS

Over the past five years, an increasing number of in vitro and in vivo studies suggest numerous possibilities for the application of MSC transplantation in treating a range of disorders. Nevertheless, further study is required to define the optimal conditions for the isolation, characterization, and expansion of MSC. More work is needed to better define the functional, physical, and phenotypic heterogeneity of the cells, we currently term MSC. Improved in vivo models designed to explore the transplantability, functionality, and plasticity of MSC are required before embarking on complicated clinical studies. The optimal route for transplantation (systemic vs. local implantation) as well as the correct dose, schedule, and source of MSC are all issues that require clarification. Answers to these questions will clearly be necessary in order to exploit the massive potential of these interesting cells and to define their ultimate clinical role. Nonetheless, it is reasonable to suggest that the initial seeds of inquiry sown by Friedenstein et al., [1968], now more than thirty years ago may begin to bear fruit within the ensuing decade.

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