Chapter 1
Mesenchymal Stromal Cells in Regenerative Medicine: A Perspective

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Abstract Multipotent mesenchymal stromal cells (MSCs) of bone marrow origin not only provide a supportive cellular niche for hematopoiesis inside the bone marrow but also differentiate into mesodermal cells such as bone, fat, and cartilage. Clinical uses of culture-expanded MSCs were originally investigated for their presumed hematopoietic-supportive activities. Their use in the clinic was later expanded to the treatment of steroid-resistant acute graft-versus-host disease based on unique immunomodulatory properties shown in a variety of in vitro experiments and in vivo models. Systemically administered MSCs participate in tissue regeneration through diverse biological activities, including paracrine effects that are not necessarily dependent on cell engraftment. Although there is an impressive record of safety in clinical trials, most outcomes have been assessed in the short term, and their efficacy has yet to be shown conclusively in randomized controlled trials. Forty years after their original description and 20 years after their use in humans, culture-expanded MSCs, and particularly their in vivo counterparts, remain poorly understood. However, unless or until better therapeutic options for debilitating disorders are found, the notion that MSCs could be potentially useful warrants further investigation to establish long-term safety and efficacy in well-designed clinical trials.

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Introduction

Friedenstein was the first to isolate fibroblast-looking cells from bone marrow (BM) and to show that they were capable of regenerating rudiments of bone and supporting hematopoiesis in vivo [1]. These cells were later shown to be capable of differentiating into fat and cartilage and thus were given the name mesenchymal stem cells (MSCs) [2]. However, to be more technically accurate and to better reflect their true biological properties, it has been suggested that the name multipotent mesenchymal "stromal" cells (with the same acronym) be used for the heterogenous population of cells that is cultured *ex vivo*, while the term mesenchymal stem cell be used only for the cells capable of both self-renewal and multi-lineage differentiation [3]. Although the correlation between *ex vivo*-generated MSCs and their in vivo counterparts still remains poorly understood, culture-expanded MSCs derived from BM or other tissues are currently being investigated for an ever-expanding number of clinical indications based on their tissue-regenerative, immunomodulatory, anti-inflammatory, and trophic paracrine effects. In this chapter, we provide an overview of the role of these cells in the nascent but exciting field of regenerative medicine.

Four decades after their original description, there is still much debate about the exact anatomical location of MSCs inside different tissues (including BM) and their true physiological role. MSCs comprise a very small population (<0.1%) of adult BM cells. It is believed that these cells, or their progeny such as osteoblasts, constitute the supportive cellular niche for hematopoietic stem cells (HSCs) inside the BM [4–7]. Derivation of cells with similar phenotypic characteristics from non-BM tissues has just added to the uncertainty surrounding the true identity and physiological roles of these cells. For example, similar populations of cells have been isolated from almost all other adult and neonatal tissues including fat [8], skeletal muscle [9], synovium [10], dental pulp [11], placenta [12], amniotic fluid [13], umbilical cord blood [14], and fetal lung, liver, and blood [15]. Importantly, MSCs isolated from these non-BM tissues share cell-surface markers similar to BM-derived MSCs and have similar differentiation potential into bone, fat, and cartilage. However, since no physiological role can be imagined for cells with bone- and cartilage-forming potential in organs such as heart and adipose tissue, it can be argued that these in vitro observations are artifacts of our experimental assays with no correlation to the true homeostatic role of MSCs in different tissues. Surprisingly, MSCs isolated from these non-BM sources possess similar immunomodulatory properties [16–18].

It should be noted that there is a vast difference between what cells do in their normal in vivo environment under physiological conditions and what they can potentially do if they are tested out of their physiological context. For example, functional properties and capabilities of BM-derived MSCs that are culture expanded may be very different from their in vivo counterparts. Furthermore, these cells are usually transplanted in very large numbers to a new location for repair of damage in a tissue different from their tissue of origin. Consequently, MSC transplantation

bears significant differences with HSC transplantation in which cells are usually transplanted with minimal manipulation. However, for clinical cell therapists, what matters most is that the transplanted cells result in some beneficial effects and do not cause harm, whatever the mechanism. This view of regenerative medicine is different from that of investigators whose primary focus is to understand the basic biology of cells and their mechanism of actions. Nevertheless, the maximum potential value of MSCs in regenerative medicine involves a swinging back and forth between bench and bedside and is in the best interest of laboratory researchers and clinicians alike.

Mechanism of Action of MSCs

Mesenchymal stromal cells have generated huge interest in both public and scientific communities because of their potential to regenerate a wide variety of tissues. The place of these cells in clinical medicine was originally thought to be due to their presumed hematopoietic-supportive activities or bone- and cartilage-forming potential. However, our view of the potential mechanisms of action of MSCs and thus of the potential indications in regenerative medicine has evolved considerably over the years. A major reason for the initial enthusiasm for MSCs in the non-bone marrow transplant field was a multitude of studies suggesting MSCs not only differentiate into other types of cells of mesodermal lineage but also into cells of endodermal and ectodermal lineages, including cardiomyocytes [19], endothelial cells [20], lung epithelial cells [21], hepatocytes [22], neurons [23], and pancreatic islets [24]. However, the degree of contribution to different tissues through trans-differentiation is now considered very unlikely given that many later studies using more sensitive and appropriate techniques could not duplicate the results of original reports [25–27]. Thus, it has now become more accepted that, despite the fact that under certain experimental conditions, these cells might assume some characteristics shared by other cells, this process, if it occurs at all, is probably a rare event in vivo and is certainly insufficient to explain the positive results observed in animal models and human studies and thus is of no clinical significance.

While under normal circumstances, we expect that MSCs will preferentially home to BM after intravenous infusion [28, 29], experimental models show that *ex vivo* culture-expanded MSCs infused intravenously can be detected at low levels in many tissues [30, 31]. Indeed, these cells preferentially home to damaged tissues, probably via the SDF1/CXCR4 axis [32, 33]. The prevailing view is that MSCs home to sites of tissue injury/inflammation, secrete trophic factors to promote recovery of injured cells, and recruit and expand resident progenitors to replace damaged cells. Likewise, they participate in tissue regeneration through matrix remodeling and exert desirable immunomodulatory and anti-inflammatory properties, making them ideal candidates for use in disorders affecting many different organs [34–36].

Originally, robust structural integration of the MSCs into patient tissue was considered a requirement for achieving the desired end points. For example, it was thought that MSCs should ideally be able to substitute affected tissues. However, the assumption that persistence of the transplanted cells in the recipient is necessary to yield a therapeutic effect is being replaced by other mechanistic paradigms that involve mainly anti-inflammatory and paracrine effects. For example, recent studies in animal transplant models have shown that infused MSCs are trapped to a significant degree in the lungs and nevertheless can exert significant beneficial systemic effects (in this case, in the heart) via paracrine effects [37]. These mechanistic insights could influence design of clinical trials, for example, choosing between intravenous delivery of MSCs and their direct intracardiac injection for repair of heart damage.

In addition to the new mechanisms of action proposed for MSCs, our view of the pathophysiology of disease processes has evolved over the years too, including many for which we contemplate using the cells. For example, we now understand that in many disease processes, inflammatory and immunological disturbances play a much bigger role than was appreciated only a few years ago. Consequently, it is no surprise that MSCs, found about a decade ago to have immunomodulatory properties, could potentially be beneficial in conditions that we now know involve immune disturbance and inflammation. Observing beneficial effects in conditions with very poorly documented engraftment of the cells is consistent with these observations. These effects could be due to transient immunomodulation, or paracrine action, including the secretion of cytokines and other trophic factors. Paracrine effects may mediate repair by protecting tissue cells from apoptosis, promoting angiogenesis, or recruiting and activating tissue progenitor cells. Alternatively, MSCs could also change the repertoire of immune and inflammatory cells present in damaged tissue to avoid further immunological damage or promote the generation of tissue-regenerating macrophages. Thus, to exert a beneficial effect, prolonged levels of engraftment might not be needed and, indeed, may be irrelevant. Nonetheless, repeated doses may be required to obtain therapeutic effects.

Clinical Experience with MSCs

Hematologists have been at the forefront of cellular therapies, as in the case of bone marrow transplantation (BMT) decades ago. Hematologists have also been the first to use MSCs clinically, given that the cells are derived from BM and support hematopoiesis in experimental models in vitro and in vivo. Thus, in the field of BMT MSCs were investigated originally to improve hematopoietic engraftment. Lazarus and his colleagues showed not only feasibility of collection and *ex vivo* culture expansion of MSCs from small BM aspirates of patients with different

malignancies but also safety of infusion of autologous MSCs alone [38] or combined with autologous peripheral blood $CD34^+$ cells [39]. They also showed that administration of culture-expanded allogeneic MSCs with their corresponding HSCs in patients undergoing myeloablative HSC transplantation for hematological malignancies was safe and not associated with an increased incidence or severity of graft-versus-host disease (GVHD) [40]. Compared with historical controls, hematopoietic engraftment was not faster, but these studies provided evidence that *ex vivo* culture expansion of MSCs was feasible and intravenous infusion did not cause toxicity. There are hints that MSCs may promote HSC engraftment based on small non-randomized clinical series [41–45]. Improvement of HSC engraftment in these settings is likely not due to a direct HSC niche effect but perhaps is more likely to be related to an immunomodulatory paracrine effect in ameliorating tissue inflammation, a major barrier to HSC engraftment. Indeed, while donor MSCs may exert an effect after BMT, many but not all studies consider them host derived in transplant recipients [46–48].

Almost a decade ago, it was suggested MSCs, including from unmatched third-party donors, may be useful in ameliorating GVHD after allogeneic HSC transplantation [49–51]. Le Blanc et al. were the first to report the treatment of GVHD with MSCs in a 9-year-old boy who received a HSC transplant from an unrelated matched donor [52]. The patient had severe refractory acute GVHD of gut and liver unresponsive to all types of immunosuppressive medications. Infusion of one dose of haploidentical MSCs resulted in an impressive response with resolution of all clinical and laboratory manifestations of GVHD. The infusion of a second dose of MSCs was also effective in treating the GVHD that soon recurred. This landmark case report was followed by another promising small case series of eight patients [53] and then by a phase II trial of 55 pediatric and adult patients, with steroid-refractory acute GVHD [54]. The latter study confirmed that the clinical responses were independent of the source of MSCs; that is, MSCs from human leukocyte antigen (HLA) identical sibling, haploidentical, and third-party HLA-mismatched donors gave similar responses. GVHD is also the only indication in which a phase III randomized double-blind controlled study has been conducted to completion [55]. In this study of refractory GVHD, subsets of patients with liver or gastrointestinal GVHD had an improved response to MSCs. However, the primary end point of the study could not be achieved. Nonetheless, pediatric patients showed a higher rate of response [56] .

Use of third-party MSCs in the context of HSC transplantation without regard to their HLA typing opened the gate to use of unmatched allogeneic MSCs for many other indications. Also, the multitude of paracrine, immunomodulatory, and anti-inflammatory properties of MSCs has been the rationale for initiating numerous phase I–III clinical trials for a wide range of human disorders. Such studies include metachromatic leukodystrophy and Hurler's disease [57], osteogenesis imperfecta [58], myocardial infarction [59], chronic obstructive pulmonary disease [60], amyotrophic lateral sclerosis [61], stroke [62], refractory wounds [63],

diabetes mellitus [64], systemic sclerosis [65], systemic lupus erythematosus [66], Crohn's disease [67], and multiple sclerosis [68]. Although unequivocal efficacy in any of these indications has yet to be shown, what we have learned is that infusion of MSCs, not only intravenously but also intra-arterially [69] and even intrathecally [68], is safe. The use of MSCs for these indications is covered in detail in many other chapters of this book.

Standardization of Culture Methodologies

Considering that more than a few hundreds MSC-related clinical research protocols are listed in www.clinicaltrials.gov and that MSCs have been given to several thousand patients worldwide, there is an urgent need to assess MSC production methodology on clinical outcomes [70]. Currently, there is no standardized culture protocol, and considerable heterogeneity exists in methods for producing MSCs [71–73]. In addition, many of the clinical trials have enrolled small number of patients for whom MSCs were generated in local hematopoietic cell processing laboratories, while some larger studies involved pharmaceutical companies in which MSCs were made under current good manufacturing practice (cGMP) standards and provided limited information on production methodology due to proprietary concerns. Thus, heterogeneity of patient-related characteristics and culture methodology in many MSC studies may prevent definitive conclusions from being drawn. Consequently, definitive studies are needed to show the efficacy of MSCs, preferably in multicenter trials with MSCs produced by a central manufacturing facility or generated according to the same protocol.

MSCs are present in the mononuclear cell (MNC) fraction of BM, and minor changes in processing, including the use of Ficoll density gradient centrifugation, can affect cell characteristics [74]. Clinical results should therefore be interpreted cautiously as the MSCs used may differ based on donor (autologous versus allogeneic, young versus old, male versus female), starting material (fresh versus frozen BM), isolation technique (Ficoll versus no Ficoll), plating density, coating material, culture medium, passage number, and cell expansion protocol specifications. Furthermore, we know that *ex vivo* culture-expanded MSCs comprise a heterogeneous population with potentially different biological characteristics. Thus, it is possible that different culture conditions may favor the growth of certain MSCs with undetermined characteristics.

It is a major challenge to determine if any changes in production methodology have an impact on the final properties in vivo. For example, one major variation is the culture medium used such as fetal bovine serum (FBS) versus synthetic serum-free medium, autologous serum, fresh frozen plasma, or human platelet lysate [75–77]. In one clinical trial, FBS was replaced by human platelet lysate to produce MSCs [78]; however, it is not known whether the generation of MSCs in platelet lysate played a role in the lower response rate observed in this small study ($n = 13$) for the treatment for steroid-refractory GVHD.

There is also no consensus on the release criteria for MSCs. However, when MSCs are used for such diverse conditions as GVHD after allogeneic HSC transplantation, bone repair, and myocardial infarction, a single potency assay is not likely to be feasible but needs to reflect the specific indication. Further work is necessary to address this important issue and will probably be managed on a case by case basis.

Unresolved Issues

Although several thousand patients have received MSCs for a wide variety of indications using different routes of administration, outcomes of most treatments have not been reported in the medical literature. Of further concern is the lack of long-term follow-up to monitor adverse events. Moreover, rare long-term adverse events are likely to be identified only from a database of a large number of treatment recipients. While analyses of blood and marrow transplant database registries have been very helpful in determining outcomes and adverse effects of specific categories of transplant recipients, a similar strategy for persons receiving cell products such as MSCs is significantly more challenging, not the least because many different and separate specialties of clinical medicine are involved that do not have a history of close interaction. Nonetheless, some issues may be possible to address with existing BMT registries, such as assessing the potential for increased relapse or opportunistic infections in allogeneic transplant recipients receiving MSCs for prevention of GVHD. Indeed, in a small open-labeled randomized trial of MSC infusion for prevention of GVHD, an increased risk of early relapse led to early termination of the study [79]. Although such risk has not been seen in similar studies, long-term outcome data collection is needed and could conveniently be collected by transplant outcomes database registries such as the Center for International Blood and Marrow Transplantation [80].

Preclinical animal models are useful in evaluating the safety and efficacy of cellular therapeutics. However, finding a relevant animal model can be challenging because of large biological differences between humans and, especially, inbreed laboratory animals. Even the evaluation of human MSCs in immune-deficient xenogeneic rodent models presents a challenge in simulating an appropriate microenvironment, in addition to accounting for the absence of an intact immune system. Conclusions from murine models have major implications in the design of human clinical trials. For example, a beneficial effect of MSCs in the NZBxNZW F1 model of SLE was not obtained [81]. However, another group, based on their promising results in an MRL/*lpr* murine model of SLE [82], showed, that a single infusion of allogeneic bone marrow-derived MSCs in four patients with lupus nephritis resulted in improvement of serologic markers and kidney function [83]. The same group later reported positive outcomes in 16 SLE cases treated with umbilical cord-derived MSCs [84]. More recently, they reported a positive outcome in 15 patients with active SLE, 14 of whom had nephritis and were refractory to conventional treatments (including the previously published four cases) [85].

All patients showed improvement in autoantibody levels, proteinuria, and non-renal manifestations of SLE after infusion of a small dose of allogeneic bone marrow-derived MSCs (1×10^6/kg by intravenous injection) with no significant acute toxicity. In contrast, in another study the injection of autologous MSCs in two patients had no effect on disease activity despite inhibition of lymphocyte proliferation in vitro [86]. The latter negative result may be due to the small number of cases treated or the possibility that MSCs derived from ill persons may not be as immunosuppressive as allogeneic MSCs from healthy individuals. This raises the possibility that the choice of autologous versus allogeneic MSCs may depend not only on the urgency of the need but also on the specific clinical indication.

The infusion of *ex vivo*-expanded MSCs without regard to HLA status has been repeatedly shown to be safe and was originally based on the assumption that the cells are non-immunogenic. However, total lack of immunogenicity is called into question given the number of studies showing minimal engraftment of these cells. Furthermore, the notion that MSCs always suppress proliferative responses of allogeneic lymphocytes is also debatable, as it has been now shown that MSCs can function as antigen-presenting cells or even activate immune responses under certain conditions [87, 88]. Also, preclinical data on the ability of MSCs to suppress these responses in vivo have been conflicting [89–91]. These discrepancies in basic research literature could be due to many factors, including the strain of mice used to derive MSCs, the culture methodology, the number of cells infused, the passage or cell doubling number, and the timing of MSC infusion. For example, in one murine study, MSCs infused on the day of BMT were ineffective in GVHD prevention, but infusion of cells on day 2 significantly reduced mortality [92]. Furthermore, this study also showed that MSCs contaminated with >3% CD45+ cells and MSCs from late passage (more than 6) did not show a significant effect on GVHD-related mortality. Results may also reflect the dose of cells used. For example, both murine [93] and human studies [94] have shown that MSCs inhibit proliferation of B-lymphocytes stimulated by various means. However, based on the Corcione et al. study [94], the inhibition was dose dependent, as more MSCs led to less inhibition. This contrasts with inhibition of T-cell proliferation, where more MSCs usually lead to greater inhibition of T-cell proliferation. Thus, it is possible that in some clinical scenarios, such as SLE in which B cells play a major pathophysiological role, a lower dose of MSCs may be more effective.

One of the inherent characteristics of cells is that, unlike pharmaceuticals, they are complex and variable. Their in vivo behavior depends on many factors, including the route of administration, autologous versus allogeneic sources, the immune system status of the patient, concomitant medications, and the microenvironment of the tissue to be augmented. Moreover, such factors can be disease specific. The potential for the accumulation of genetic mutations after long-term culture [95–98] theoretically exists and mandates vigilance, especially if cells of multiple doublings are used. However, more important than the theoretical possibility of malignant transformation of MSCs is the possibility of the promotion of the growth of existing tumors or an enhancement of their metastatic potential, as previously documented in some murine models [99, 100]. Nevertheless, it is reassuring that no tumor formation has been found to date in human recipients of MSCs [101].

Conclusion

MSCs were originally isolated from bone marrow and provided a critical step in the in vitro and in vivo study of hematopoiesis. The cells were later found to possess intriguing immunomodulatory and trophic properties both in vitro and in preclinical models, in addition to supporting hematopoiesis. Numerous clinical studies followed investigating the role of MSCs for a wide range of clinical conditions. Currently, MSCs are at the forefront of regenerative medicine and offer the potential to ameliorate serious or debilitating diseases with limited or no other therapeutic options. Many issues remain to be addressed, including mechanisms of action, the best methods for cell production, the optimal dose, frequency and route of administration, and, in particular, appropriate indications for use. The collaborative efforts of scientists and clinical researchers are essential to advance our understanding of the biology and clinical applicability of these intriguing cells.

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