Mesenchymal Stem Cell Transplantation for Tissue Repair

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ABSTRACT

There are several characteristics of stem cells that make them unique in comparison with other mammalian cells. First, they exist as unspecialized cells lacking tissue-specific characteristics and they maintain this undifferentiated phenotype until exposed to appropriate signals. Second, they have the capacity for extensive self-renewal. Third, under the influence of local biological signals they can differentiate into specialized cells with a phenotype fully distinct from that of the precursor. Mesenchymal stem cells in the bone marrow apparently conform to this definition. These cells, as their name implies, are the precursors of cells of mesenchymal lineage, including cartilage, bone, fat, muscle, and tendon. They are easily isolated from bone marrow and adipose tissue and from several other sources. At this point we have an incomplete understanding of the regulation of differentiation, commitment, and plasticity of the mesenchymal cell population isolated from marrow. We can identify several of the signals that activate the cells to differentiate along specific cell pathways and we can describe the phenotype of the fully differentiated cells, but we understand little of the intermediate steps. In addition, we know nothing about the reversibility of these pathways or the ability of differentiated cells to revert to a stem cell phenotype. Nor do we understand transdifferentiation or the ability of cells to differentiate horizontally from one lineage to another. Furthermore, there is little clarity surrounding the niche, or tissue-specific microenvironment, in which the cells reside. Despite the lack of understanding of these cells and their natural history, it is clear that they have therapeutic potential in a broad variety of clinical applications. There are many disease targets for which mesenchymal stem cell therapy is being assessed in both preclinical and clinical studies. This article assesses our current understanding of the natural history of mesenchymal cell populations in marrow and other tissues,

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their control, proliferation, and differentiation, and attempts to assess accurately the status of their therapeutic evaluation in different diseases.

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Mesenchymal stem cells (MSCs) were first isolated from rat bone marrow by Friedenstein and coworkers.[1] They described the isolation and expansion in culture of adherent fibroblastic cells and their differentiation to osteocytes. They noted the appearance of colonies of cells in culture and deduced that each colony was derived from a single precursor, or colony-forming unit. The term colony-forming unit–fibroblastic (CFU-F) is often used in this context. Friedenstein's work has received somewhat less attention than it deserved, and it was some time before the magnitude of his contribution was realized. Now, however, we fully acknowledge the debt we owe and appreciate his insight into progenitor cell populations in bone marrow. Over the past decade there has been an explosion of information that has added to our understanding of stem cell biology. In many ways it was possible to learn from the efforts of hematopoietic stem cell (HSC) biologists two decades earlier, especially in the technical and experimental approaches used. However, at this point MSCs are still less well understood than HSCs and in some ways these cells have not been fully defined.

MSCs reside within the stromal compartment of bone marrow. They have the capacity to differentiate into cells of connective tissue lineages, including bone, fat, cartilage, and muscle. They also provide the stromal support system for hematopoiesis. Although they represent a very small fraction of the total population of nucleated cells in marrow, they can be isolated and expanded with high efficiency and induced to differentiate under well-defined culture conditions. These cells have generated a great deal of interest because of their potential use in regenerative medicine. Although the therapeutic testing of these cells has progressed well, there are still many questions to be addressed concerning the role of endogenous populations of stem cells in the adult and the function of various stem cell niches. In addition, there are several aspects of the implanted cell-host interaction that need to be addressed as we attempt to understand the mechanisms underlying these therapies. Of particular importance are the host immune response to implanted cells, homing mechanisms that direct these cells to a site of injury, and their differentiation under the influence of host-derived signals. This article describes the characteristics of MSCs and provides some examples of clinical approaches in MSC therapy that are being evaluated.

ISOLATION AND EXPANSION OF MESENCHYMAL STEM CELLS

MSCs represent about 0.0001% of the total population of nucleated cells in marrow. Despite their rarity, they can be isolated and expanded with efficiency and induced to differentiate to multiple lineages under defined culture conditions. They are generally isolated from an aspirate of bone marrow harvested from the superior iliac crest of the pelvis in humans. In larger animals[2–5] marrow is often obtained from the same site, and in rodents it is generally harvested from the mid-diaphysis of the tibia or femur. Although they represent a minor fraction of the total nucleated cell population in marrow, MSCs can be plated and enriched using standard cell culture techniques. Frequently, the whole marrow sample is subjected to fractionation on a density gradient solution such as Percoll, after which the cells are plated at a density of about 1.6×10^5 cells/cm^2. Cells are generally cultured in

basal medium such as Dulbecco's modified Eagle's medium (high glucose) in the presence of 10% fetal bovine serum.[6] One of the technical challenges regarding the isolation and culture expansion of human MSCs (and those of other species) is this dependence on fetal bovine serum. This has a more serious element because it is also batch specific. This means that certain batches of sera give better results than others. Little is known about the detailed composition of positive sera, but a serum screen is often recommended. Because the large-scale culture of cells depends on the availability of suitable batches of sera, this is a strategic impediment in the commercialization of stem cell therapy. The second major obstacle in the use of MSCs for human therapy arises because of the risk of disease transmission, such as transmission of the variant form of Creutzfeldt-Jakob disease.

Because of these problems, several investigators have looked at alternatives to bovine serum for MSC expansion. Lennon et al[7] reported on the development of a serum-free medium for rat MSCs, but little advance has been made in the development of a serum-free medium for human cells. An alternative approach in the clinical delivery of cells has been the use of autologous patient serum. A study performed by Stute et al[8] indicated that the addition of 10% autologous serum allowed the expansion and osteogenic activation of bone marrow–derived MSCs in a manner that was comparable with fetal bovine serum. This is an encouraging result, and this question will certainly be studied by others. In the meantime, the issue of serum-free media conditions for the preparation of cells for human use is one that will continue to affect the development of clinically acceptable protocols.

MSCs in culture have a fibroblastic morphology and adhere to the tissue culture substrate (Fig. 1). In fact, their adherent nature serves as a functional definition of phenotype. Primary cultures are maintained for 12 to 16 days, during which time the HSC population is depleted. The addition of growth factor supplements such as fibroblast growth factor-2 (FGF-2) to primary cultures of human MSCs was reported by Martin et al[9] to lead to an enhanced osteogenic potential. Although this effect was not observed with murine MSCs,[10] the addition of FGF-2 is associated with the selection of cells with increased telomere length.[11]

Phinney et al[10] reported substantial variation in the yield and level of expression of alkaline phosphatase in MSCs prepared from different strains of inbred mice. They also noted the persistence of $CD45^+$ and $CD11b^+$ pre–B cell progenitors and granulocytic and monocytic precursors in these cultures. Nonetheless, a fraction of these adherent cells represented true MSCs, as shown by osteogenic and adipogenic activity. These observations led to the development of useful methods involving CD34/CD45/CD11b immunodepletion to generate purified MSC preparations.[12]

Further characterization of the conditions required for culturing progenitor cells from murine and rat bone marrow was performed by Jiang et al.[13] These authors found that murine, but not human, cells required leukemia inhibitory factor (LIF) for expansion. Further, they reported that rat cells required epidermal growth factor (EGF) and platelet-derived growth factor-BB (PDGF-BB) in addition to LIF, conditions similar to those required for embryonic stem cells. The cells, referred to as multipotent adult progenitor cells (MAPCs), were found to have the capacity to differentiate into cells with mesodermal, neuroectodermal, and endodermal characteristics in vitro and, when injected into an early blastocyst, gave rise to most somatic cell types. These observations indicated that the plasticity of cell populations in the marrow is greater than previously understood. In an attempt to understand the effect of different culture protocols on cell phenotype, Lodie et al[14] performed a systematic comparison of cells isolated from human marrow and cultured in either 10% FBS, 0.5% FBS supplemented with FGF-2, or 2% FBS supplemented with EGF and PDGF-BB. These authors reported little functional difference between the cells isolated by any protocol, in terms of surface marker expression and differentiation potential. Taken together, these results illustrate the complexity of subpopulations of bone marrow cells, the need to evaluate

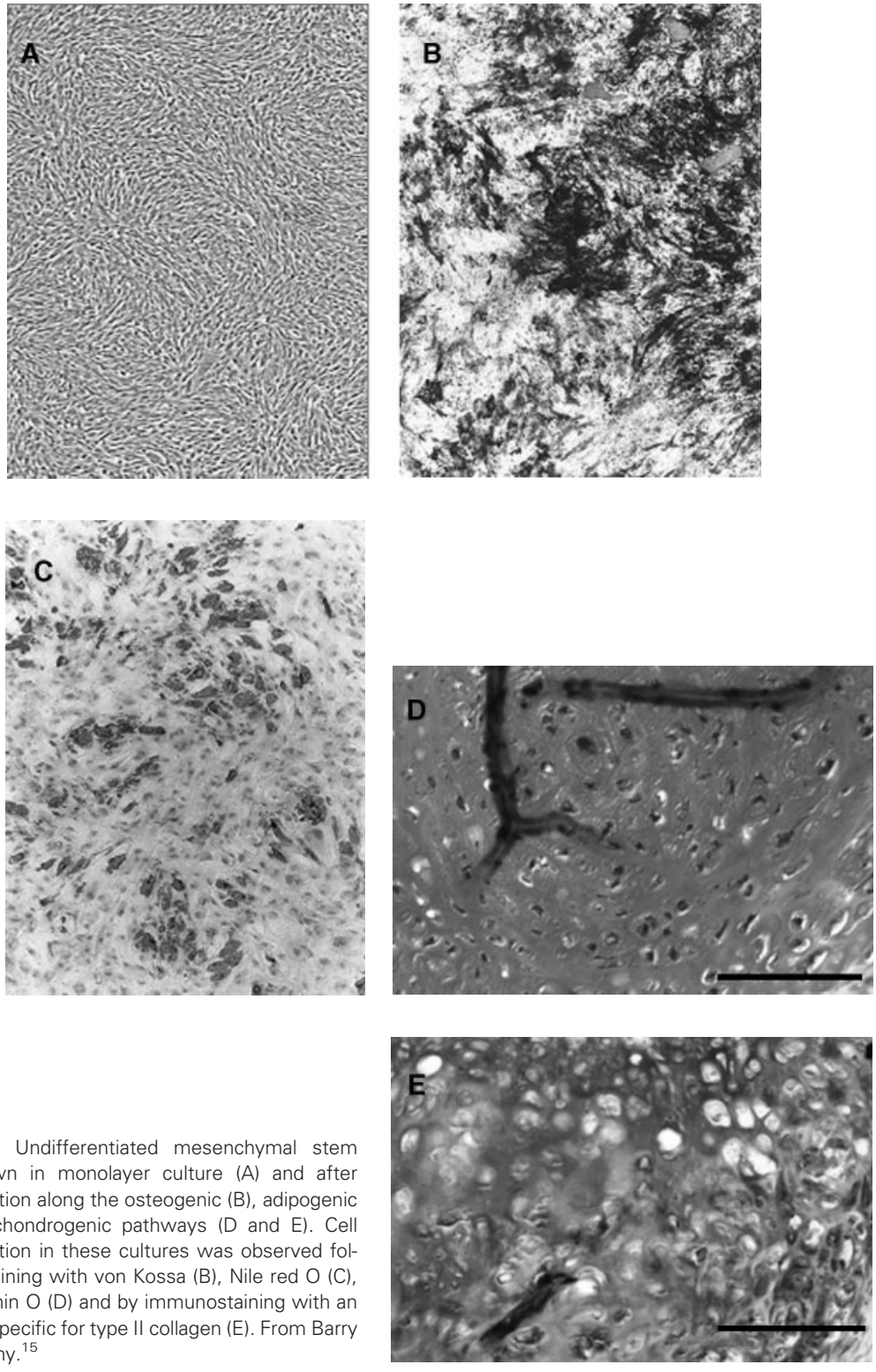

Figure 1 Undifferentiated mesenchymal stem cells grown in monolayer culture (A) and after differentiation along the osteogenic (B), adipogenic (C), and chondrogenic pathways (D and E). Cell differentiation in these cultures was observed following staining with von Kossa (B), Nile red O (C), and Safranin O (D) and by immunostaining with an antibody specific for type II collagen (E). From Barry and Murphy.[15]

isolation techniques with care, and the need to identify new cell-specific markers.

DIFFERENTIATION

The differentiation of MSCs into bone, cartilage, and fat has been well described. Osteogenic differentiation occurs when cells are cultured in a monolayer in the presence of β-glycerol-phosphate, ascorbic acid-2-phosphate, dexamethasone, and fetal bovine serum (Fig. 1B). Differentiation is accompanied by an alteration in morphology, upregulation of alkaline phosphatase activity, and the elaboration of a mineralized extracellular matrix.

The ideal culture environment for the induction of chondrogenesis of MSCs consists of a three-dimensional culture format, serum-free medium, and the presence of a member of the transforming growth factor-β (TGF-β) superfamily (Fig. 1D–E). Under these conditions, the cells undergo a rapid and dramatic morphological change and begin to express several cartilage-specific extracellular matrix components. This is accompanied by a rapid accumulation of glycosaminoglycan. TGF-β1, β2, and β3 have the ability to induce this response, and TGF-β2 and β3 are more effective than β1 in promoting chondrogenesis.[16] This may relate to the abundance of these isoforms in bone and their role in fracture callus formation and wound healing.

MSCs cultured in monolayer in the presence of isobutylmethylxanthine become adipocytes with the production of large lipid-filled vacuoles (Fig. 1C). Adipogenic differentiation of MSCs is induced by the nuclear receptor and transcription factor, peroxisome proliferator-activated receptor-γ (PPAR-γ), as well as fatty acid synthetase. Both interleukin-1 and tumor necrosis factor-α suppress adipogenesis, and this is mediated through nuclear factor-κB (NF-κB) activated by the TAK1/TAB1/NF-κB induction kinase cascade.[17] The effect of inhibition by these cytokines is to direct differentiation toward osteogenesis.

Differentiation toward a myoblast phenotype, induced by 5-azacytidine, was reported by Taylor and Jones for embryonic and adult cells[18] and by Wakitani et al for rat stromal cells.[19] Phinney et al found that exposure of mouse MSCs to amphotericin B, but not 5-azacytidine, resulted in the formation of multinucleated fibers resembling myotubes.[10] There are other examples of in vivo differentiation associated with therapeutic delivery. Gussoni et al[20] showed that murine MSCs, injected into the quadriceps muscle of *mdx* mice, expressed dystrophin in association with the muscle fiber sarcolemma, and they pointed toward a potential therapy for muscular dystrophy. Toma et al[21] injected β-galactosidase–expressing human MSCs into the left ventricle of CB17 SCID/*beige* adult mice and found the labeled cells dispersed throughout the myocardium and expressing desmin, cardiac-specific troponin T, α-actinin and phospholamban, all indicative of differentiation of the engrafted cells to a mature myocardial phenotype.

Induction of mouse, and human MSCs along the neurogenic pathway has been described.[22–27] Treatment of MSCs with isobutylmethylxanthine and dibutyryl cyclic AMP induced expression of early markers of neuronal differentiation,[26] as did EGF or brain-derived neurotrophic factor (BDNF) in a neuronal growth medium.[25] Transdifferentiation of mouse marrow stromal-derived mature osteoblasts and the stromal cells themselves to a neural phenotype was achieved by treatment with 5-azacytidine in the presence of nerve growth factor, BDNF, and neurotrophin-3.[27] Treatment of rat cells with dimethyl sulfoxide/butylated hydroxyanisole in the presence of basic FGF and PDGF was also successful in inducing a neural phenotype.[22,23]

THERAPEUTIC APPLICATIONS OF MESENCHYMAL STEM CELLS

Stem cell therapy involves the transplantation of autologous or allogeneic stem cells into patients,

through either local delivery or systemic infusion. There is a precedent in HSC transplantation, which has been used for some years in the treatment of leukemia and other cancers.[28] Some striking examples of the therapeutic use of marrow-derived MSCs have been reported. These address a broad spectrum of indications, including cardiovascular repair, treatment of lung fibrosis, spinal cord injury, and bone and cartilage repair. Orlic et al[29] showed that locally delivered bone marrow cells can generate de novo myocardium, indicating that stem cell therapy can be useful in treating coronary artery disease. Stamm et al[30] demonstrated the practical utility of this approach in a study involving the delivery of bone marrow cells into the infarct zone in patients following myocardial infarction. The result of this treatment was a dramatic improvement in global heart function. Deb et al[31] have also shown engraftment of bone marrow–derived cardiomyocytes in the adult heart following bone marrow transplantation. Saito et al[32] demonstrated that MSCs are tolerated in a xenogeneic environment while retaining their ability to be recruited to the injured myocardium and undergo differentiation to a cardiac phenotype.

MSCs have also been shown by Ortiz et al[12] to engraft at high levels in lung tissue following exposure to bleomycin and to offer protection against bleomycin-induced lung injury, including inflammation and collagen deposition. These observations have broad implications in the area of lung disease associated with environmental damage.

Stem cells with the ability to differentiate into neurons, astrocytes, and oligodendrocytes have been isolated from rat spinal cord,[33] and implantation of neural stem cells in an adult rat model of spinal cord injury resulted in long-term functional improvement.[34] Embryonic stem cells are capable of forming dopamine neurons in an animal model of Parkinson's disease.[35] The ability of bone marrow–derived stem cells to differentiate into neural lineages in vitro and after transplantation in both mice and rats has been evaluated by Sanchez-Ramos,[24] leading to the conclusion that they may be useful in the treatment of stroke, traumatic injury, and Parkinson's disease. Furthermore, it was demonstrated by Mezey et al that adult human bone marrow cells can enter the brain and generate neurons after transplantation.[36] These and other equally dramatic observations underlie much of the current excitement and optimism about the use of stem cell therapy in the treatment of neuronal injury.

In the area of orthopedic medicine there are also many examples of applications involving local delivery of marrow stem cells. These include spine fusion,[37] the repair of segmental bone defects,[38] and craniotomy defects.[39] Similar approaches have also been described for the repair of focal defects in articular cartilage.[40,41] In an animal model of osteoarthritis (OA) involving injury to the meniscus, delivery of stem cells by intra-articular injection resulted in engraftment of those cells on the meniscus, fat pad, and synovium with regeneration of meniscal tissue and protection of the cartilage.[5] The chondroprotective effects seen in these studies apparently derive from the regenerated meniscus because there is no evidence of direct engraftment of the implanted cells on the fibrillated cartilage.

There is accumulating evidence of the hypoimmunogenic nature of MSCs, and this has broad implications in terms of allogeneic therapy or the delivery to a recipient of cells derived from an unmatched donor. There are several reports describing the clinical use of allogeneic donor–mismatched cells with little evidence of host immune rejection or graft-versus-host disease (GVHD). For example, allogeneic bone marrow transplantation in children with osteogenesis imperfecta resulted in engraftment of donor-derived MSCs and an increase in new bone formation.[42] Infusion of allogeneic MSCs into patients with Hurler's syndrome or metachromatic leukodystrophy showed no evidence of alloreactive T cells and no incidence of GVHD.[43] Engraftment of allogeneic MSCs has also been demonstrated in a patient with severe idiopathic aplastic anemia with improvement of marrow stromal function.[44] Tables 1 and 2 summarize in vivo studies involving preclinical or clinical evaluation of MSC therapy in several disease targets.

Table 1 Testing of Mesenchymal Stem Cell Therapy (MSC) in Animal Models of Disease

Indication	Animal Model/Route of Delivery	MSC Source	Result	Reference
Myocardial infarction	Mouse/direct injection	$Lin^{-}c\text{-}kit^{+}$ bone marrow cells	De novo myocardium	29
Myocardial infarction	Immunocompetent Lewis rats/IV injection I week before infarction	C57B1/6 mouse MSCs	Donor-derived cardiomyocytes and angiogenesis	32
Muscular dystrophy	mdx mouse/IV injection	Normal mouse muscle-derived MSCs	Partial restoration of dystrophin expression in affected muscle	20
Lung fibrosis	Bleomycin (BLM)-sensitive C57BL/6 mouse/ IV injection	BLM-resistant BALB/c mouse	Reduced inflammation and collagen deposition	12
Spine fusion	Canine bone marrow–derived cells/cancellous bone matrix	Autologous	Improved bone grafting	37
Segmental bone defects	Athymic rat/ceramic carrier	Human MSCs	Enhanced bone formation and improved biomechanics	45
	Canine/ceramic carrier	Autologous MSCs		
	Canine/ceramic carrier	Allogeneic MSCs	Enhanced bone formation	46
			Enhanced bone formation	47
Craniotomy defect	Immunocompromised mouse/gelatin sponge	Alloplastic transgenic mouse marrow stromal cells	>99% repair within 2 weeks	39
Tendon defect	Rabbit/contracted collagen gel	Autologous MSCs	Improved tendon biomechanics, structure, and function	48
Meniscus	Caprine/intra-articular injection	Autologous MSCs	Enhanced tissue formation and reduced osteoarthritis	5

From Barry and Murphy.[59]

STEM CELL EXHAUSTION IN DISEASE

It is interesting to consider that certain degenerative conditions, where there is progressive tissue damage and an inability to repair, may be due to the fact that stem cell populations are depleted or functionally altered. This has been considered in the case of OA, a disease of the joints in which there is progressive and irreversible loss of cartilage, with changes also

Table 2 Clinical Evaluation of Mesenchymal Stem Cells (MSCs)

Indication	Source/Route of Delivery	Result	Reference
Myocardial infarction	AC133+ bone marrow cells/direct injection	Function enhanced in 4 of 6 and tissue perfusion improved strikingly in 5 of 6 patients	49
Osteogenesis imperfecta	Allogeneic bone marrow transplantation/infusion	New dense bone formation and engraftment of donor-derived cells in 3 patients	42
Large bone defect	Autologous bone marrow stromal cells/scaffold	Enhanced bone repair in 1 of 1 patient	38
Metachromatic leukodystrophy (MLD) and Hurler syndrome	Allogeneic MSCs/infusion	Significant improvements in nerve conduction velocities in 4 of 6 MLD patients. No graft-versus-host disease.	43
Severe idiopathic aplastic anemia	Allogeneic MSCs/infusion	Improved stroma in 1 of 1 patient	44

From Barry and Murphy.[59]

in the underlying bone. In a study described by Murphy et al,[50] MSCs were prepared from marrow taken from patients with end-stage OA undergoing joint replacement surgery. The marrow samples were harvested both from the site of surgery (either the hip or the knee) and from the iliac crest. It was found that the proliferative capacity of the cells was substantially reduced in the osteoarthritic patients, and this was independent of the site of harvest. In addition, the chondrogenic and adipogenic activity of the cells was significantly reduced, again independent of the site of marrow harvest. These effects were apparently disease related and not age related, but additional studies are necessary to confirm these preliminary observations. However, the data lead to an attractive notion that susceptibility to OA or other degenerative diseases may be due to the reduced mobilization or proliferation of stem cells, and, even if mobilized, the cells may have a limited capacity to differentiate, leading to defective tissue repair. This may be a simple interpretation, and the altered stem cell activity may also be interpreted as a response of the cells to the disease environment, specifically the elevated levels of inflammatory cytokines seen in OA. Further, the effect of anti-inflammatory drugs on cell activity needs to be taken into account.

HOST IMMUNE RESPONSE TO ALLOGENEIC TRANSPLANTATION

Several studies have suggested that MSCs are immunosuppressive or hypoimmunogenic[51–56] with allogeneic transplantation to an immunocompetent host possible. MSCs may in fact play a role in enabling alloantigen tolerance.[42,43] Present at low levels in HSC transfers, they are considered to be immunosuppressive, and human MSCs can promote unrelated HSC survival and suppress T cell activation in a nonobese diabetic–severe combined immunodeficiency (NOD-SCID) model.[57] Numerous therapeutic possibilities based on this immunosuppressive effect have been proposed.[58] Using bone marrow–derived allogeneic Flk-1 Sca-1 MSCs in a mouse model, Deng et al not only demonstrated long-term tolerance of allogeneic skin grafts but also provided data suggesting the induction of hematopoietic chimerism.[59] Aggarwal and Pittenger demonstrated that human MSCs modulate allogeneic immune responses[60] and supported the tolerance of allogeneic MSCs. There is a broad body of data using different cells, different species, and diverse readouts all supporting the description of MSCs as being exceptions to the regular allogeneic rejection mechanisms. These findings have been seen with human cells, with rat and mouse cells, and even in xenogeneic mixed lymphocyte reaction.[51,61–64]

It is clear that a broad body of data supports the description of MSCs as being exceptions to the regular allogeneic rejection mechanisms. Two questions arise out of these observations. First, how do MSCs mediate this effect? This gap in our understanding has prompted the focus of research to the discovery of mechanisms by which hypoimmunogenicity is maintained. Two mechanisms have been evoked, one involving direct cell-cell interaction, the other requiring the secretion of immunomodulatory factors. The second question relates to the mechanism by which allogenic MSCS escape immune detection and is more difficult to approach. Nonetheless, these are questions of critical importance that must be addressed before we can proceed with the widespread use of allogeneic MSCs in therapy.

CONCLUSION

It seems that MSC therapy is likely to be both effective and safe for use in several disease targets, but there are still many questions to be answered regarding the fate of transplanted cells and the mechanism of repair. It is necessary to continue to study the pharmacokinetics of transplanted cells and mechanisms of engraftment, homing, and in vivo differentiation. Chronic toxicology studies will also

be necessary as the clinical use of these cells becomes widespread. It is clear that MSCs present many fascinating opportunities for the study of stem cell differentiation, plasticity transdifferentiation, engraftment, and homing. Another exciting development is the use of stem cells as vehicles for the delivery of therapeutic genes. As the safety profile of stem cells is more fully understood and more advanced and safer gene vectors are developed, it may be that the combination of stem cell and gene therapy is a powerful new approach for the treatment of several diseases that are currently untreatable.

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