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Mesenchymal Stem Cell treatment for autoimmune diseases: a critical review

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ABSTRACT

Mesenchymal stem cells (MSCs) are now known to display not only stem cell multipotency, but also robust antiinflammatory and regenerative properties. After widespread *in-vitro* and *in-vivo* preclinical testing, autologous and allogeneic MSCs have been applied in a range of immune mediated conditions, including graft versus host disease, Crohn´s disease, multiple sclerosis, refractory systemic lupus erythematosus and systemic sclerosis. Current data suggests that MSCs may not only replace diseased tissues, but also exert several trophic, regenerative and antiinflammatory effects. While the clinical outcome in case reports and phase I-II trials seems occasionally striking, these limited results point to the need to perform controlled multicenter trials. Future advances from stem cell science can be expected to pinpoint significant MSC subpopulations and/or stem cell markers for improved regenerative or immunoregulatory properties.

Key words: Mesenchymal stem cells, autoimmune diseases, cellular therapy.

1. INTRODUCTION

Mesenchymal Stromal Cells, originally described in the 1960s as bone forming cells in the bone marrow (Friedenstein et al., 1996), are more accurately called Multipotent Mesenchymal Stromal Cells, though they are often named Mesenchymal Stem Cells (MSCs) since they display adult stem cell multipotency. Although they differentiate to bone, cartilage and other connective tissues at the single cell level *in vitro* (Pittenger et al., 1999), debate persists regarding their true multipotential capacity *in vivo*. Unlike hematopoietic stem cells originating from bone marrow, MSCs can be isolated from a variety of other sources including placenta, umbilical cord, adipose tissue, teeth and menstrual fluid (Hass et al., 2011). Their ability to differentiate into classical mesodermal tissues led to an early emphasis on the regenerative potential of MSCs; however, the findings of Bartholomew and colleagues in 2002 (Bartholomew et al., 2002) pointed to new features of these progenitor cells, the consequences of which are presently being elucidated in several areas of medicine; MSCs were found to escape T-cell recognition, suppress T cell response to mitogens and also to prolong skin graft survival in baboons. In spite of this array of effects that were proven subsequently to affect T and B lymphocytes, natural killer (NK) cells and also antigen/presenting cells (Uccelli et al., 2006; Tyndall et al., 2007), MSCs remain immune privileged. Since they exhibit low levels of major histocompatibility (MHC) class I molecules, rarely express cell surface MHC class II and do not express co-stimulatory molecules (CD40, CD40L, CD80, CD86), they escape T cell recognition (Chamberlain et al., 2007). Furthermore, their effects on immunocompetent cells are not MHC restricted, allowing allogeneic MSCs to be used with no need to match with host human leukocyte antigens (HLAs). These properties have provided the basis for the development of "off the shelf" cellular therapy when needed. In this review we analyze data on MSC treatment in immune-mediated diseases with emphasis on systemic lupus erythematosus (SLE), we discuss possible mechanisms of action of MSCs and address some areas of concern regarding stem cell treatment with MSCs.

2. USE OF MSCS IN AUTOIMMUNE AND INFLAMMATORY DISEASES

Given their vast proliferative potential, immunosuppressive properties and the ease of access to available tissue sources, therapies with autologous or allogeneic MSCs have been tested in a variety of immune-mediated disease models, including experimental allergic encephalomyelitis (Rafei et al., 2009; Bai et al., 2009) –a model of multiple sclerosis–, diabetic NOD/scid mice (Lee et al., 2006), collagen induced arthritis (Augello et al., 2007; González et al., 2009), and several lupus murine models (Zhou et al., 2008; Sun et al., 2009; Gu et al., 2010; Yamaza et al., 2010; Youd et al., 2010; Chang et al., 2011; Schena et al., 2010). Results have been mainly encouraging, but not altogether consistent, particularly in the case of arthritis (Schurgers et al., 2010), and lupus mice (Youd et al., 2010; Chang et al, 2011).

At the time of this review, 228 MSC registered human trials were found at the **National Institutes of Health** (NIH) website, including 19 for graft-versus-host disease (GVHD), 18 for diabetes, 11 for Crohn´s disease or ulcerative colitis, 7 for multiple sclerosis, 3 for amyotrophic lateral sclerosis, one each for Sjögren syndrome, rheumatoid arthritis and systemic sclerosis and 3 for SLE (http://clinicaltrials.gov/). Some of these trials point to non immune-mediated conditions associated with tissue injury such as hepatic cirrhosis, myocardial infarction or congestive heart failure. In several of these instances it has become apparent that MSCs are not necessarily replacing diseased tissues or differentiating into separate cell lineages but rather exert a complex pattern of

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trophic, regenerative and antiinflammatory effects, as we discuss later (Block et al., 2009; Chen et al., 2008).

3. CLINICAL TRIALS IN HUMAN DISEASE

Autoimmune disease is one of the top 10 leading causes of death in women up to 64 years of age (Walsh and Rau, 2000) and the second leading cause of chronic illness in the United States (Faustman, 2010). Commonly used immunosuppressant treatments lead to devastating long-term side effects; thus the NIH has recently recognized the need for "*Translation of… knowledge into new, broadly applicable strategies for treatment and prevention of [such] diseases*" (Biennial Report of the Director, NIH). Cell therapy indeed appears to be one of these broadly applicable translational strategies for autoimmune diseases.

A. Graft versus Host Disease (GVHD)

In humans, the most studied application for MSCs is GVHD, a complication of hematopoietic stem cell transplantation in which donor T cells attack an immunocompromised and genetically disparate recipient (English et al., 2010). In 2004, Le Blanc *et al,* treated a 9-year-old boy with severe treatment-resistant acute GVHD of the gut and liver with third party haploidentical mother-derived MSCs (Le Blanc et al., 2004). The clinical response was striking, with improvement of liver and intestinal function. A later phase II clinical study from the same group involved 55 steroid-resistant patients (25 children and 30 adults) with severe acute disease. Treatment with HLA-identical and haploidentical sibling donor bone marrow or third-party mismatched bone marrow MSCs induced a 70% initial response rate that was not related to age or HLA match. None of the patients had side effects either during or immediately after the MSC infusion (Le Blanc et al., 2008). The most recent placebo controlled trials have confirmed the significant improvement in liver and gastrointestinal GVHD, but did not reach significance for durable complete responses or other primary endpoints. (http://investor.osiris.com/releasedetail.cfm?releaseID=407404)

B. Crohn´s Disease (CD)

CD is a disorder of uncertain etiology that can involve the entire gastrointestinal tract with persistent transmural inflammation and fistulization. The first report of a phase I clinical trial of cell therapy using autologous adipose-derived MSCs was published in 2005. Local injection led to healing of fistulas (6/8) with no adverse effects (Garcia-Olmo et al., 2005). These results were confirmed by the same group in 2009 in a phase II multicenter in a randomized controlled trial including 49 patients with complex perianal fistulas (Garcia-Olmo et al., 2009). However, the intravenous infusion of MSCs in CD patients has produced mixed results. Onken et al. reported a clinical response (≥100 point reduction in the Crohn Disease Activity Index) in 3/9 (33.3%) patients, while Duijvestein et al. observed no efficacy in a small group of patients treated with allogeneic Bone Marrow Derived MSCs (BM-MSC) (Onken et al., 2006; Duijvestein et al., 2010). Currently, there is a phase III, multicenter, placebo-controlled, randomized and blind study to evaluate the safety and efficacy of allogeneic BM-MSCs, conducted by Osiris Therapeutics. (http://www.clinicaltrials.gov/ct2/show/NCT00482092).

C. Multiple Sclerosis (MS)

MS is the most common autoimmune inflammatory demyelinating disease of the central nervous system, often resulting in major disability. In the first report of a pilot study injecting autologous MSCs intrathecally, no significant clinical response or adverse events were observed in 10 patients with non-responsive disease, indicating the feasibility of autologous MSC for treatment of MS (Mohyeddin Bonab et al., 2007). Further phase I/II studies involving 10-15 patients each (Yamout et al., 2010; Karussis et al., 2010) confirmed the absence of adverse effects during follow-up (6-28 months). An increase in the proportion of CD4+CD25+ regulatory T cells with decreased proliferative responses of lymphocytes and activation markers on dendritic cells was detected hours after MSC transplantation (Karussis et al., 2010). Connick et al. recently reported a proof-of-concept study including 10 patients with MS treated with an intravenous infusion of autologous MSCs (Connick et al., 2012). Patients improved on measures of visual function, without evidence of significant adverse events. Progression of general disability was also reduced after treatment. The reproducibility and clinical significance of these findings remains to be confirmed. An international MSC Transplant Study Group (MSCT) has recently derived guidelines on the utilization of MSCs in MS, along with protocols for the culture of the cells and the treatment of patients (Freedmann et al., 2010)

D. Systemic Lupus Erythematosus (SLE)

Perhaps the most remarkable results of human MSC therapy emerge now from clinical trials aimed at severe, treatment-refractory SLE (Sun et al., 2010; Liang et al., 2010). While these are still uncontrolled surveys, the recent report of successful MSC treatment for other renal conditions akin to the SLE spectrum (Tögel and Westenfelder et al., 2010, Lee et al., 2010) lend support to these notoriously favorable outcomes. Taken together, these results highlight the need to advance the clinical science of stem cell therapy, identifying specific mechanisms of action and also promoting the development of safe but accessible controlled clinical protocols (Singer and Caplan, 2011).

Prompted by the positive results in the Fas-deficient MRL/lpr mice treated with human MSCs from healthy individuals (Zhou et al., 2008), Sun et al. treated four patients with active disease and lupus nephritis that was unresponsive to monthly i.v. cyclophosphamide and oral prednisone (≥ 20 mg/day) (Sun et al., 2009). The Disease Activity Index (SLEDAI) improved significantly at one, six and twelve months follow-up, as did urinary protein. CD4+ Foxp3 (T regulatory) cells increased at 3 months follow-up, and treatments were tapered and even suspended in two patients. None had complications after 12-18 months follow-up. These encouraging results led to a larger phase I trial in 15 patients also with refractory disease, including the first 4 cases reported. In this trial one third of the patients had previously failed oral mycophenolate mophetil (1-2 gr/day x 3 months) (Liang et al., 2010). Non-renal manifestations were prominent, including arthritis, severe skin disease, serositis and non-responsive cytopenias. Patients received one infusion of allogeneic BM-MSCs from passage 3-5 from non HLA matched healthy family members. Clinical and serological changes were quite dramatic for

those patients with truly severe disease as gauged by a high baseline SLEDAI, in spite of treatment with glucocorticoids and immunesuppressants. Follow-up reached 17.2 (3-36) months, with no adverse effects, deaths or ensuing GVHD. Quite surprisingly, 24 h proteinuria decreased significantly as early as one week after MSC therapy, even preceding changes in anti-dsDNA antibodies, which decreased significantly at one month and three months post transplant. T regulatory (Treg) cells, found to be quantitatively and qualitatively deficient in active SLE, (La Cava, 2008; Valencia et al., 2007), were restored at week one as judged by the percentage of CD4+ Foxp3+ cells among peripheral blood mononuclear cells.

A second trial from this group in Nanjing, China followed, reporting the use of umbilical cord-derived MSCs (UC-MSCs) also in severe lupus patients (n=16) (Sun et al., 2010). This time 5 of 15 renal cases had histological confirmation of proliferative nephritis, and 11 were preconditioned with cyclophosphamide prior to MSC infusion. Cords for MSC culture were derived from normal deliveries, minced and cultured with 10% bovine serum through passages 2-5 before use. Follow-up was only 8.25 months, but significant improvement was verified for SLEDAI score, serum albumin, 24 h urinary protein, serum creatinine, serum complement and anti-dsDNA antibodies. A decrease in serum IL-4 (with a nonsignificant increase of IFN-γ) was interpreted by the authors as a hint of improvement of pathogenic Th2 imbalance, though animal lupus models have shown rather the opposite cytokine change (Chang et al., 2011). These trials, although with shorter follow-up (8-17 months) seem to compare favorably with hematopoietic stem cell transplants in SLE that still exhibit 4-12% mortality (Burt et al.,2006; Jayne et al., 2004). Undoubtedly MSC therapy must be further explored in SLE. The EULAR Stromal Cell Group is now conducting a prospective, double-blind, comparative, multicenter trial of renal lupus treated with allogeneic MSCs (Tyndall, 2011).

More than 50 years ago Dr. Paul Klemperer suggested that the histopathological connective tissue changes found in SLE were common to *"connective tissue or collagen diseases"* (Klemperer, 1962). Little did he know that a cure for such diseases might be found within connective tissues!

E. Systemic Sclerosis (SS)

SS is an immune mediated disease with a prominent vascular and microvascular component often leading to ischemic complications (Guiducci et al., 2007) Since MSCs can differentiate to endothelial cells *in vitro* and also participate in blood vessel formation in adult tissues (Martens et al., 2006), therapy both with autologous and haploidentical third party donor MSCs has been reported, leading to striking improvement in two separate case reports (Christopheit et al., 2008; Guiducci et al., 2010). In a most interesting investigation by Akiyama, transplantation of allogeneic MSCs in 5 patients with SS triggered the induction of T cell apoptosis, lymphopenia and Treg induction, leading to skin ulcer healing in one case, and significant improvement in the Skin Score, Health Assessment Questionnaire and autoantibody titer in the whole group. In this report MSCs from SS patients were found to be deficient in the expression of FAS and FAS-L, the main molecules mediating the immunoregulatory effects described by the authors (Akiyama et. al., 2012).

4. MECHANISMS OF THE THERAPEUTIC EFFECT OF MSC TREATMENT

Despite the *in vitro* and *in vivo* evidence for therapeutic effects of MSCs, the mechanisms by which MSCs exert their immunomodulatory and reparative effects are still incompletely understood, but most likely involve multiple pathways (Figure 1).

4.1. Proinflammatory "licensing" of MSCs

In contrast to therapies that cause global immune suppression, MSCs have been dubbed as ''smart" immune modulators since their suppressive effects require a previous *licensing* step that occurs in the presence of an inflammatory environment and is mediated by the secretion of specific cytokines (Jones et al., 2007, Jorgensen, 2010). Thus, IFN-γ, alone or together with tumor necrosis factor (TNF)-α, IL-1α or IL-1β, are required to trigger the expression by MSCs of high levels of soluble factors involved in immunosuppression such as IDO, HGF, TGF-β, and NO (Aggarwal and Pittenger, 2005; Ryan et al., 2007; Krampera et al., 2006, Ren et al., 2008). The need for this activation step has been confirmed in a model of GVHD, since recipients of IFNγ−/− T cells did not respond to MSC treatment, evolving into fatal GVHD (Polchert et al., 2008). Others have attributed the immunomodulatory function of MSCs mainly to IL-6-dependent secretion of prostaglandin E2 (PGE2) (Boufii et al., 2010).

4.2. Tipping of the Th1/Th2 balance

Although still controversial, an imbalance in IFN-γ and IL-4 cytokine levels suggestive of a pathogenic T helper 2 (Th2) response has been reported in SLE. Accordingly, experimental data suggests that MSC therapy might ameliorate disease by promoting the conversion from a Th2 humoral response to a Th1 cellular immune response through modulation of IL-4 and IFN-γ levels in effector T cells. Zhou et al. showed that intraperitoneal infusion of human BM-MSCs in MRL/lpr mice decreased production of IL-4 and increased IFN-γ in peripheral blood T cells (Zhou et al., 2008). Sun et al. reported similar findings with UC-MSCs transplantation in patients with refractory SLE 3 months after treatment, also suggesting a polarization toward the Th1 phenotype, that was associated with clinical improvement (Sun et al., 2010). In gastrointestinal models of disease, pretreatment of MSCs with IFN-γ markedly inhibited dextran sodium sulfate (DSS) induced colitis in mice, leading to improvement of body weight, colitis scores and better survival rates compared to untreated mice (Duijvestein et al., 2011). Recent studies have suggested that Toll Like Receptor (TLR) signaling regulates the proliferation, differentiation and immune function of MSCs. Waterman et al. provided evidence that human MSCs polarize into distinct phenotypes following specific TLR-activation. TLR3-priming would favor an immunosuppressive (MSC2) phenotype, expressing CCL10, CCL5 and to a lesser degree IL-4 and IL-10, while TLR4-priming triggered a pro-inflammatory phenotype (MSC1), secreting IL-6 and IL-8 and even reversing MSC-established suppressive effects (Waterman et al., 2010). The *in vivo* effects of these polarized MSCs in autoimmune or proinflammatory diseases remain to be clarified.

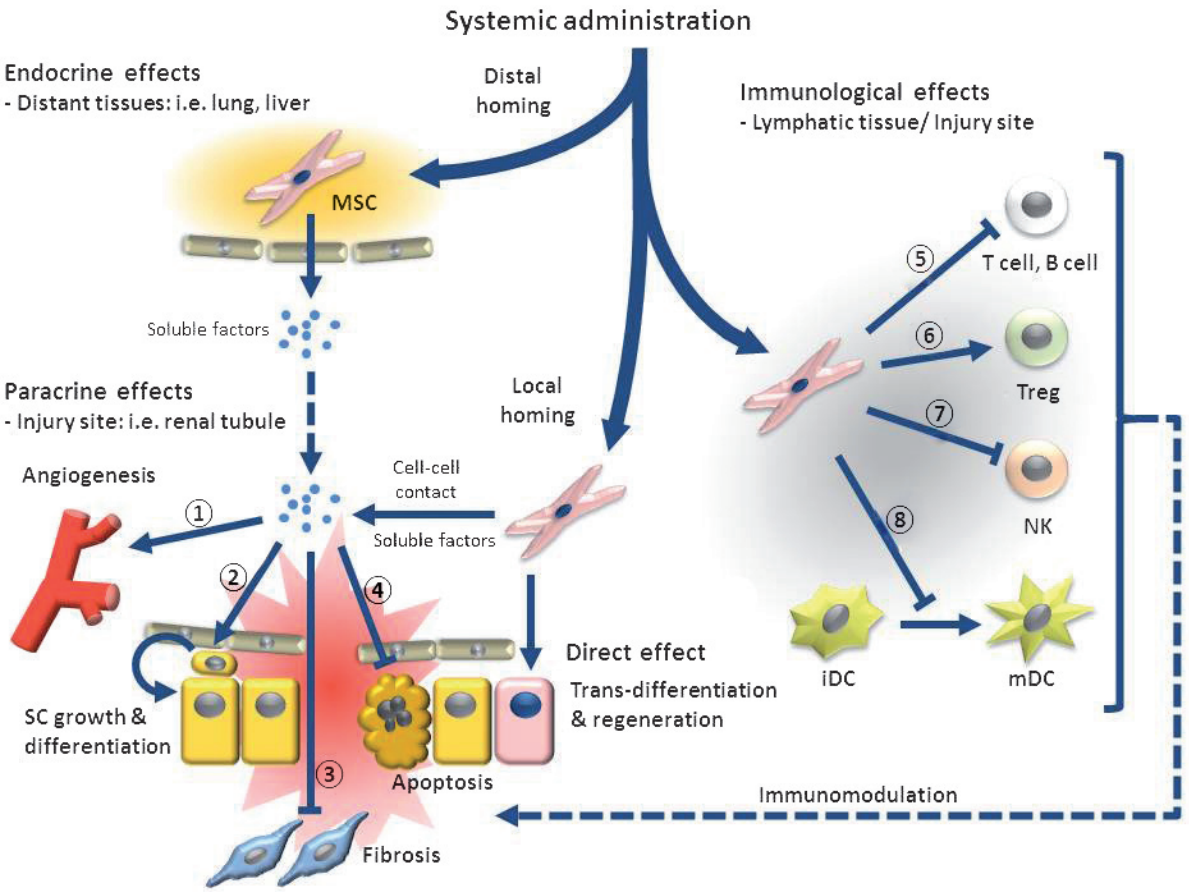

Figure 1. Systemic administration of mesenchymal stem cells can: trigger distal (endocrine) or local (paracrine) effects that include cellmediated actions. 1) Promotion of angiogenesis: vascular endothelial growth factor (VEGF), insulin like growth factor 1 (IGF-1), monocyte chemoatractant protein 1 (MCP1), basic fibroblast growth factor (bFGF) and interleukin 6 (IL6). **2)** Stem cell growth and differentiation: stem cell factor (SCF), leukemiainhibitory factor (LIF), macrophage colonystimulating factor (MCSF), stromal derived factor 1 (SDF1), angiopoietin1 and activin A. **3)** Inhibition of fibrosis: hepatocyte growth factor (HGF), bFGF, adrenomedullin (ADM). **4)** Inhibition of apoptosis: VEGF, HGF, IGF1, transforming growth factor (TGF)β, bFGF, granulocyte macrophage colonystimulating factor (GMCSF), activin A and thrombospondin1. Immune mediated effects include the following (5 to 8). **5)** Suppression of T and B cells: human leukocyte antigen G5 (HLAG5), HGF, inducible nitric oxide synthase (iNOS), indoleamine2,3dioxygenase (IDO), prostaglandin E2 (PGE2), bFGF and TGFβ. **6)** Induction of regulatory T cells (Treg) differentiation and expansion by TGFβ expression. **7)** Inhibition of natural killer (NK) cells by secretion of IDO, PGE2 and TGFβ. 8) Inhibition of dendritic cell (DC) maturation by secretion of PGE2. Figure reproduced from Carrión and Figueroa, Stem Cell Res Ther 2011 May 11;2(3):23.

4.3. Effects on CD4 + T cell populations: Treg/Th17 ratio

Several studies have reported a quantitative and/or qualitative defect of Treg cells in human SLE, as well as increased production of Th17 proinflammatory cells (La Cava, 2008; Valencia et al., 2007; Crispin and Tsokos, 2010). MSCs have also been shown to induce the generation of functional Tregs both *in vitro* and *in vivo* (González et al., 2009; Prevosto et al., 2007; Gonzalez-Rey et al., 2010). In MLR/lpr mice, transplantation of MSCs from many sources (bone marrow, umbilical cord or exfoliated deciduous teeth), can restore Treg cells and induce a significant reduction in Th17 levels, consequently up-regulating the ratio of Treg/Th17 cells (Sun et al., 2009; Gu et al., 2010; Yamaza wet al., 2010). Recently we have shown that MSCs also generate functional active CD4+CD25+Foxp3+ T-regulatory cells during the *in vitro* differentiation phase of Th1 and Th17 cells (Luz-Crawford 2012). In human SLE the transplantation of either allogeneic or autologous MSC derived from bone marrow or UC has also increased Treg cells, suggesting that this may be one of the mechanisms of the MSC-mediated improvement of disease (Sun et al., 2009; Sun et al., 2010; Liang et al., 2010). However in two patients with active, but not highly inflammatory SLE, we reported that the infusion of autologous MSCs induced no amelioration, in spite of generating a marked increase in Treg cells (Carrión et al., 2010). In experimental autoimmune encephalomyelitis (EAE), considered a model of human MS, the administration of *ex vivo* culture-expanded MSCs has been shown to reverse neuroinflammation (Rafei et al., 2009). The effect seems to be dependent on the MSC-driven proteolytic processing of CC Chemokine Ligand 2 (CCL2) to an antagonistic derivative that interferes with CD4 Th17 cell function, thus suggesting that the therapeutic effects of MSCs in EAE occur via the paracrine conversion of CCL2 from agonist to antagonist of inflammatory cell recruitment (Rafei et al., 2009).

4.4. MSC homing and survival

One of the most relevant ongoing debates in the field of cell therapy is whether the engraftment of MSCs at the target site of injured tissues is mandatory, or can be replaced by systemic or paracrine effects. Local delivery and homing of cells toward the injury site is beneficial due to the cell-to-cell interaction with host tissues, accompanied by an increased concentration of the secreted trophic factors. However, in some preclinical models of disease, cell homing to the damaged tissue (i.e. infarcted heart or kidney) following systemic infusion remained largely inefficient. This is mainly due to the limited homing capacity of MSCs, and their entrapment in the microvasculature and other organs such as the liver and lungs (Karp and Leng Teo, 2009). Moreover, the migration of the cells was shown to be negatively affected following their *ex vivo* expansion, probably because of a lower expression of migratory and adhesion ligands such as CXCR4 and CCR1; a genetic modification of MSCs to overexpress CXCR4 was necessary to re-establish their homing properties (Cheng et al., 2008).

Long-term engraftment is another hallmark for showing the beneficial effect of stem cell-based therapies. The long-term persistence of autologous or allogeneic MSCs after a single intravenous infusion has been described in baboons, with levels of tissue engraftment ranging from 0.1% to 2.7% (Devine et al., 2003). Long-term engraftment of MSCs that differentiated to form myogenic cells in dogs with Duchenne muscular dystrophy has been recently reported (Nitahara-Kasahara et al., 2012). In NZB/W F1 lupus mice treated with $1x10^6$ human UC-MSCs via the tail vein, Chang and colleagues (Chang et al., 2011) found evidence of MSCs in kidney tissues at week 2 of infusion, but no long-term engraftment. Even if MSCs protect and improve recovery from several models of acute and chronic myocardial and renal injury (Humphreys and Bonventre, 2008; Choi et al., 2009), paracrine and endocrine effects seem most important, since conditioned medium from MSCs has been able to mimic the beneficial effects of stem cell therapy (Bi et al., 2007).

4.5. MSC paracrine factors in the repair mechanism

Cellular regeneration of an ischemic tissue necessitates massive cell supply, on the order of a billion for an infarcted heart, for example (Laflamme and Murry, 2005). Experimental studies and clinical trials have revealed that MSC-mediated therapeutic benefit might largely rely on the contribution of the secreted amounts of growth factors and cytokines rather than on their potential for differentiation into cardiomyocytes, vascular or renal cells (Bi et al., 2007). The panel of regulatory and trophic factors secreted by MSCs include a large number of growth factors, cytokines and chemokines. This MSC secretome was shown to be responsive to stress, including physiological changes (hypoxia or anoxia), small molecule stimulation and cytokine treatments (Kamota et al., 2009). Despite the absence of *in vivo* profiling of the MSC secretome and its response to disease, current MSC-based therapies have shown results largely related to a paracrine effect. Nguyen et al. showed that the injection of MSC-derived factors (MDFs) achieved protection by paracrine effects rather than direct cardiac regeneration in a swine model of myocardial infarction (Nguyen et al., 2010). The array of potential therapeutic mechanisms offered by MDFs includes antiapoptotic (Shabbir et al., 2009), anti-inflammatory (Bartosh et al., 2010), antifibrosis (Mirotsou et al., 2011), angiogenic (Kinnaird et al, 2004) and also regenerative effects. The intricacy of such factors *in vivo* has been also illustrated by Lee et al. in a model in which the reduced size of myocardial infarction in response to the infusion of human MSCs was due to the secretion of the anti-inflammatory protein (TSG-6), triggered by the entrapment of MSCs in the lung (Lee et al., 2009).

MDFs also appear to contribute to improved function and renal repair in response to MSCs (Tögel and Westenfelder, 2010). In an effort to address the mechanisms involved in the amelioration of renal disease induced by MSCs, we recently evaluated several functional and molecular markers of kidney damage and regeneration in rats subjected to 5/6 nephrectomy (NPX) (Villanueva et al., 2011). In this well known model of chronic kidney disease, a single intravenous infusion of 0.5×10^6 MSCs was associated with significant reduction of serum creatinine and inflammatory markers including macrophage infiltration and interstitial α-SMA (α-smooth muscle actin). Treated rats exhibited a significant induction in epitheliogenic molecules [Pax-2, bFGF (basic fibroblast growth factor) and BMP-7 (bone morphogenetic protein-7)], and increased expression of transcription factors Tie-2 and VEGF –involved in angiogenesis–, with respect to sham operated animals or NPX animals treated only with culture medium (Figure 2). These results are in agreement with *in vitro* studies (Tögel and Westenfelder, 2010), suggesting that there is a pathway related to vascular protection induced by MSC.

Finally, the importance of epigenetic regulatory factors in the control of biological processes and in the immune response has also been stressed. Common miRNA patterns of expression have been found in three different murine models of SLE (Dai et al., 2010), suggesting these might be targeted therapeutically. Since MSCs have been shown to secrete microparticles enrichened in miRNAs (Chen et al., 2010), several authors have suggested that microvesicle-mediated transfer of mRNA from MSC to target tissues might also participate in some of the processes involved in immunoregulation or in the recovery from kidney injury in response to stem cell treatment (Camussi et al., 2010).

5. CONCLUSIONS

A wealth of information has now accumulated linking the biology of MSCs to beneficial effects in a number of animal and human diseases. Even if the *in vivo* role of endogenous MSCs remains speculative, harnessing the therapeutic effects of *ex vivo* expanded MSCs for tissue repair and regeneration seems to have a significant clinical potential. Initial hypotheses centered on the role of the differentiation of MSCs into healthy cells and tissues have led to a wider view of MSC mediated effects, including immune modulation, paracrine and endocrine effects and even genetic regulatory mechanisms. In the midst of these scientific developments, the results of the first clinical trials with MSC therapy are undoubtedly encouraging in some cases. However, the heterogeneity of MSCs as defined today and the intricate circuitry of cellular, humoral and regenerative factors that mediate their presently known effects still point to many issues to be resolved. Long term safety concerns remain an issue, given the description of *in-vitro* malignant MSC transformation (Miura et al., 2006)

and the unknown interaction of regular immunosupressants with single or repeated MSC therapy (Spaeth et al., 2009). Regulatory and technical conditions must be defined, allowing the development of better clinical surveys. Along with the need for larger randomized controlled clinical trials, future advances from stem cell science can be expected to pinpoint significant MSC subpopulations and/or stem cell markers for regenerative or immunoregulatory properties, as well as new mechanisms of action (Psaltis et al., 2010). Thus assays for *in vitro* or *in vivo* MSC potency could be developed, leading to the use of more potent stimulated or primed pretreated MSCs. This is an exciting era for the development of safe and effective regenerative therapies, but special efforts will be required to build both the basic and clinical foundation for stem cell applications.

LIST OF ABBREVIATIONS

bFGF, basic fibroblast growth factor; BM-MSCs, bone marrow-derived mesenchymal stem cells; BMP-7, Bone morphogenetic protein 7; CD40L, CD40 ligand; dsDNA, double-stranded DNA; EGF, epidermal growth factor; GVHD, graft versus host disease; HGF, hepatocyte growth factor; HLA, human leukocyte antigen; HLA-G, human leukocyte antigen G; HRCT, high resolution computed tomography; i.v., intravenous; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; IL-1RA, Interleukin 1 receptor antagonist; MHC, major histocompatibility complex; MSC, mesenchymal stem cell; NK, natural killer cells; NO, nitric oxide; NPX, nephrectomized; Pax-2, paired box protein Pax-2; PGE-2, prostaglandin E2; SDF-1, stromal cell-derived factor-1; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus disease activity index; Th2, T helper 2; Tie-2, angiopoietin-1 receptor; TNF, tumor necrosis factor; Treg, T regulatory cells; UC-MSCs, umbilical cord-derived mesenchymal stem cells; VEGF, vascular endothelial growth factor.

COMPETING INTEREST

The authors declare that they have no competing interests. MK holds a consulting position with "Cells for Cells" S.A.

AUTHORS' CONTRIBUTIONS

All authors contributed to the writing of the manuscript and read and approved the final version.

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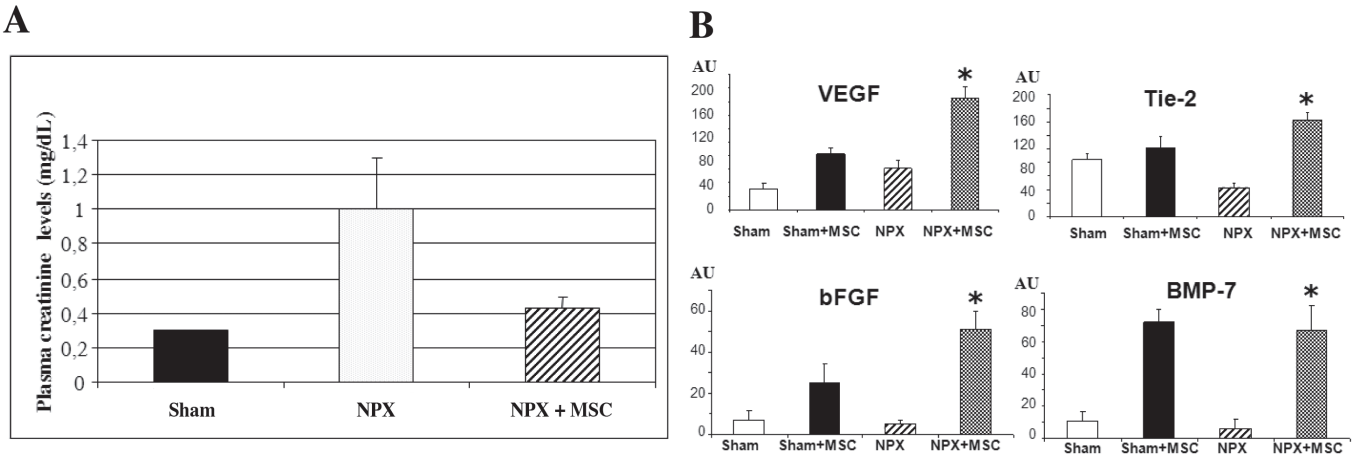

Figure 2. Functional renal damage: A. Renal function was assessed by plasma creatinine levels in sham animals (Sham) infused with fresh XVivo medium, 5/6 nephrectomized (NPX) rats infused with fresh XVivo medium and NPX injected with MSC (NPX+MSC). Sham rats injected with XVivo had normal plasma creatinine levels (0.3 mg/dL). In NPX animals, creatinine levels increased to 1.0±0.3 mg/dL which was significantly higher than both Sham groups ($p<0.05$). In rats submitted to NPX+MSC, creatinine level was reduced to 0.4±0.1 mg/dL, ($p<0.05$ *vs*. NPX group). Furthermore, creatinine values of the NPX+MSC were not significantly different from those in Sham animals.
B. Five weeks after nephrectomy, the presence of transcriptional factors involved in angiogenesis, VEGF, Tie2, and epithelial markers bFGF, BMP7 were analyzed. VEGF and Tie2 levels were minimal in Sham (31±7, 84±9 Arbitrary Units (AU) respectively) and NPX animals (61±12, 43±6 AU respectively) measured 35 days after damage. However, Sham+MSC had augmented angiogenic proteins (VEGF: 83±8, Tie2: 102±16 AU). All markers were elevated in NPX+MSC animals (VEGF: 165±16, Tie2: 142±11 AU). The differences between NPX and NPX+MSC were significant ($p<0.05$). On the other hand, a significant difference was found in epithelial markers among controls and MSC kidneys treated. The expression of bFGF and BMP7 in Sham kidneys was scarce, whereas in NPX animals the expression level was even lower (8±4 and 9±3 AU respectively). However, in Sham+MSC an increased expression of epitheliogenic markers was observed (bFGF: 25±3, BMP7: 68±13 AU) that was higher in NPX+MSC rats (bFGF: 52±7, BMP7: 67±15 AU). These differences were significant ($p<0.05$). The expression of alpha tubulin was used to correct for variation in sample loading.

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