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C. Herrero and J.A. Pérez-Simón

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Immunomodulatory effect of mesenchymal stem cells

C. Herrero and J.A. Pérez-Simón

Hospital Clínico Universitario de Salamanca and
Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León,
Salamanca, Spain

Abstract

Mesenchymal stem cells (MSC) are multipotential nonhematopoietic progenitor cells capable of differentiating into multiple mesenchymal tissues. MSC are able to reconstitute the functional human hematopoietic microenvironment and promote engraftment of hematopoietic stem cells. MSC constitutively express low levels of major histocompatibility complex-I molecules and do not express costimulatory molecules such as CD80, CD86 or CD40, thus lacking immunogenicity. Furthermore, they are able to suppress T- and B-lymphocyte activation and proliferation and may also affect dendritic cell maturation. Based on these properties, MSC are being used in regenerative medicine and also for the treatment of autoimmune diseases and graft-versus-host disease. On the other hand, MSC from patients diagnosed with myelodysplastic syndromes or multiple myeloma display abnormalities, which could play a role in the physiopathology of the disease. Finally, in patients with immune thrombocytopenic purpura, MSC have a reduced proliferative capacity and a lower inhibitory effect on T-cell proliferation compared with MSC from healthy donors.

Key words: Mesenchymal stem cells; T-lymphocytes; B-lymphocytes; Dendritic cells; Graft-versus-host disease; Allogeneic transplantation

Introduction

In 1968, Friedenstein et al. (1) isolated clonogeneic spindle-shaped cells in monolayer cultures, which they called colony-forming unit fibroblasts. These cells showed the ability to self-renew and to differentiate towards a mesodermal lineage as adipocytes, chondrocytes, osteocytes, and connective stromal cell. More recently, several studies have reported that multipotential stromal precursor cells can also differentiate into other lineages such as ectodermal (epithelial cells and neuroglial-like cells) and endodermal (muscle cells, lung cells, gut epithelial cells, and hepatocyte-like cells) ones (2,3). For this reason, bone-marrow-derived stromal cells were first considered to be stem cells by Caplan (4) and named mesenchymal stem cells (MSC). MSC are defined as a non-homogenous population of multipotent cells, which are classically expanded *in vitro* from the plastic-adherent cell population and lack specific markers. Because of this, in 2006 the International Society of Cellular Therapy established minimal criteria to define them (5): ability to stick to plastic and ability to differentiate at least into adipocytes, chondrocytes, osteocytes, and having the following phenotypes: CD90+, CD73+, CD105+, CD34-, CD45-, CD14-, CD19-, HLA-DR-, CD11b-, and CD79α.

Stromal cells in the hematopoietic-stem-cell niche

Several studies, both in animal models and clinical assays, have shown the ability of MSC to reconstitute the functional human hematopoietic microenvironment and to promote engraftment of hematopoietic stem cells (HSC) (6,7). Through direct cell-to-cell contact and release of cytokines and growth factors, stromal cells support the maintenance and self-renewal of HSC, as well as their proliferation and differentiation and the release of mature progeny into the vascular system (8). MSC express a wide variety of cell adhesion molecules (STRO-1, VCAM-1, ICAM-1/2, ALCAM-1, L-selectin, CD105, CD44), integrins, growth factor receptors (bFGFR, PDGFR, EGFR, TGFβIR/IIR), chemokine receptors (some interleukins, CC and CXC receptors) and produce a vast array of matrix molecules

Correspondence: J.A. Pérez Simón, Servicio de Hematología, Hospital Clínico Universitario de Salamanca, Paseo de San Vicente, s/n, 37007 Salamanca, Spain. E-mail: pesimo@usal.es

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including fibronectin, type I, III and IV collagen, laminin, hyaluronan, and proteoglycans (9). The presence of these molecules leads to the activation of numerous pathways such as Notch, Tie-2, bone morphogenetic protein pathways, or Wnt (10). In addition, there is interaction and signaling through the CXCR4 and stromal-derived factor-1 or CXCL12 axis, which plays a crucial role in the regulation of HSC proliferation and cycling (11).

Immunomodulatory effect of MSC

Human MSC (hMSC) are characterized by a low expression of major histocompatibility complex (MHC) class I and the absence of costimulatory molecules such as CD80, CD86 or CD40 (12). Moreover, hMSC fail to induce proliferation of allogeneic or xenogeneic lymphocytes. These characteristics support the possibility of exploiting universal donor MSC for therapeutic applications. MSC constitutively express low levels of MHC-I molecules while, as a general rule, they do not constitutively express MHC class II molecules (13). However, recent evidence indicates that MSC can function as antigen-presenting cells and activate immune responses under appropriate conditions (14). Although one study reported constitutive MHC class II expression on MSC (15), several groups reported that both MHC class I and class II molecules are up-regulated following interferon-γ (IFN-γ) treatment, thus inducing a T-cell response to recall antigens (13,14,16).

Effect of MSC on T cells

The earliest indications of the immunosuppressive nature of MSC were derived from studies with human, baboon and murine MSC demonstrating that MSC were able to suppress T-lymphocyte activation and proliferation *in vitro* (17-19).

Different studies have demonstrated that MSC inhibit T-cell proliferation thanks to the arrest of T cells in the G0/G1 phase (20). In this regard, Benvenuto et al. (21) showed that MSC inhibit T-cell proliferation but also support the survival of T cells that are subjected to overstimulation through the T-cell receptor and are committed to undergo CD95-CD95 ligand-dependent activation-induced cell death. MSC also inhibit the production of IFN-γ and increase the production of interleukin-4 (IL-4) by T-helper 2 cells. This indicates a shift in T cells from a pro-inflammatory (IFN-γ) state to an anti-inflammatory (IL-4 production) state (22,23).

Regarding the pathways involved in the inhibition of T-cell proliferation, Glennie et al. (20) reported that this effect is due to inhibition of cyclin D2 and up-regulation of p27kip1 induced by MSC, which arrest T-lymphocytes in the G1 phase.

The mechanisms underlying these effects are unknown but are probably mediated by both direct cell-cell contacts and soluble factors such as nitric oxide and indoleamine 2,3-dioxygenase, which are released by MSC after being triggered by IFN-γ produced by target cells (24). Other soluble factors such as transforming growth factor-β1, hepatocyte growth factor, prostaglandin E2 (PGE2), IL-10, haem oxygenase-1, IL-6, and HLA-G5 are constitutively produced by MSC (22,25-28).

Effects of MSC on B cells

As regards the effect of MSC on B-lymphocyte function, most studies (see, e.g., 29) indicate that MSC inhibit immunoglobulin production and arrest B-lymphocytes in the G0/G1 phase of the cell cycle.

In our experience MSC promote B-lymphocyte survival while, under highly proliferative conditions, they arrest the B-cell cycle, inhibiting differentiation of B cells into plasma cells (30).

In order to define the pathways involved in the effects of MSC on B-lymphocytes, we evaluated by Western blot analysis the pathways involved in cell survival, proliferation and differentiation. With regard to cell survival and proliferation, under conditions of low B-cell proliferation we observed that MSC increased ERK1/2 and inhibited p38 MAPK phosphorylation, which may explain the increased viability of B cells in the presence of MSC. Surprisingly, the opposite effect was observed under culture conditions, which favored B-cell proliferation, so that MSC inhibited ERK1/2 phosphorylation and induced activation of p38 MAPK in B cells (30).

Effect of MSC on dendritic cells

MSC have been demonstrated to interfere with dendritic cell differentiation, maturation and function (31-34).

Transwell experiments suggest that these effects are mediated by soluble factors (33) such as IL-6 and M-CSF, although blocking studies indicate that these factors are not solely responsible for the inhibitory effect. Alternatively, PGE2 might be an intriguing candidate factor, which is released by MSC after cell-to-cell contact. Aggarwal and Pittenger (22) proved that the inhibition of PGE2 synthesis restored the secretion of TNF-α and IFN-γ by dendritic cells cultured in the presence of MSC (22,31). Similarly to what was observed in T cells, Ramasamy et al. (34) reported that the cell cycle of dendritic cells was arrested in the G0/G1 phase upon interaction with MSC.

Clinical applications

Hematopoietic stem cell transplantation

The goal of hematopoietic stem cell transplantation is to replace the hematopoietic system of the patient with a healthy one (hematopoietic cell donor) after high doses of chemotherapy and radiotherapy. Transplanted donor T-lymphocytes recognize and exert cytotoxic effect against tumor cells from the host inducing a graft-versus-leukemia (GVL) effect. Unfortunately, the use of this kind of treatment is limited because it is difficult to find an HLA-matched relative donor and a transplant from a non-relative donor or a

non-HLA-matched donor increases the risk of morbidity and mortality for three principal reasons: rejection of the graft, infections, and graft-versus-host disease (GVHD).

MSC use in the clinical setting for the treatment of graft-versus-host disease

Koc et al. (35) reported a clinical study on 28 breast cancer patients who received an autologous hematopoietic stem cell transplantation together with autologous MSC. In 62% of the patients, clonogenic MSC were detected in peripheral blood up to 1 h after infusion. To achieve >500 granulocytes/mm^3 and >20,000 platelets/mm^3, median time was 8 and 8.5 days, respectively. All this suggests that infusion of MSC after myeloablative conditioning seems to be safe and to have a positive impact on hematopoiesis for a quick hematopoietic recovery.

In 2005, Lazarus et al. (36) carried out a study with 46 patients who underwent allogeneic stem-cell transplantation after a myeloablative conditioning regimen. These patients received HSC and culture-expanded MSC from their HLA-identical sibling donors. Moderate to severe acute GVHD was observed in 28% of patients and chronic GVHD was observed in 61% of patients, with a 2-year progression-free survival of 53%. In comparison with historical controls, no MSC-associated toxicities were reported and MSC accelerated the hematopoietic engraftment. Moreover, MSC prevented graft rejection and did not increase the incidence or severity of GVHD. But MSC are difficult to detect after infusion, even in immunocompromised patients, since stromal cell chimerism was only found in 2 of 19 examined patients 6 and 18 months after transplantation.

Le Blanc et al. (37) suggested that MSC may not only exert preventive effects on the development of GVHD, but also exhibit therapeutic effects on established GVHD of the gut after allogeneic stem-cell transplantation. A patient with severe treatment-resistant grade IV acute GVHD of the gut and liver was transplanted with haploidentical MSC. The clinical response was striking, with normalization of stool and bilirubin on two separate occasions. Later, the same investigators were able to prove that 6 of 8 patients with steroid refractory grade III-IV acute GVHD treated with MSC showed a complete disappearance of acute GVHD, with a survival curve better than that of 16 patients not treated with MSC (P = 0.03) (38).

In Europe, these studies have been further extended including 55 patients with steroid-resistant acute GVHD. These patients had previously failed to respond to a first line (N = 55), second line (N = 33), third line (N = 14) or fourth or more lines of treatment (N = 6). The median dose of bone marrow-derived MSC was 1.4 x 10^6 cells per kg body weight. Twenty-seven patients received one dose, 22 patients received two doses, and 6 patients received three to five doses of MSC obtained from HLA-identical sibling donors (N = 5), haploidentical donors (N = 18) or third-party HLA-mismatched donors (N = 69). Overall, 30 patients achieved a complete response and 9 patients a significant clinical improvement of GVHD. Median time from first MSC infusion to clinical response was 18 days (39).

Although MSC are immunosuppressive and may alleviate GVHD, it is not known whether they will increase the risk of leukemic relapse by abrogating the GVL effect. In a prospective randomized trial, HLA-identical sibling-matched HSC were transplanted alone (non-MSC group, N = 15) or co-transplanted with MSC (MSC group, N = 10) in patients suffering from hematopoietic malignancies. The median number of MSC infused was 3.4 x 10^5/kg. MSC infusions were tolerated and the median time to neutrophil engraftment was 16 days for the MSC group and 15 days for the non-MSC group. The median time to platelet engraftment was 30 and 27 days, respectively. Grade II-IV acute GVHD was observed in 10 and 53% of patients from the MSC and non-MSC groups, respectively. Similarly, chronic GVHD was observed in 14 and 28% of patients from the MSC and non-MSC groups, respectively. However, the proportion of patients who relapsed was 60 and 20%, and 3-year disease-free survival was 30 and 66% in the MSC and non-MSC groups, respectively (40). According to these results, co-transplantation of MSC and HSC may have prevented GVHD, but the relapse rate was significantly higher in the group where MSC were co-infused along with HSC. This clearly indicates that the use of MSC should be handled with extreme caution.

Traditionally MSC have been expanded in a medium supplemented with fetal calf serum (FCS), and some of these MSC have been used in clinical trials. But the administration of animal products to humans might cause the transmission of prions and of still unidentified zoonoses. In addition, hMSC expanded in the presence of FCS have been implicated in anaphylactic or arthus-like immune reactions (41). Although it has been reported that after expansion of hMSC in the presence of FCS the contamination is lower than 0.01%, the risk of disease transmission still persists (42). In view of these considerations, different studies have focused on the identification of a serum-free medium appropriate for the growth of the large number of MSC required for clinical application. As an alternative to FCS, platelet lysate (43-46) and both autologous and allogeneic human serum have been proposed (47,48). The use of allogeneic human serum has resulted in MSC growth arrest and death in some studies, while autologous serum allows a faster proliferation compared to FCS at least during the first passages and, in addition, avoids the exposure to allogeneic antigens and minimizes the risk of infection (48). Human autologous serum would be the safest choice since it would avoid the exposure not only to FCS but also to allogeneic products such as platelet lysate or allogeneic serum. Nevertheless, the amount of autologous serum required for a sufficient expansion used to be a matter of debate. We have reported a method for obtaining large amounts of autologous serum suitable for

culture on a clinical scale (skeletal myoblasts) (49), which would allow us to obtain the amount of autologous serum required for sufficient expansion of MSC.

Furthermore, MSC expanded with human serum inhibit the expression of CD40L and the production of IFN-γ to a higher extent than MSC cultured with FCS (50).

MSC involvement in hematopoietic malignancies and autoimmune disorders

Myelodysplastic syndromes (MDS) are a group of clonal disorders of pluripotent HSC characterized by ineffective hematopoiesis and an increased potential to progress to acute myelocytic leukemia (51). The microenvironment has been proposed to play a key role in MDS development and new treatment strategies are being developed for these targets (52).

We have characterized bone marrow-derived MSC from patients with MDS. Interestingly, the growth kinetics of MSC from MDS patients showed an impaired expansion potential. MSC from healthy donors build confluent layers after a median of five passages (range: 2-7), and time to reach confluence is 15.5 days (range: 11.6-30). However, MSC from MDS patients need more time to achieve confluence, with a median of 23.5 days (range: 12-90), and are only able to survive until the third passage. Moreover, in 6 MDS cases (one patient with refractory anemia with excess blasts-1 (RAEB-1), one patient with RAEB-2, 2 patients with 5q- syndrome, and 2 unclassifiable patients), their MSC did not reach confluence and could not be expanded.

In addition, karyotypic abnormalities were observed in MSC from 13 patients, the regions most commonly involved being 19p13.3, 11q13.1, and 20q13.33 (53).

Multiple myeloma (MM) is a B-cell neoplasia characterized by infiltration and accumulation of clonal plasma cells in bone marrow. This causes a build-up of monoclonal immunoglobulin in blood and/or urine, and the presence of a peak in the gamma zone of serum and/or urine protein electrophoresis. MSC may play a role in the development of bone disease in MM patients. Based on this hypothesis, our group performed an array-based comparative genomic hybridization analysis showing that, while healthy donor MSC were devoid of genomic imbalances, several non-recurrent chromosomal gains and losses (>1 Mb in size) were detected in MM-MSC (54). These abnormalities were not found in the myelomatous cells from the same patient.

Regarding the involvement of MSC in the development of autoimmune disorders, we have analyzed MSC from patients diagnosed with immune thrombocytopenic purpura (ITP). In this bleeding disorder there is a destruction of platelets and a reduction of their production because of the existence of autoimmune reactive antibodies. In addition, patients with ITP possess activated platelet-autoreactive T cells (55) and cytokine imbalance, which have been related to decreased levels and abnormal function of regulatory T cells (56,57). Previous reports have shown that the immunomodulator effect of MSC can be attributed, at least in part, to their capability to expand regulatory T cells (21,28). With this background, we decided to analyze the potential role of MSC in the pathogenesis of ITP.

We isolated and expanded MSC from bone marrow of healthy donors (N = 13) and of ITP patients (N = 26) (58). While MSC were expanded in 7 of 7 controls, only in 7 of the first 20 ITP patients analyzed was it possible to expand MSC until the third passage (P = 0.006). Interestingly, unlike MSC from healthy donors, MSC expansion from bone marrow of ITP patients was slower and the cells did not acquire spindle-shape morphology. Their phenotype and differentiation capacity were similar to controls, although MSC from ITP patients required a longer period of incubation in differentiation medium.

Furthermore, unlike MSC from healthy donors, MSC from patients do not down-regulate p27 after culture with PDGF. Interestingly, p27 not only inhibits cell-cycle progression but also acts as a promoter of apoptosis (59,60). The persistence of p27 up-regulation after stimulation with PDGF explains the poorer proliferative capacity of MSC from ITP patients. Also, RQ-PCR assays were performed showing that caspase 9 expression was higher in MSC from ITP patients compared to controls. Finally, MSC from ITP patients were unable to block proliferation of T cells to the same extent as MSC from healthy donors (58).

In conclusion, MSC have an immunomodulatory effect, which is currently being exploited in the clinical setting for the treatment of GVHD. Furthermore, MSC from patients diagnosed with MDS or MM display abnormalities, which could play a role in the physiopathology of the disease. Finally, in ITP patients, MSC have a reduced proliferative capacity and a lower inhibitory effect on T-cell proliferation compared with MSC from healthy donors. This defect is associated with a reduced down-regulation of suppressor genes involved in cell-cycle control as well as an overexpression of caspase 9. These abnormalities suggest a role of MSC malfunction in the physiopathology of the disease.

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