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Mesenchymal Stromal Cells: New Directions

Armand Keating[1,*]
[1]Cell Therapy Program, Princess Margaret Hospital, Toronto, ON M5G 2M9, Canada
*Correspondence: armand.keating@uhn.on.ca
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Research into mesenchymal stromal/stem cells (MSCs) has been particularly exciting in the past five years. Our understanding of mechanisms of MSC-mediated tissue regeneration has undergone considerable evolution. Recent investigation of the primary in situ counterpart of cultured MSCs has led to fresh insights into MSC physiology and its role in the immune system. At the same time, the clinical application of MSCs continues to increase markedly. Taken together, a reappraisal of the definition of MSCs, a review of current research directions, and a reassessment of the approach to clinical investigation are timely and prudent.

Introduction

Few cell types have captivated so many biomedical researchers over the last 10 years as have mesenchymal stromal/stem cells (MSCs). PubMed, in 2012, identifies over 17,000 references for "mesenchymal stem cells" and more than 4,500 for "mesenchymal stromal cells." There have been several comprehensive recent reviews on MSCs (Uccelli et al., 2008; Bianco et al., 2008; Tolar et al., 2010; Ranganath et al., 2012). Hence, rather than cover all of the work in this field, in this perspective I will focus on some areas that have seen notable advance in the past 5 years and others that warrant further investigation to improve our insight into the properties and potential of this intriguing cell population.

First, a case can be made to revisit the nomenclature and definition of MSCs, not as a semantic exercise, but to better define the direction of research. The availability of new molecular tools makes the need for rigorous definitions increasingly important. Moreover, the differences between MSC populations derived from different tissues are becoming more apparent, presenting an additional challenge to devising a universal definition. MSCs as currently defined are a phenomenon of in vitro culture, suggesting that extrapolating the function of these cells to activity in vivo must be done with caution. This limitation highlights the need for direct in vivo studies with endogenous MSCs or an equivalent physiological population as an essential next step in establishing their true biological role. It is encouraging in this regard that recent studies have employed transgenic animal models to enable the tracking and assessment of MSC-like cells in vivo. The mechanisms underlying tissue regeneration and immune modulation by therapeutic doses of MSCs also require further elucidation, particularly the extent to which the two processes intersect. The more recent appreciation that MSCs may not mediate tissue regeneration by direct cell replacement is also likely to redirect investigation into more fruitful directions. Finally, in view of the extraordinarily rapid and extensive use of MSCs clinically, a reappraisal of the approach to the development of clinical protocols based on confirmed laboratory and preclinical observations would be timely and helpful.

Background

MSCs were initially identified as a subpopulation of bone marrow cells with osteogenic potential as shown by heterotopic transplantation and subsequently were confirmed to contain clonal, plastic adherent bone-marrow derived nonhematopoietic cells in the mouse and guinea pig (Friedenstein et al., 1968, 1970, 1976). An in vitro colony assay developed by Friedenstein and coworkers to detect the clonogenic cell among this population (the colony-forming unit-fibroblast [CFU-F]) was also adapted for human marrow (Castro-Malaspina et al., 1980). Subsequent studies in the 1980s focused on the role of a similar population of bone marrow stromal cells derived from the adherent layers of long-term bone marrow cultures in supporting hematopoiesis (Dexter et al., 1977, reviewed in Clark and Keating, 1995). Caplan's proposal that these cells were mesenchymal "stem" cells (Caplan, 1991) capable of differentiation to all cells of mesodermal lineage stimulated investigation into their role in mediating tissue regeneration. Although the multilineage differentiation potential of MSCs was later shown (Pittenger et al., 1999), in vivo demonstration that these cells possess the hallmark stem cell characteristics of self-renewal and differentiation had not been accomplished.

Confusion arising from the definition of the MSC population made comparisons among published studies in the 1990s and 2000s problematic and led to the proposal of new terminology and criteria by the International Society for Cellular Therapy (ISCT) (Horwitz et al., 2005; Dominici et al., 2006). According to these widely adopted proposals, the cells were more appropriately considered mesenchymal stromal cells given that not all were stem cells (Horwitz et al., 2005). The minimum criteria for MSCs included plastic adherence and in vitro trilineage differentiation to adipogenic, chondrogenic, and osteogenic cells (Dominici et al., 2006). Additional requirements included cell surface expression of CD105 (endoglin, SH2), CD73 (ecto-5′-nucleotidase), and CD90 (Thy1) and the absence of the hematopoietic markers, CD45, CD19, CD19 or CD79, CD14 or CD11b, and HLA-DR. A particular challenge for the field has been the absence of a specific marker to define MSCs, although a large number of different determinants have been associated, albeit not exclusively, with them (reviewed by Lindner et al., 2010 for human MSCs), including CD271 (low-affinity nerve growth factor receptor) (Jones et al., 2002) and CD146 (Sacchetti et al., 2007). MSCs are also highly active metabolically, secreting not only components of the extracellular matrix (Wight et al., 1986) but also a vast array of cytokines (reviewed by Horwitz and Dominici, 2008). More recent work has documented

extensively the secretome and proteome of MSCs (Ranganath et al., 2012).

In addition to bone marrow, MSC populations can be obtained readily from adipose tissue (Zuk et al., 2002) and also from a variety of tissues including placenta (In 't Anker et al., 2004), skin (Shih et al., 2005), umbilical cord blood (Erices et al., 2000), umbilical cord perivascular cells (Sarugaser et al., 2005), umbilical cord Wharton's jelly (Wang et al., 2004), dental pulp (Gronthos et al., 2000), amniotic fluid (Nadri and Soleimani, 2007), synovial membrane (De Bari et al., 2001), and breast milk (Patki et al., 2010).

Revisiting the Definition of MSCs

The minimum criteria for defining MSCs established earlier (Horwitz et al., 2005; Dominici et al., 2006) may now be unduly constraining for a number of reasons. First, the characteristics of MSCs may vary according to the source of tissue. In an effort to define an MSC-like product, scientific entrepreneurs and biotechnology companies have focused on differences in surface marker profile to optimize intellectual property protection of relatively similar cell types. The recognition of species-specific differences in cell characteristics and generation of a variety of transcriptional and secretomic signatures for the cells also indicate diversity. Moreover, panels of reagents (especially antibodies) equivalent to those available for characterizing human MSCs are still not in place for a number of other species, so the criteria recommended by the ISCT (Dominici et al., 2006) may be difficult to meet.

The challenge is to devise an appropriate definition without losing the benefit that the current criteria provide in enabling evaluation of different studies of similar, if not identical, cell populations. A major hurdle is the absence of a single characteristic or marker with which to define MSCs. Nonetheless, a re-evaluation is timely and will require consensus among leading investigators in the field. In addition to standard methods of cell characterization of which surface marker profile and differentiation potential are the mainstays, the relative benefits of more advanced molecular tools including assessments of the cell transcriptome, proteome, and secretome (Ranganath et al., 2012) should be evaluated in creating this new definition. Moreover, the need to demonstrate trilineage differentiation, especially toward the chondrogenic lineage by MSCs derived from tissues other than bone marrow, also requires reassessment.

It is possible that a global definition of MSCs may now be overly simplistic or unnecessary. Specific definitions of particular MSC subsets may suffice, provided that they accurately and reproducibly define the cells under study. For example, the so-called stromal vascular fraction (SVF) of adipose-derived cells represents a highly heterogenous cell population and contains cells that express CD90 but not CD105 until they become plastic adherent (Yoshimura et al., 2006). Nonetheless, the cells have been considered to be MSC like. This issue is of additional significance because SVF cells have been extensively applied in clinical settings, despite a paucity of reported trials. It is unclear whether these cell products are uniformly defined prior to clinical administration.

Some general concepts of a new approach to the nomenclature, definition, and characterization of MSCs may provide a framework for discussion. The rationale is to help inform the investigation of these cells rather than to serve merely as a classification:

(1) The general population of MSCs should continue to be identified as mesenchymal stromal cells, although this is not an ideal term.
(2) The term "mesenchymal stem cell" should be used to specifically describe a cell with documented self-renewal and differentiation characteristics.
(3) MSCs should be categorized as cultured or primary—this is an important distinction (see below) because the characteristics are likely to be different and should avoid confusion when comparisons are made between studies.
(4) The source of MSCs should be specified (e.g., adipose, BM, cord blood, etc.); differences in cell characteristics are likely to be encountered.
(5) Species should be identified—this information is not always explicitly stated in the text of publications (except in the Methods section) and has led to confusion in the past.
(6) Minimum criteria for a surface marker profile need to be revisited and are likely to vary among species.
(7) The need to document the in vitro differentiation potential of the cells should be re-examined.
(8) The in vitro clonogenic capacity of MSCs should be enumerated.
(9) The reproducible representation of transcriptome, proteome, and secretome of MSCs should be evaluated and the major factors influencing the signatures should be identified and specified.
(10) Consideration should be given to characterizing the cells according to tissue specificity (e.g., the differentiation potential of human umbilical cord perivascular cells is more extensive than for BM MSCs).

Stem Cell Properties of Cultured MSCs

Despite numerous reviews attesting to the stem cell nature of MSCs from their ability to undergo differentiation along at least three lineages, there appear to be only three studies that can lay claim to identifying stem cells among human cultured MSCs, on the basis of rigorous clonal analysis. Muraglia et al. (2000) showed by limiting cell dilution that clones arising from single cells of bone marrow stromal cultures displayed multilineage differentiation potential and exhibited self-renewal. These authors proposed a hierarchical model in which there was sequential loss of lineage potential from the most primitive osteo-chondroadipogenic to osteo-chondrogenic, and finally to osteogenic precursors. Notably, osteo-adipogenic and chondro-adipogenic precusors were not detected, nor were purely chondrogenic or adipogenic clones. Lee et al. (2010) conducted single-cell studies of GFP-marked human MSCs (using irradiated stromal feeder layers to facilitate growth) and demonstrated that a minor subpopulation with high proliferative potential exhibited differentiation along osteogenic, chondrogenic, and adipogenic lineages and could self-renew from colony replating assays.

Analyzing the clonogenic differentiation capacity of another MSC population, human umbilical cord perivascular cells

(HUCPVCs), Sarugaser et al. (2009) documented the self-renewal and multipotent capacity of an infrequent mesenchymal stem cell able to differentiate to myogenic, osteogenic, chondrogenic, adipogenic, and fibroblastic lineages and proposed a hierarchical stem cell lineage relationship for these cells. These examples highlight the differences in differentiation potential between cells obtained from different tissues. This is an important area of investigation because as in the case of hematopoietic stem cell lineage relationships, much can be learned from studies of MSC clones that may be lost by an investigation of a heterogeneous MSC population, even one enriched for clonogenic cells.

Immunomodulatory Properties of Cultured MSCs

At this point, there is a considerable body of literature documenting the pleotropic effects of MSCs on the immune system. MSCs act on both the adaptive and innate immune systems by suppressing T cells, suppressing dendritic cell maturation, reducing B cell activation and proliferation, inhibiting proliferation and cytotoxicity of NK cells, and promoting the generation of regulatory T cells via an IL-10 mechanism. The role of MSCs in mediating these processes by affecting the expression of inflammatory cytokines is well established. This topic has been covered extensively in several reviews (Nauta and Fibbe, 2007; Le Blanc and Ringdén, 2007; Uccelli et al., 2008; Tolar et al., 2010; Chen et al., 2011, among others), and I will therefore focus on drawing attention to a few key issues.

One major area of MSC-mediated activity is T cell suppression (Yang et al., 2009). Several recent studies have identified pathways that are involved, including downregulation of NF-κB signaling and cell cycle arrest at G0/G1 (Jones et al., 2007; Choi et al., 2011). However, it is still somewhat unclear to what extent these pathways will have physiological significance. Some of the confusion in the literature in this area may be alleviated by the appreciation that there are major differences in the mechanisms of T cell suppression among species. For example, in humans and Rhesus monkeys, indoleamine 2,3- dioxygenase (IDO) is predominantly involved in T cell suppression, whereas nitric oxide is the main mediator in mice (Ren et al., 2008; Ren et al., 2009).

One emerging area of investigation involves studies of Toll-like receptors (TLRs) on MSCs and their contribution to immune modulation. These receptors respond to so-called danger signals consisting of molecules released by injured tissue or microbial invasion (e.g., endotoxin, LPS, dsRNA, and heat shock proteins). At least ten human TLRs are known and are expressed on innate immune effector cells (Kawai and Akira, 2011). Surprisingly, functional TLR3 and TLR4 are abundantly expressed on human BM-derived MSCs. Ligation of these TLRs induces activation of proinflammatory signals and prevents the suppression of T cell proliferation, possibly by MSC-mediated downregulation of Notch ligand (Liotta et al., 2008; Tomchuck et al., 2008). MSC-associated TLR signaling appears to not only involve a direct immune stress response but also the promotion of MSC migration (with TLR3 ligation). Interestingly, TLR3 and TLR4 stimulation does not appear to suppress IDO activity or PGE2 levels that decrease inflammatory responses (Liotta et al., 2008) and raises important implications for the role of MSCs in host defense. These observations suggest that activation of the TLRs on MSCs may maintain antiviral host defense.

TLR-mediated proinflammatory responses by MSCs could potentially have additional functional implications. On the basis of the divergent patterns of TLR3 versus TLR4 ligation in a short-term assay with respect to cytokine and chemokine secretion, cell migration capabilities, TGF-β secretion, and expression of the downstream effectors, SMAD3/SMAD7, Waterman et al. (2010) proposed a novel paradigm for MSC action. In their model, MSCs can polarize to a proinflammatory MSC1 type (TLR4-primed) or an immunosuppressive MSC2 (TLR3-primed) phenotype, analogous to the action of M1 versus M2 monocyte/macrophages (Dayan et al., 2011). Thus, the classical monocyte/macrophage responses to injury are reprised with the MSC1 (response to acute injury)/MSC2 (anti-inflammatory/healing) model (Figure 1).

It is also possible that through TLR signaling, MSCs play a pivotal role in both initiating the clearance of pathogens and promoting the repair of injured tissue, raising the possibility that MSCs could be employed clinically to augment host defense (Auletta et al., 2012). For future applications, the challenge will be to discover the key factors that contribute to achieving a balance that functions effectively in the best interests of the host. The next steps include confirmatory studies using different assays for further testing in animal models. In that regard, investigators will need to deal with the additional level of complexity from MSC-mediated augmentation of IL-10 production by macrophages via TLR4 ligation (Németh et al., 2009).

As is true for most studies of MSCs, the bulk of these immune modulation experiments were conducted with cultured MSCs. Data generated in vivo from putatively equivalent primary MSCs (MSCs in situ) remain lacking. Unfortunately, an assessment of immune interactions of uncultured MSCs in vivo has the same limitations as those for other MSC studies: the low frequency of primary MSCs in vivo, a lack of appropriate animal models, and interspecies variation in mechanisms of action (Ren et al., 2009). Differing results may also be reconciled by taking into account opposing mechanisms to maintain immune homeostasis. Alternative explanations include differences in cell dose, assay methodology, and MSC source. Given these limitations, an attempt to extrapolate in vitro data by Uccelli et al. (2008) is laudable and possibly amenable to testing. These authors provide several intriguing potential explanations for effects in vivo. For example, the effect of infused cultured MSCs on NK and dendritic cells may result in potentially opposite interactions that eventually will be resolved by predominant microenvironmental cytokine levels.

Evolving Concepts of Tissue Regeneration by MSCs

Over the past decade, there has been considerable evolution in our understanding of the mechanisms underlying tissue regeneration from MSCs. Progress may have been limited to some extent by the concept of the mesenchymal "stem" cell and the implicit idea that the objective was cell "replacement" therapy. For example, the concept of transdifferentiation of hematopoietic progenitors into cardiac cells was difficult to dislodge, despite rigorous studies failing to support the idea (Murry et al., 2004; Balsam et al., 2004). It was interesting that this notion was displaced by the phenomenon of cell fusion, another

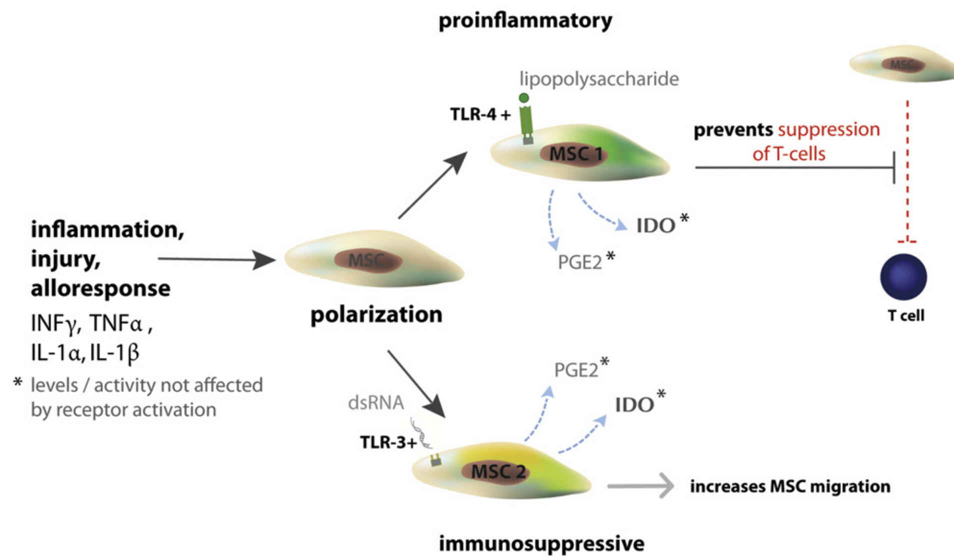

Figure 1. Proposed Immunomodulatory Mechanisms of Cultured MSCs
MSC-mediated immune interactions shown here include a proposed polarization of MSCs into MSC1 and MSC2 cells as a result of activation of Toll-like receptors (based on work by Waterman et al., 2010). Activation of MSC-resident TLR4 leads to a MSC1 or M1 type cell with a proinflammatory response, whereas activation of TLR3 gives rise to a M2 type MSC with an anti-inflammatory/immunosuppressive response. Overall outcome will depend on the balance between the cytokines/chemokines released into the microenvironment.

biological process also unlikely to account for documented improvements in preclinical models of cell treatment of injured tissue (if only because of its very low frequency).

However, the possibility that partial cellular reprogramming, leading to the acquisition of some characteristics of the desired lineage, could contribute to the tissue regeneration capacity of MSCs (Rose et al., 2008) remains to be investigated. A recent example of high throughput screening using human MSCs to identify small molecules that promote chondrogenic differentiation suggests an approach that may be more fruitful (Johnson et al., 2012). These investigators showed that the small molecule kartogenin induces chondrocyte differentiation of MSCs, protects articular cartilage in vitro, and promotes cartilage repair after intra-articular injection in an osteoarthritis animal model. Whether the administration of exogenous MSC-derived chrondrocytic cells will be superior to local treatment with the heterocyclic molecule alone is not yet known. Nonetheless, a more extensive drug discovery approach to identify molecules that mediate the differentiation/reprogramming of MSCs along mesodermal lineages is an exciting prospect.

Other explanations for the varying degrees of efficacy mediated by MSCs have been extensively reviewed elsewhere and are often characterized as "paracrine" effects. The cells are perceived to exert their effects by the release of factors that stimulate tissue recovery on many potential levels, including stimulation of endogenous stem/progenitor cells, suppression of apoptosis of vulnerable cells, remodeling of extracellular matrix, and stimulation of new blood vessel formation. Investigating MSCs as cytokine "factories" will likely uncover new mechanisms and identify compounds that may in some cases supplant the cells themselves (Ranganath et al., 2012). For example, tumor necrosis factor-inducible gene 6 protein (TSG6) is an immunosuppressive molecule produced by MSCs that partially recapitulates the hemodynamic improvement after intravenous infusion of the cells following experimental acute myocardial infarction in mice (Lee et al., 2009). This study serves to further underscore the shift toward the importance of the immunomodulatory properties of MSCs in regenerating injured tissue. Another example is the association between cardiac improvement and an MSC-mediated switch in macrophages/monocytes infiltrating ischemic tissue from the M1 to M2 phenotype (Dayan et al., 2011). Of interest, the switch was observed among circulating monocytes but not in the bone marrow, raising the possibility of a potentially useful distinction between more commonly accepted paracrine phenomena versus an allocrine effect produced by exogenous cells in a remote location.

How MSCs communicate with endogenous cells requires further study and the contribution by which cell-cell contact mediates the biological effects needs further clarification. In this regard, exploring the role of exosomes, secreted vesicles potentially involved in intercellular communication may provide novel insights (Lai et al., 2011).

Physiological Role of Primary In Situ MSCs

The study of culture-expanded MSCs is unlikely to help establish the physiological role of native in vivo cells. Progress in dealing with this limitation has initially been slow, partly because potentially useful experimental tools have been employed only recently and the frequency of putative native MSCs is very low. However, momentum is growing as the importance of these studies becomes more evident.

McGonagle and others have shown that the in vivo counterpart of MSCs has the following immunophenotype: $CD45^{-}$ or low, $CD271^{+}$ (Jones et al., 2002). More recent data show that the cells within this population have greater transcriptional activity than cultured MSCs or dermal fibroblasts, reflecting

broader differentiation potential and a marked increase in the transcription of osteogenic and Wnt-related genes (Churchman et al., 2012). $CD105^+$ cells can also be isolated in situ from human bone marrow and exhibit high levels of CFU-F activity, generate $CD105^+$ $CD90^+$, and $CD106^+$ cells that undergo trilineage differentiation (adipogenic, chondorgenic and osteogenic lineages) after culture, and differentiate into osteoblasts in vivo in response to BMP-2 (Aslan et al., 2006).

Other evidence indicates that the human in situ MSC in vivo is $CD146^+$, gives rise to CFU-F, and exhibits self-renewal in vivo. These cells are also capable of forming both bone and heterotopic hematopoiesis-associated MSCs from single clones in immune-deficient murine experiments. The $CD146^+CD45^-$ cells are subendothelial and localize in vivo as adventitial reticular cells (Sacchetti et al., 2007). More recent work from another group has confirmed that CFU-F activity resides exclusively in the $CD271^+$ cell population enriched directly from human marrow cells and shown that both $CD271^+CD146^+$ or $CD271^+CD146(^-)$ cells can give rise to stromal clones that form bone ossicles and hematopoiesis-associated stromal cells (Tormin et al., 2011). The Frenette group has shown that a small proportion of MSCs are $Nestin^+$, can self-renew in vivo, contain all the CFU-F activity of the bone marrow, and undergo osteogenic, chondrogenic, and adipogenic differentiation (Méndez-Ferrer et al., 2010). The relationship of these mesenchymal stem cells and CXCL12-abundant reticular (CAR) cells (Sugiyama et al., 2006), which also have osteoprogenitor capacity, requires further investigation. However, short-term ablation of CAR cells in vivo impaired the ability of BM cells to undergo adipogenic and osteogenic differentiation (Omatsu et al., 2010). The Scadden group has further examined osteolineage progenitors in the MSC pool. Their recent elegant study of bone maintenance and repair (Park et al., 2012) highlights the importance of genetic tools that better define the in vivo role of BM MSCs. They showed that a subset of $Nestin^+$ osteolineage-restricted MSCs present in vivo are able to replace short-lived mature osteoblasts to maintain homeostasis and respond to bone injury (Park et al., 2012).

Taking an innovative approach involving phage display and cell sorting, Daquinag et al. (2011) screened combinatorial libraries for peptides that target adipose stromal cells in vivo in the mouse based on the immunophenotype profile, $CD34^+CD31^-CD45^-$. They found a cell surface marker, the N-terminally truncated proteoglycan, δ-decorin highly expressed on the cells in vivo and identified resistin, a known protein adipokine, as its endogenous ligand. They hypothesized that signaling by resistin via the δ-decorin receptor regulates the fate of adipose stromal cells. Although observed almost in passing, the authors note that the δ-decorin is localized on the cell surface that faces away from blood vessels, suggesting an opportunity to interact with extracellular matrix components. In addition, they found that culturing the stromal vascular fraction (SVF) of adipose cells under standard conditions for generating MSCs led to loss of cell surface δ-decorin. These data underscore the challenges associated with identifying unique cell markers on cultured MSCs. Nonetheless, a similar approach for identifying an analogous receptor/ligand on bone marrow-derived MSCs may also yield valuable information regarding the nature and biology of the native MSC in vivo.

Clinical Application

At this point, there is extensive clinical activity involving MSCs, and many available treatments are outside the oversight of national regulatory bodies or clinical trial sites such as ClinicalTrials.gov. Moreover, the outcomes of a large number of these treatments are not documented in peer-reviewed journals. Unfortunately, the rationale for the clinical application of MSCs, particularly in regenerative medicine, has lagged behind laboratory observations. It is important to optimize the design of MSC trials based on the most current preclinical observations to maximize their scientific rigor. Several protocols involving systemic administration of MSCs to treat injured tissue are still in progress because of the notion of cell replacement therapy rather than on the more recently accepted paracrine and anti-inflammatory effects of these cells. The study outcomes are unlikely to be optimal if the major effect is actually an anti-inflammatory one and may arise from number of factors including inappropriate dose, scheduling, or route of administration. Furthermore, the coadministration of anti-inflammatory agents may be a confounding factor.

A second issue is the difficulty in fully evaluating completed clinical trials for which the results have not been formally published in international peer-reviewed journals. Valuable insights into trials design, patient selection, underlying rationale, and potential improvements would be gained by rigorous peer review.

Nevertheless, the results of several phase II trials with MSCs show promise. Le Blanc's phase II trial using MSCs to treat steroid-resistant aGvHD (Le Blanc et al., 2008) indicates that a multicenter randomized controlled trial should be conducted. Because several transplant centers already routinely employ MSCs for that indication on the basis of only the phase II data, the need for a randomized controlled trial seems quite urgent.

MSCs were also tested for their ability to support kidney transplantation on the basis of the promising data treating aGvHD. In an open label prospective trial, 159 patients undergoing a living related donor kidney transplant were registered for randomization to receive IL-2 receptor antibody induction therapy versus autologous BM-MSCs to assess rejection rate (Tan et al., 2012). Although patient and graft survival were similar, patients receiving MSCs had a lower incidence of acute rejection, decreased probability of opportunistic infections, and better kidney function 1 year later.

In addition, preclinical data have suggested that MSCs may have a role in the management of acute myocardial infarction. An industry-sponsored randomized double-blind placebo-controlled dose escalation study of systemically administered MSCs after acute myocardial infarction in 53 patients provided safety and preliminary efficacy data (Hare et al., 2009). Adverse events were similar between the test and placebo groups over a 6 month period. Ventricular arrhythmias were reduced ($p = 0.025$) and pulmonary function improved ($p = 0.003$) in patients receiving MSCs. In a subset analysis, patients with an anterior acute myocardial infarct had improved ventricular function (ejection fraction) compared with the placebo cohort. These are encouraging data and a prospective randomized trial with clinically significant endpoints is awaited with interest.

Well-designed clinical trials will be critical for determining whether MSCs can be effective in treating tissue injury or

immune disorders. Success is more likely if clinician investigators work very closely with laboratory researchers to design better clinical trials. Particular attention should be paid to factors that may be overlooked but could affect the efficacy of MSCs, including culture conditions/medium, oxygen tension, time from thawing after cryopreservation to administration, the tissue source of the MSCs, priming or activation prior to administration, the use of gene engineered versus unmodified cells, MSC subsets, and autologous versus allogeneic cells. Given the serious limitations associated with testing human MSCs in animal models, detailed analysis of immune and other perturbations in patients participating in prospective trials should be undertaken to help optimize subsequent protocols. Two other areas also require further attention. The importance of cell tracking and persistence of the exogenous MSCs in vivo in affecting clinical outcome can only be addressed when the cells are safely and effectively labeled and monitored. Although several studies have looked at the persistence of MSCs in animal models, similar correlative studies are required in human study recipients. Currently, the only viable option is superparamagnetic iron nanoparticles and magnetic resonance imaging, but even this approach has very limited availability. Finally, given the underreporting of MSC treatments and the paucity of publications describing long-term follow-up after MSC administration, a convincing case can be made for establishing a database registry of cell therapy recipients to track treatment outcomes and monitor for long-term adverse events. Two additional aspects suggest that clinical correlative studies are warranted. Although the recent observation of the acquisition of chromosomal aberrations in cultured human adult stem cells (Ben-David et al., 2011) is of uncertain significance (Sensebé et al., 2012; Prockop and Keating, 2012), it will be important to correlate assays of genetic instability prospectively with clinical outcome. The other aspect relates to the interaction of MSCs with cancer. Although there is a growing body of literature in this area (Djouad et al., 2003), the outcomes of experimental models appear to be conflicting. A spectrum of responses has been observed with different tumor models, from tumor suppression to stimulation. Clinical correlation with studies of the signaling pathways involved in stromal-tumor interactions is an important goal that should also accompany the establishment of a cell therapy patient registry.

Conclusions

In summary, the past 5 years have been remarkably active for MSC studies. Several initiatives can be undertaken to further accelerate the process of enhancing our understanding of MSC biology and improve access to well-designed clinical trials. Current definitions of this cell population need to be revisited given the wide range of tissue sources and the recognition of subpopulations with specific properties. Extending the availability of an international reference MSC repository for access to all investigators is also a priority. Additional animal models need to be developed to better identify and study the biology of primary in situ MSCs. The design of optimal clinical trials requires the close cooperation of laboratory and clinical investigators. Future studies need to be designed that also include assaying perturbations in patients in vivo and are therefore best positioned to overcome some of the inherent limitations of xenogeneic animal models with human MSCs.

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