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Review

Mesenchymal stem cells and their use in therapy: What has been achieved?

V.B. Fernández Vallone [a,1], M.A. Romaniuk [b,2], H. Choi [c,3], V. Labovsky [a,1], J. Otaegui [a,1], N.A. Chasseing [a,*]

[a] Experimental Biology and Medicine Institute, CONICET, Buenos Aires, Argentina
[b] Thrombosis I Laboratory, Hematological Research Institute, National Academy of Medicine, CONICET, Buenos Aires, Argentina
[c] Institute for Regenerative Medicine, Texas A&M Health Sciences Center, Temple, TX, USA

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ABSTRACT

The considerable therapeutic potential of human multipotent mesenchymal stromal cells or mesenchymal stem cells (MSCs) has generated increasing interest in a wide variety of biomedical disciplines. Nevertheless, researchers report studies on MSCs using different methods of isolation and expansion, as well as different approaches to characterize them; therefore, it is increasingly difficult to compare and contrast study outcomes. To begin to address this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed minimal criteria to define human MSCs. First, MSCs must be plastic-adherent when maintained in standard culture conditions (α minimal essential medium plus 20% fetal bovine serum). Second, MSCs must express CD105, CD73 and CD90, and MSCs must lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. Third, MSCs must differentiate into osteoblasts, adipocytes and chondroblasts *in vitro*. MSCs are isolated from many adult tissues, in particular from bone marrow and adipose tissue. Along with their capacity to differentiate and transdifferentiate into cells of different lineages, these cells have also generated great interest for their ability to display immunomodulatory capacities. Indeed, a major breakthrough was the finding that MSCs are able to induce peripheral tolerance, suggesting that they may be used as therapeutic tools in immune-mediated disorders. Although no significant adverse events have been reported in clinical trials to date, all interventional therapies have some inherent risks. Potential risks for undesirable events, such as tumor development, that might occur while using these stem cells for therapy must be taken into account and contrasted against the potential benefits to patients.

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Contents

Abbreviations: ASCs, adult stem cells; BM, bone marrow; CFU-F, fibroblast colony-forming units; FACS, fluorescence activated cell sorter; FBS, fetal bovine serum; HSCs, hematopoietic adult stem cells; HSPC, hematopoietic stem cells/progenitors cells; ISCT, International Society for Cellular Therapy; MSCs, mesenchymal stem cells

* Corresponding author. Present address: 2490 Vuelta de Obligado, Ciudad Autónoma de Buenos Aires (1428), Buenos Aires, Argentina. Tel.: +54 11 47832869x233; fax: +54 11 47862564.
E-mail addresses: valeria.fernandezvallone@ibyme.conicet.gov.ar (V.B. Fernández Vallone), albertinar@gmail.com (M.A. Romaniuk), hchoi@medicine.tamhsc.edu (H. Choi), jlnotaegui@gmail.com (V. Labovsky), jlnotaegui@gmail.com (J. Otaegui), achasseing@ibyme.conicet.gov.ar (N.A. Chasseing).
[1] Present address: 2490 Vuelta de Obligado, Ciudad Autónoma de Buenos Aires (1428), Buenos Aires, Argentina. Tel.: +54 11 47832869x233; fax: +54 11 47862564.
[2] Present address: 3092 Las Heras Avenue, Ciudad Autónoma de Buenos Aires (1425), Buenos Aires, Argentina. Tel.: +54 11 48055759.
[3] Present address: 5701 Airport Road, Module C. Temple, TX 76502, USA. Tel.: +1 254 771 6810.

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1. Stem cells

Stem cells are undifferentiated cells characterized by their self-renewal capacity, high potential for proliferation and their differentiation into non-self-renewable committed progenitors (Aranda et al., 2009; Wagers and Weissman, 2004).

Stem cells have been classified by their development potential as *totipotential* (capable of giving rise to all embryonic and extraembryonic cell types), *pluripotential* (capable of giving rise to all embryonic cell types), *multipotential* (capable of giving rise to a great number of cellular lineages), *oligopotential* (capable of giving rise to a more limited number of cellular lineages than multipotential cells) and *unipotential* (capable of giving rise to only one specific cellular lineage) (Prindull, 2005; Wagers and Weissman, 2004). An example of a totipotential cell is a zygote; cells derived from the internal mass of blastocysts are an example of pluripotential cells; finally, adult stem cells (ASCs) such as hematopoietic adult stem cells (HSCs) and mesenchymal stem cells (MSCs), from post-natal organisms are examples of multipotential cells (Wagers and Weissman, 2004), Fig. 1.

Multipotent ASCs have been described in different tissues, and some of these have been characterized in recent years, including the following examples: HSCs and MSCs in bone marrow (BM) and peripheral blood, neural stem cells in the central nervous system, hepatic stem cells in the Canals of Hering, pancreatic stem cells inside pancreatic islets, skin stem cells in the basal lamina of the epidermis and hair follicle, epithelial stem cells in lung, epithelial stem cells in intestine and skeletal muscle stem cells in muscle fibers (Korbling and Estrov, 2003).

The aim of this work is to briefly review the nature, biology and perspectives of the possible therapeutic roles of a special ASC: the MSC, particularly from BM.

2. A special ASC: MSC from BM

2.1. MSCs and BM

BM is composed of two compartments: the hematopoietic compartment (HSCs and committed progenitors of different specific hematopoietic lineages) and the stromal or hematopoietic microenvironment (stromal cells themselves, accessory cells, extracellular matrix components and soluble factors) (Janowska-Wieczorek et al., 2001). Among the stromal cells, we find MSCs, stromal precursors, stromal progenitors, fibroblasts, macrophages, adipocytes and endothelial cells (Dexter et al., 1977a), Fig. 2. Almost every BM stromal cell is derived from a MSC, with the exception of the macrophages, which come from HSCs, and endothelial cells that can be derived from both types of stem cells (Mayani et al., 1992; Wang et al., 2011). MSCs established in the BM have been observed in both postnatal and adult periods, and their presence declines with age (Caplan, 1994). At birth, the frequency of MSCs has been reported as 1 MSC/10^4 BM-mononuclear cells, decreasing to 1 MSC/2×10^6 mononuclear cells in 80-year-old individuals (Fibbe and Noort, 2003).

MSCs are also known as mesenchymal stromal cells or fibroblast colony-forming units (CFU-F), Fig. 3A. They are quiescent cells; however, they can proliferate *in vitro* in the presence of adequate stimuli: PDGF, FGF-2, TGF-β, EGF, SDF-1 and Dkk-1, among others (Gregory et al., 2003; Kortesidis et al., 2005; Ng et al., 2008; Rougier et al., 1996; Tamama et al., 2006; Yamada et al., 2000; Zorn, 2001), Fig. 3B. MSCs, stromal precursors and progenitors are plastic adherent cells, non-phagocytic and capable of differentiating *in vivo* and *in vitro* into specific cell lineages of mesoderm origin, such as osteocytes, chondrocytes, adipocytes, tenocytes, muscle cells and stromal cells (Alhadlaq and Mao, 2004; Baksh et al., 2004; Bianco et al., 2010;

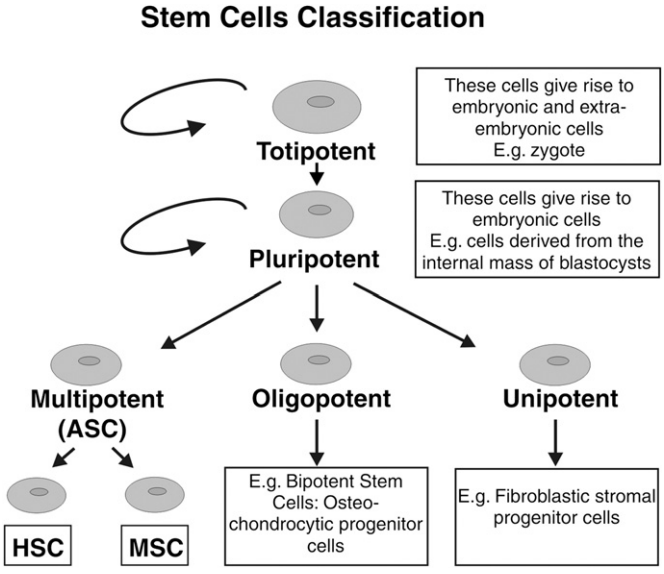

Fig. 1. Stem cell classification.

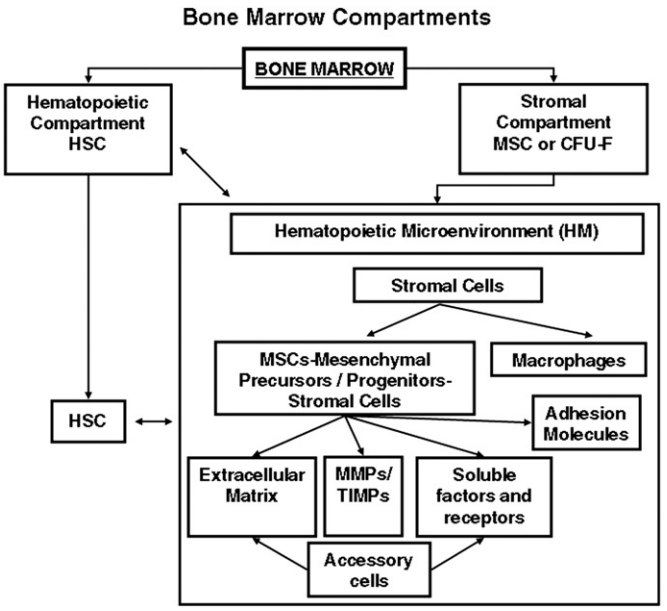

Fig. 2. Bone marrow compartments.

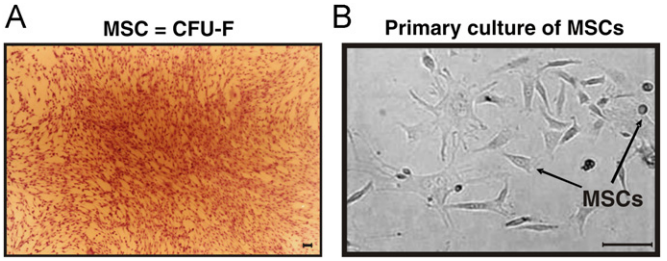

Fig. 3. (A) Human normal bone marrow colony forming units-fibroblastic (CFU-F) assay gives information on the number of MSCs and their differentiation capacity *in vivo*. A CFU-F with a larger size originates from an MSC with major multipotentiality capacity (100 ×). Scale bar 100 μm. (B) MSCs growth morphology in human normal bone marrow primary cultures (400 ×). Scale bar 100 μm.
Source: this figure corresponds to that published in Differentiation Journal by our group (Labovsky, V., Hofer, E.L., Feldman, L., et al., 2010. Cardiomyogenic differentiation of human bone marrow mesenchymal cells: role of cardiac extract from neonatal rat cardiomyocytes. Differentiation 79 (2), 93–101).

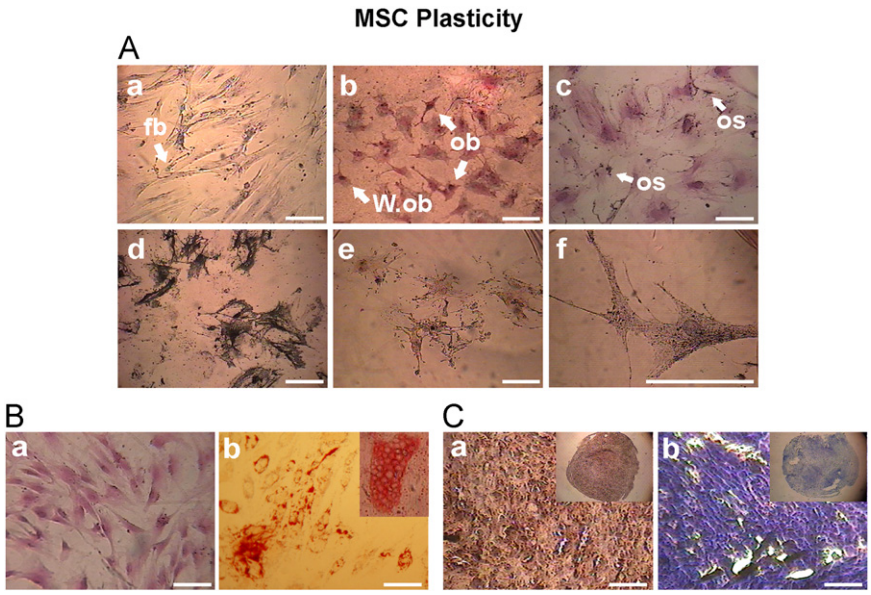

Fig. 4. Multilineage differentiation of normal hMSCs. Cells were cultured under osteogenic (A), adipogenic (B) and chondrogenic (C) differentiation mediums. (A) (a) hMSCs cultured with control medium showed fibroblast-like cell morphology (fb) and were Von Kossa (−). (b) hMSCs cultured with osteogenic differentiation medium, cells on the way to osteoblasts (W.ob) and osteoblasts (ob) and (c) osteocytes (os) both Von Kossa-Giemsa staining (+) (400×). Normal hMSCs cultured with osteogenic differentiation medium showed (+) staining for Von Kossa (d), Alizarin Red-S (e) (400×) and Osteocalcin (f) (600×). Scale bars 100 μm. (B) (a) hMSCs with control medium, Giemsa and Oil Red-O (−) staining (400×); hMSCs with adipogenic differentiation medium (b) Oil Red-O (+) staining revealed the presence of numerous oil droplets in the cytoplasm (400×). Scale bars 100 μm. (C) Typical differentiation into chondrocytes was shown by (a) type II Collagen and (b) Toluidine blue staining when hMSCs were cultured in chondrogenic differentiation medium (400×). Scale bars 100 μm. Stain revealed evidence of matrix production. This was not present in normal hMSCs cultured in control medium (absence of chondrogenic pellet).
Source: this figure corresponds to that published in Differentiation Journal by our group (Labovsky, V., Hofer, E.L., Feldman, L., et al., 2010. Cardiomyogenic differentiation of human bone marrow mesenchymal cells: role of cardiac extract from neonatal rat cardiomyocytes. Differentiation 79 (2), 93–101).

La Russa et al., 2002; Pountos and Giannoudis, 2005), Fig. 4. Additionally, MSCs are capable to transdifferentiate into different cell types of diverse origin (endodermal, ectodermal and mesodermal), such as neuronal, hepatic, pancreatic, renal and myoblast cell types (Baksh et al., 2004; Jiang et al., 2002; La Russa et al., 2002; Zipori, 2004). Concerning this property, the cells obtained have some morphological, phenotypic and functional characteristics similar to the cells of the specific tissue in a matter; so, they could be considered neuronal-like cells, hepatic-like cells, etc.

Under the appropriate culture conditions, different types of CFU-Fs are formed, each of which is derived from a single MSC (Castro-Malaspina et al., 1980). In BM tissue cultured with α minimal essential medium plus 20% fetal bovine serum (FBS), the mesenchymal stromal cell shape of the CFU-Fs is predominately fusiform which is characteristic of stromal cells of a fibroblastic nature (Prolyl 4 hydroxylase+, CD44+, CD105+, stro-1+) (Anonymous, 1984; Castro-Malaspina et al., 1980; Krebsbach et al., 1999).

The frequency of human BM CFU-Fs is extremely low, ranging from 1/10,000 to 1/100,000 BM-mononuclear cells. This amount is significantly lower than the frequency of CD34+ hematopoietic progenitor cells/HSCs, which includes approximately 1% of the mononuclear cell fraction (Castro-Malaspina et al., 1980).

All CFU-Fs are formed from stromal differentiated cells, committed progenitors, precursors and MSCs of diverse proliferation and differentiation capacity (multi-, tetra-, tri-, bi- and uni-potential stem cells). Therefore, taking this heterogeneous population of cells into account, it is important to choose the best methodology to isolate, characterize and study the functionality of MSC, in order to select the type of interest before it is used in tissue repair or gene therapy (Baksh et al., 2004; Sekiya et al., 2002; Ylostalo et al., 2008).

The long-term BM culture assay (Dexter et al., 1977a; Sacchetti et al., 2007) is the principal system in which we can describe the roles of these mesenchymal stromal cells in creating an appropriate hematopoietic microenvironment through the release of cytokines (IL-6, IL-7, IL-8, IL-11, IL-12, IL-15, etc.), growth factors (LIF, G-CSF, GM-CSF, M-CSF, Flt-3, SCF, PDGF, thrombopoietin, etc.), chemokines (SDF-1, RANKL, CCL2, etc.), metalloproteinases (MMP-2, MMP-3, MMP-9, MMP-13, etc.), MMP inhibitors (TIMP-1, TIMP-2, etc.) and extracellular matrix components (fibronectin, collagen I, III and IV, laminin, heparan sulfate, dermatan sulfate, chondroitin sulfate, proteoglycans and hyaluronic acid) (Charbord, 2010; Dexter et al., 1977b; Hofer, 2002; Majumdar et al., 1998). Some factors are involved at various levels of hematopoiesis, at the same time acting as negative or positive regulators of proliferation (TGF-β, MIP-1α, etc.), according to the targeted cells; these factors may also be involved in the control of the proliferation of mesenchymal stromal cells from the BM and proliferation of other tissues (Boiret et al., 2005; De Becker et al., 2007; Kasper et al., 2007; Minguell et al., 2001). However, the mechanisms implicated in hematopoietic support are not fully characterized. Van Overstraeten-Schlogel et al. (2006) suggested that human MSCs support hematopoiesis in Dexter-type cultures through activation of the SDF-1/CXCR4 axis during the interaction of MSCs with HSCs/progenitors cells (HSPC). Their data indicated that the chemokine SDF-1 stimulates retention of HSPCs in human BM–MSC niches, resulting in a situation that increases HSPCs exposure to stimulatory and inhibitory factors in a paracrine manner. It is now accepted that MSCs play a role as organizers of the HSC niche *in vivo* (Mendez-Ferrer et al., 2010; Sacchetti et al., 2007).

2.2. Phenotypic characterization of MSCs

Much progress has been made on the phenotypic characterization and isolation of BM–MSCs using the fluorescence activated cell sorter (FACS) and magnetic separation techniques.

Studies in primary cultures of normal human BM–MSCs that were tested for the presence of surface markers indicated the following: Stro-1+, CD73+, CD49a+, CD49b-low, CD49c+, CD49d-low, CD49f+, CD44+, CD105+, CD106-low, CD166+, CD29+, CD90+,

PODXL+, CD13+, HLA-ABC+, CD146+, CD147+, CD271-low or (−), CD117 (c-kit)-low or (−); and lack of expression of CD34, CD31, CD45, CD14, CD133, CD11b, CD113, HLA-DR, CD80 (B7-1), CD86 (B7-2), CD40, CD40 L, CD36, CD19, CD3, CD79, CD184 and c-met. However, non-expanded cultures are positive for both CD184 (also known as CXCR4) and c-met (also known as HGF receptor) (Baksh et al., 2004; Buhring et al., 2007; Buhring et al., 2009; Jones and McGonagle, 2008; Zhou et al., 2003). In recent years, it has been reported that SSEA-4, an early embryogenic glycolipid antigen commonly used as a marker for undifferentiated pluripotent human embryonic stem cells, also identifies the adult BM–MSC population (Gang et al., 2007). Furthermore, several additional studies performed with non-expanded progenitor mesenchymal cells derived from fresh human BM have led to the identification of two cellular subsets: CD45− CD14−/CD73+ and CD45− CD14−/CD49a+ (Boiret et al., 2005). The early antigenic expression of CD73 and CD49a is an essential characteristic that defines MSCs, although its functional significance is not yet well known. As CD73 is an adhesion molecule, it might be a signal transduction activator during the interaction of the MSCs with the rest of the stromal microenvironment components; in this way CD73 might favor the proliferation and differentiation processes (Barry et al., 2001). The expression of CD49a (VLA-1 α chain) could possibly allow MSCs to interact with extracellular matrix components, such as collagen IV and laminin; both such interactions are involved in migration. Moreover, the interaction between CD49a and collagen would induce cell cycle progression and survival of quiescent MSCs (Barry et al., 2001; Minguell et al., 2001; Wang et al., 1998).

Another recent interesting finding is that the expression of CD146 in MSCs is a marker of their origin from the BM-vascular niche; increased expression of CD146 surface molecule per MSC may be related to tripotentiality, greater capacity for self-renewal and regulation of hematopoiesis. Alternatively, compatively less expression of the CD146 surface molecule per MSC corresponds to the unipotential MSCs with low cloning efficiency (Bianco et al., 2011; Russell et al., 2010; Sorrentino et al., 2008).

In 2006, the International Society for Cellular Therapy (ISCT) proposed minimum criteria to define human MSCs. First, MSCs must be adherent to plastic when maintained in standard culture conditions. Second, MSCs must express CD105, CD73 and CD90, and they must lack expression of CD34, CD45, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. Third, MSCs must differentiate *in vitro* into osteoblasts, chondroblasts and adipocytes (Dominici et al., 2006). This last proposal from the ISCT was a consequence of the wide range of results on the characterization of the BM–MSCs observed by different researchers from around the world by that time. The heterogeneity of data were a direct result of the use of different methods for isolation, expansion and differentiation of MSCs, making it increasingly difficult to compare and contrast study outcomes.

In vitro, BM–MSCs represent a phenotypically heterogeneous population of stem cells. With this concept in mind, Jones and McGonagle (2008) (Buhring et al., 2007) tried to find the best positive marker for the *in vivo* identification of BM–MSCs based on the following criteria: the highest expression of the marker in MSCs and the lowest expression of it in all other BM cell populations. Data demonstrated that the low-affinity nerve growth factor receptor (LNGFR), today clustered as CD271, was the most differentially expressed marker. Furthermore, CD271 and CD106 (VCAM-1) antigens provide a versatile marker for prospective isolation and expansion of multipotent MSCs with immunosuppressive and lymphohematopoietic engraftment-promoting properties (Kuci et al., 2010; Ren et al., 2010). Co-transplantation of CD271 and/or CD106+ MSCs together with HSCs in patients with hematologic malignancies may be valuable in the prevention of impaired/delayed T-lymphocyte recovery and graft-versus-host disease (Kuci et al., 2010; Ren et al., 2010).

2.3. Isolation, expansion and use of MSCs

BM–MSCs isolation can be achieved using different methodologies, but the most commonly used method resulting in high efficiency is through an initial isolation of mononuclear cells by a Ficoll-Hypaque gradient ($\delta = 1.075$ gr/cm^3) and then adherence to plastic for 24 h, using α minimal essential medium supplemented with 20% FBS. After this period, the non-adherent hematopoietic cells are eliminated, but stromal cells are incubated again in the same medium until 70–80% confluence is reached. After that, mesenchymal stromal cells (80–90% fibroblast-like cells) are isolated by treatment with a Trypsin–EDTA solution (0.05 − 0.02% in PBS) and re-plated (Castro-Malaspina et al., 1980).

MSC cultures grown at low cell density have 50% of their cells in the S-phase at early passages, compared with 10% of cells observed at day 7. These variations were consequence of MSC expansion through distinct lag, exponential growth, and stationary phases (Larson et al., 2008). Some authors suggest that the initial plating density is not critical for maintaining a well-defined, multipotent MSC population (Tocci and Forte, 2003). Moreover, some authors reported that up to 40 subcultures could be created without losing the plasticity of mesenchymal stromal cells (MSCs and stromal progenitors); others affirm that the amount of time in culture affects cell characteristics, suggesting that cell expansion should be limited (from 1 to 5 passages), especially until the specific characteristics of different MSC subpopulations are better understood (Neuhuber et al., 2008; Tocci and Forte, 2003).

Bernardo et al. (2007) found that human BM–MSCs had a progressive decrease in proliferative capacity until reaching senescence but did not show chromosomal abnormalities. Moreover, telomerase activity and human telomerase reverse transcriptase transcripts were not expressed, and telomeres shortened during the culture period. Similar data was reported from other authors (Kim et al., 2009), who suggested that long-term culturing of expanded human MSC resulted in the cells becoming aged above a population doubling of 30; their telomerase activity was unchanged, whereas telomeres length decreased. Karyotypes were not changed. They also observed that *in vivo* transplantation of long-term cultured human MSCs to nude mice did not result in tumor formation. Expression of genes related to tumorigenesis decreased in proportion as the population doubling of human MSCs increased (Kim et al., 2009). Therefore, it is very important to work with non-senescent MSC cultures, especially when the plan is to then use these stem cells for clinical therapy (Prockop, 2010; Prockop et al., 2010a).

All of these findings suggest that diverse tests for cellular therapy should be considered during the *ex vivo* culture of human BM–MSCs, particularly when a prolonged and extended propagation period is required. The process of malignant initiation *in vivo* and the exact characteristics of the cancer-initiating cells still remain to be investigated. Moreover, the quality of preparations from different laboratories varies tremendously, and the cell products are notoriously heterogeneous. Consequently, there is an urgent need for the development of reliable reagents, common guidelines and standards for MSC preparations; in addition, precise molecular and cellular markers to define subpopulations with diverse pathways of differentiation and divergent potentials are necessary (Ho et al., 2008; Kasten et al., 2008).

2.4. Bone, BM and MSCs

A decrease in the number and cloning capacity of the BM–MSCs to generate CFU-Fs *in vitro* (colony-forming efficiency, CFE) could indicate an alteration of the osteogenic differentiation capacity of MSCs from BM, so CFE may provide useful insights

into pathogenetic mechanisms of bone/BM disorders (Galotto et al., 1999; Li et al., 2007).

It has to be mentioned that the bone and the BM are anatomically contiguous sites and that they exhibit a pronounced functional relationship. Although the BM and bone work as a simple unit, generally they are considered as separate systems. MSCs not only regulate osteogenesis but also osteoclastogenesis through the release of multiple factors, such as IL-1β, IL-6, IL-11, TGF-β, FGF-2, PDGF, PGE2, Dkk-1, Wnt 2, 4, 5, 11, 16, RANKL, LIF, OPG, M-CSF, MIP-1α and hyaluronic acid (Kim et al., 2005; Majumdar et al., 1998).

Osteoclasts are derived from BM mielo-monocytic progenitors; however, their differentiation is regulated in an autocrine and/or paracrine way, not only by MSCs, but also by osteoblasts through the release and expression of multiple factors, such as cytokines, growth factors, hormones and transcriptional regulators (Compston, 2002). Similarly to BM mesenchymal stroma cells, osteoblasts release multiple soluble factors that regulate the hematopoietic process (IL-11, IL-6, GM-CSF and M-CSF), and in particular, the myelopoietic process (Taichman and Emerson, 1998). From these last observations, the concept arises that many disorders of BM significantly affect the composition and function of the bone, including interactions between normal and pathological BM cells and those that exist in the bone compartment. In spite of the huge advances that have been made in the area of MSC differentiation into osteoblast/osteocytes, as well as the influence of this type of stem cell in osteoclastogenesis, there is still a lack of research on the mechanisms responsible for the synergistic function of BM and bone in the regulation of normal and pathologic bone remodeling.

2.5. MSC plasticity

The regulation of self-renewal and differentiation processes in MSCs is very complex; they depend on multiple factors: intrinsic (genetic) and extrinsic (microenvironment of the specific tissue). The loss of the balance between self-renewal and differentiation brings an uncontrolled cell growth and/or an increase in the maturation of different committed progenitors. Moreover, the ability to differentiate declines with age and because adult BM–MSCs are not a homogenous population, the plasticity may vary depending on the donor (Chang et al., 2006; Mueller and Glowacki, 2001). Therefore, a better understanding of MSC biology is necessary to establish a secure discernment of its potential clinical use.

The expansion possibility of MSCs and their multipotentiality increased the clinical interest in using them for tissue repair and gene therapy. The differentiation of MSCs into osteocytes, chondrocytes, adipocytes and stromal cells depends on a limited number of growth factors and nutrients; however, the transdifferentiation into, for example, cardiomyocytes, neurons and hepatocytes, is very intricate because it has many stages and requires the presence of specific pre-conditioned growth factors and very defined conditions (Gregory et al., 2005b).

Notable advances have been made in the study of mesenchymal *in vitro* differentiation into adipocytes, osteocytes and chondrocytes in recent years (Gregory et al., 2005a; Honczarenko et al., 2006; Ng et al., 2008). The presence of soluble factors in culture media is essential. For example, TGF-β and BMP are required for cartilage development, an organic phosphate source is necessary for osteogenesis, and hormonal stimuli are also needed for adipogenesis (Gregory et al., 2005b). Nevertheless, an appropriate medium is not enough to achieve the differentiation because daughter cells could exist within a clone of MSCs with different potentiality, such as multipotential, oligopotential or unipotential. Therefore, some of them could give rise to osteocytes, chondrocytes and adipocytes *in vitro*, while others could give rise to only 2 cell types, or a daughter MSC could possibly give rise to only one cell type (Baksh et al., 2004; Pevsner-Fischer et al., 2011; Ylostalo et al., 2008). Moreover, the number of passages for MSCs also produces an effect on the plasticity (Neuhuber et al., 2008). This last observation probably depends on cell density, which influences the MSC space distribution and components of the extracellular matrix present in the culture. For example, the three-dimensional distribution of MSCs in culture is critical for the development of the chondrogenic pellet, in which MSC suspensions of 100,000–200,000 cells are centrifuged, and then the micromass culture is exposed to TGF-β and BMP (Mackay et al., 1998; Tuli et al., 2003). The presence of both factors and the proximity of cells to one another in the micromass begin the chondrogenic cascade that is coordinated by extracellular molecules that develop signals at the junction of Wnt glycoproteins and the membrane, as well as signals induced by adhesion molecules, such as N-cadherin and connexin (Gregory et al., 2005b). Furthermore, cell seeding density plays a role in the expansion capacity of MSCs. For example, Colter et al. (2000) demonstrated that higher expansion profiles of MSCs can be attained when plated at low density (1.5–3 cells/cm^2) but not at high density (12 cells/cm^2), resulting in a dramatic increase in the fold expansion of total cells (2000-fold versus 60-fold expansion, respectively).

The commitment and differentiation of an MSC into a specific mature cell type is a process that involves the activity of various transcriptional factors, cytokines, growth factors and components of the extracellular matrix. During differentiation, expression of specific genes increases. The gene expression of osteocyte, adipocyte and chondrocyte lineages was studied, and an increase in the expression of 914, 947 and 52 genes was found for each differentiation, respectively. Eight genes are shared by the 3 lineages, 235 genes are common between adipogenesis and osteogenesis processes, 10 genes are common between adipogenesis and chondrogenesis and 3 genes are common between chondrogenesis and osteogenesis (Gregory et al., 2005b). The fact that osteocytes and adipocytes share this broad number of genes during their phenotypic acquisition was an indication that they could come from a common precursor (Gregory et al., 2005b). Nevertheless, there are contradictory data that describe an osteocytic–chondrocytic common precursor (Muraglia et al., 2003).

2.6. Migration and tissue repair

Different experiments demonstrate the migration of the BM–MSCs to different damaged organs, but few of those studies show the engraftment of transplanted MSCs from allogenic BM in the host's BM. Karyotype studies performed in long-term BM cultures of the host, after allogenic CD34+ and MSCs transplantation, showed that stromal cells were from the host's karyotype (Dickhut et al., 2005; Simmons et al., 1987).

MSCs' homing from endogen or exogen (by local or systemic infusion) sources toward a specific niche is a process that involves MSC migration and incorporation into the microenvironment of the damaged tissue or inflammation site (Yagi et al., 2010). During this process, several migration factors, such as SDF-1, TRAIL, RANKL, PDGF, IL-17, bFGF, INF-γ, IGF, TGF-β, EGF and EPO, are involved. These factors are released at the site of injury by different cells (endothelial cells, tumor cells, cells derived from the affected tissue, etc.). To respond to these factors, the presence of specific receptors expressed by MSCs is essential. These receptors include CXCR4, TRAIL receptors (DR5 and DcR2), RANK, PDGF receptor type α and β, IL-17 receptor, bFGF receptor, INF-γ receptor, IGF receptor, TGF-β receptor, EGF receptor and EPO receptor, among others (Alphonso and Alahari, 2009; Fox et al., 2007; Honczarenko et al., 2006; Kidd et al., 2009; Koh et al., 2009; Ponte et al., 2007; Schmidt et al., 2006; Yagi et al., 2010).

Moreover, it is well known that integrins play a fundamental role in relation to circulating MSCs and their adhesion to the vascular

endothelium and, after that, migration and chemotaxis toward the damaged site. Among them, β-1 and α-4 integrins, as well as other factors such as VCAM-1 and MMP-2, are the most important, but it is possible that their expression levels decrease in subsequent cultures (Karp and Leng Teo, 2009; Ruster et al., 2006; Steingen et al., 2008; Yagi et al., 2010). Some authors have demonstrated that α4/β1 (VLA-4) integrins might be important for the initial capture of MSCs, rolling and transmigration through the endothelial surface after BM transplantation (Jacobsen et al., 1996; Yagi et al., 2010).

The transplantation of MSCs, as well as their engraftment and differentiation into the cells of the multiple damaged organs, have been demonstrated in many animal models and human clinical assays (Jiang et al., 2002; Muraglia et al., 2003; Simmons et al., 1987). These studies indicate that MSCs are functionally prepared to recognize the place of injury and to transform it into an appropriate functional tissue. However, the exact mechanism that leads to the MSCs homing into damaged tissue and their differentiation and/or repair of damaged tissue through the release of different chemokines and soluble factors (such as FGF, EGF, PDGF, VEGF, SDF-1, IL-6, TGF-β), matrix components (such as fibronectin and hyaluronic acid), MMPs (such as MMP-2 and MMP-9), etc., was unknown until now (Horwitz and Dominici, 2008; Matthay, 2010; Prockop, 2007, 2009; Prockop et al., 2010b). However, emerging evidence suggests that most of the beneficial effects could be explained by secretion of therapeutic factors that have multiple effects, including modulation of inflammatory and immune reactions, protection from cell death and stimulation of endogenous progenitor cells (Lee et al., 2011; Prockop et al., 2010b). More importantly, as Lee RH et al described (Lee et al., 2011) MSCs can be activated to express high levels of additional therapeutic factors by crosstalk with injured cells or the microenvironment.

The actual situation concerning regenerative therapy with MSCs has been well described by Helmy et al. They reported the use of MSC for human transplant in over 101 cases registered by the FDA in "clinical trials" [*US Food and Drug Administration, www.clinicaltrials.gov*] (Helmy et al., 2010). As phases I and II studies are currently being developed, they include a low number of patients. The preliminary results are satisfactory and do not suggest major risks, but we have to wait for more time to pass to have definitive conclusions. MSCs used for the transplants reported in this study were from the BM (51%), adipose tissue (7%), umbilical cord blood (5%) and other sources such as peripheral blood or liver (3%). Approximately 48% of these studies have been performed with autologous MSCs, 42% with allogenic MSCs and the remaining 10% have not been registered. Helmy et al. reported that in those cases, MSCs were tested for the treatment of different pathologies such as skeletal-muscle disorders (24 trials), cardiac (16 trials), GVHD (14 trials), inflammatory disorders such as Crohn's disease (9 trials), neurologic diseases (8 trials), liver disorders such as cirrhosis (7 trials), diabetes type I and II (7 trials) and other diseases (16 trials).

Therefore, MSCs are useful for transplantation when certain criteria are carried out: differentiation into a specific cell, survival in the host after the transplant, integration into the microenvironment of the specific tissue that is need in of repair, fulfillment of an adequate function in the host during its life, lack of a graft reaction against the host, and a high potential for proliferation to generate sufficient quantity of undamaged tissue.

Therefore, a better and deeper understanding of MSCs' biology is necessary to establish efficient criteria for their potential clinical use (Matthay, 2010).

2.7. General functions and characteristics of BM–MSCs

1. *Hematopoiesis regulation*: MSCs support self-renewal, proliferation and differentiation of BM–HSCs and committed progenitors through the development of a healthy hematopoietic microenvironment. They are important in neutrophil and platelet recovery after high doses of chemo and/or radiotherapy treatments (Eaves et al., 1991; Koc et al., 2000; Prockop et al., 2010b; Sacchetti et al., 2007; Whetton and Dexter, 1993). Koc et al. (2000) proposed that infusion of autologous MSCs along with a peripheral blood progenitor cell transplantation could improve the BM microenvironment and, as a consequence, the rate and quality of hematopoietic recovery after myeloablative therapy.
   Multipotentiality or plasticity: BM–MSCs have the capacity for self-renewal, differentiation and transdifferentiation; properties that are essential for using these stem cells in the repair of tissues. Many experimental pre-clinical and clinical reports indicate that local administration as well as systemic administration of MSCs resulted in at least a transitory improvement for a large percentage of cases of pathologies, such as osteogenesis imperfecta, stroke, spinal medullar injury, Parkinson, acute lung and renal injury, diabetes and gastrointestinal disorders such as Crohn's disease (Alaiti et al., 2010; Bruno et al., 2009; Chopp et al., 2009; Ezquer et al., 2009; Helmy et al., 2010; Horwitz et al., 2002; Krause et al., 2007; Lee et al., 2009, 2006; Matthay, 2010; Pereira et al., 1998; Prockop et al., 2010b; Schwarz and Schwarz, 2010; Shaker and Rubin, 2010; Sueblinvong and Weiss, 2010; Togel and Westenfelder, 2009). Cell therapy with MSCs for the pathologies previously described is not restricted only to differentiation and transdifferentiation mechanisms or to the release of paracrine factors, but also to the anti-inflammatory effects of MSCs over the niches of injured tissues (Abrams et al., 2009; Waterman et al., 2010). So two paradigms are involved in tissue repair mechanisms; it was believed for a long period of time that these cells repaired tissues by engraftment and differentiation to replace injured cells (paradigm I). However, the recent studies showed that MSCs engage in cross-talk with injured tissues and thereby generate microenvironments or "quasi-niches" that enhance the repair tissues (paradigm II) (Horwitz and Dominici, 2008; Matthay, 2010; Parekkadan and Milwid, 2010; Prockop, 2009; Prockop et al., 2010b). During this cross-talk, MSCs release soluble factors that can modulate cell proliferation (SDF-1, HGF, VEGF, IGF-1), apoptosis (STC-1), angiogenesis (VEGF, SDF-1), and immune responses (LIF, iNOS, IDO, TGF-β1, PGE-2, TSG-6), etc. in the damaged tissue (Lee et al., 2011; Prockop et al., 2010b).
   The risk that cannot be forgotten when using this type of therapy is that MSCs, as we mentioned before, could favor *in vivo* tumoral growth (Kidd et al., 2008; Wong, 2011).
2. *Immunogenic potential:* MSCs are considered to be hypoimmunogenic because they exhibit minimal constitutive expression of HLA class I and HLA class II are expressed on a small subsets of MSCs (Potian et al., 2003). The expression of class II is modified with the degree of the inflammation process. At low IFN-γ levels, HLA II expression is maintained in the specific MSC subset but is downregulated at high levels (Chan et al., 2006). This suggests that the degree of inflammation within an anatomical region would determine whether HLA II is expressed on MSCs or its differentiated progenies. There is also an absence of the co-stimulating molecules: CD80 (B7.1) and CD86 (B7.2), CD40 and CD40 L (Nauta and Fibbe, 2007; Patel et al., 2008).
   MSC properties described under **1**, **2** and **3** could be favored by genetic modifications of this ASC, combining the best of both therapies cellular and genetic for the treatment of multi- or mono-genic disorders (Myers et al., 2010).
3. *Immunosuppressive function*: MSCs favor the engraftment of different organ transplantations, decreasing the graft reaction against the host and the symptoms of autoimmune illnesses such as like autoimmune encephalitis, diabetes type 1, etc.

(Ghannam et al., 2010; Le Blanc et al., 2008, 2004b; Le Blanc and Ringden, 2007; Li et al., 2008; Uccelli and Prockop, 2010; Yagi et al., 2010). MSCs decrease the production of TNF-α from dendritic cells 1 (DC1) and increase the release of IL-10 production from DC2 (Aggarwal and Pittenger, 2005). Moreover, MSCs have been shown to interfere with DC differentiation, maturation and function interfering in the expression of co-stimulatory molecules such as CD40 and CD86 over mature DC (Djouad et al., 2007). In addition, MSCs inhibit dendritic differentiation of monocytes through the release of IL-6, M-CSF, GM-CSF and PGE2 (Chen et al., 2007; Jiang et al., 2005; Kruse et al., 2000; Nauta et al., 2006). Moreover, MSCs inhibit differentiation of both CD34+ progenitors and monocytes into CD1a+-DCs, skewing their differentiation toward macrophage-like cells (Jiang et al., 2005; Kruse et al., 2000; Nauta and Fibbe, 2007). DCs produced in the presence of MSCs were impaired in their response to maturation signals and exhibited no expression of CD83 or upregulation of HLA-DR and costimulatory molecules (Djouad et al., 2007; Kruse et al., 2000; Nauta and Fibbe, 2007; Patel et al., 2008). Immature DCs generated in the presence of MSCs were strongly modified in their ability to induce activation of T lymphocytes (Nauta and Fibbe, 2007). Additionaly, MSCs decrease IFN-γ released from T-helper 1 and NK cells, decrease TNF-α released from T-helper 1 cells, and increase IL-4 released from T-helper 2 cells (Aggarwal and Pittenger, 2005; Nauta and Fibbe, 2007). Inhibition of NK functionality is mediated by IDO, PGE2 and TGF-β released by MSCs (Aggarwal and Pittenger, 2005; Yagi et al., 2010). Moreover, IL-6 and VEGF released by MSCs mediate the inhibitory effects over the proliferation of T cells (CD4+ and CD8+), as well as other soluble mediators such as galectin-1, semaforin-3A, IDO, PGE2, TGF-β, IL-10, MMP-2/9 and membrane molecules such as VCAM-1 (Ding et al., 2009; Djouad et al., 2007; Le Blanc et al., 2004a; Le Blanc and Ringden, 2007; Lepelletier et al., 2010; Ren et al., 2009, 2010).

On the contrary, it has been shown that MSCs can promote *in vitro* and *in vivo* development of regulatory T cells CD4+ CD25+, which present immunosuppressive activity (Le Blanc et al., 2004b; Le Blanc and Ringden, 2007). MSCs can also promote the proportion of T cells CD4+/CD25+/CTLA4+ and T cells CD4+/CTLA4+ in the presence of IL-2 or in a lymphocyte mixed culture (Aggarwal and Pittenger, 2005; Maccario et al., 2005). Furthermore, MSCs can produce BMP-2, which mediates immunosuppression through the production of regulatory T cells (Djouad et al., 2003). However, some authors found contradictory data with respect to regulatory T cell induction by MSCs *in vivo* (Parekkadan et al., 2008; Zappia et al., 2005).

With regard to the inhibitory effect of MSCs on B lymphocytes, it was recently shown that it occurs through an arrest of the G0/G1 phase of the cell cycle and through the induction of apoptosis (Corcione et al., 2006). MSCs decrease the expression of chemokine receptors on B cells, suggesting blunting effects on B cell migration to sites of inflammation (Corcione et al., 2006).

4. *Immunostimulatory function*: observations have indicated that low numbers of MSCs stimulate the immune response, whereas excess MSCs have an inhibitory effect (Le Blanc et al., 2003). MSCs have been shown to mildly increase IgG and IFN-γ production (Rasmusson et al., 2007).

With regard to previous observations, Waterman et al. (2010) have recently described that human MSCs can be polarized because of downstream Toll-like receptor (TLR) signaling into two types of homogeneous subpopulations with a different active phenotype. They classified those MSCs as MSCs1 and MSCs2. MSCs1 release pro-inflammatory mediators such as IL-6, IL-8, etc., and MSCs2 release more immunosuppressive mediators such as IL-10, IDO, TSG-6, etc., through the action of TLR4 and TLR3, respectively. This difference between MSCs1 and MSCs2 is not only related to soluble mediators (cytokines) but also to extracellular matrix components because TLR4 favors collagen deposits from MSCs1, and TLR3 favors fibronectin deposits from MSCs2. Moreover, activation of TLRs has the following repercussions for MSC differentiation: TLR3 activation inhibits MSCs triple plasticity (osteogenic, adipogenic and chondrogenic), and TLR4 activation inhibits adipogenic differentiation, stimulates osteogenic plasticity and has no effect on chondrogenic differentiation. Both activated TLRs favor migration and invasive capacity of MSCs1 and 2, but the observation that the release of chemokines is increased after TLR3 activation (CCL5 or RANTES and CCL10 or IP-10) must be addressed. Therefore, taking all of these observations into account, TLR3 and TLR4 may be considered for regenerative or immunosuppressive therapy at the time of MSCs selection.

3. Final comments

Many experimental and pre-clinical assays have been developed during the last decade; however, a great number of questions related to MSC biology are still unsolved. These questions are related to MSC survival, homing capacity after transplant, the relationship between MSC immune phenotype and function, MSC route of administration (local or systemic), autologous versus allogenic MSCs, and whether some properties of MSC such as self-renewal, differentiation and transdifferentiation are maintained after transplantation.

Therefore, out of the previous questions emerges the necessity to continue to advance MSC knowledge, in order to close the gap between the hope of MSC potential use in the clinic and the real therapeutic application of MSC.

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