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Role of mesenchymal stromal cells in solid organ transplantation

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Abstract

Mesenchymal stromal/stem cells (MSCs) originally isolated from bone marrow (BM) have been derived from almost every tissue in the body. These multipotent cells can be differentiated in vitro and in vivo into various cell types of mesenchymal origin, such as bone, fat, and cartilage. Furthermore, under some experimental conditions these cells can differentiate into a wider variety of cell types. Upon systemic administration ex vivo expanded MSCs preferentially home to damaged tissues and participate in regeneration processes through their diverse biological properties. In vitro and in vivo data suggest that MSCs have low inherent immunogenicity and modulate/suppress immunological responses through interactions with different immune cells. Ease of isolation and ex vivo expansion of MSCs, combined with their intriguing differentiation and immunomodulatory potential, and their impressive record of safety in clinical trials make these cells prime candidates for cellular therapy. MSCs derived from BM are currently being evaluated for a wide range of clinical applications including for treatment of immune dysregulation disorders such as acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. In the future, MSCs might potentially provide novel therapeutic options for treatment/prevention of rejection and/or repair of organ allografts through their multifaceted properties.

Keywords

mesenchymal stromal cells; mesenchymal stem cells; solid organ transplantation; immunomodulation

1. Introduction

Bone marrow (BM) stromal cells were first described by Friedenstein and colleagues, who identified an adherent, fibroblast-like population in the adult BM capable of regenerating rudiments of bone in vivo (1). These cells were later shown to be capable of differentiating into other cells of mesenchymal lineage such as fat and cartilage. Additionally, it is believed these cells produce matrix elements of the stromal tissue to provide physical support, give rise to the cellular elements of hematopoietic microenvironmental niche, and secrete a variety of cytokines and growth factors to support HSC maintenance and differentiation in the BM (2, 3). Caplan was the first to use the term mesenchymal stem cells (MSCs) (4) to reflect the multipotent capabilities of this population of BM resident cells and estimated that MSCs

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compromise 0.01% to 0.1% of total adult BM cells (5). However, three decades after their original description still there is no single definitive marker for their direct isolation from BM, and their exact anatomical location inside BM and true physiological role is not clear. It is also not clear if MSCs per se or some of their derivatives comprise the HSC cellular niche in BM. Based on recent data, osteoblasts (6,7) which originate from MSCs, may play a major, although not exclusive (8), role as the true HSC niche.

MSCs are most commonly isolated by gradient centrifugation of BM aspirates to isolate mononuclear cells, followed by in vitro culture and serial passage. Although MSC preparations generated ex vivo appear homogenous under the light microscope, they probably comprise a heterogeneous group of progenitor cells, and on most occasions do not fulfill strict criteria for a stem-cell entity at a single cell level (i.e. self renewal and multilineage differentiation capacity). Thus, the term multipotent mesenchymal stromal cells (with the same acronym as MSCs) was recently proposed (9) as the preferred terminology. The criteria set forth for MSCs include adherence to plastic in standard culture conditions, and a combination of phenotypic and functional characteristics. Culture-expanded MSCs are considered to be negative for hematopoietic markers such as CD14, CD45, and CD34; although some strains of mice express CD34 antigen on a portion of their MSCs (10). On the other hand, MSCs are generally, but not homogeneously, positive for a number of cell surface molecules, including CD73 (SH3 or SH4), CD90, CD105 (SH2 or endoglin), CD44, CD29, and CD166. Finally, the biologic property that most uniquely identifies MSCs is their capacity for tri-lineage differentiation potential in vitro into bone, fat, and cartilage upon addition of proper exogenous growth factors (11). Further characterization of these cells has shown they possess a spectrum of matrix receptors and integrins, and secrete a variety of growth factors, chemokines, cytokines and extracellular matrix proteins (11,12). However, the physiological correlation between ex vivo generated MSCs and their in vivo counterparts still remains unclear (13).

Despite the large amount of unknown information about MSC biology, MSCs have generated a lot of excitement in the field of regenerative medicine over the last decade. Enthusiasm about these cells was originally fueled by a flurry of studies suggesting MSCs not only differentiate into other types of cells of mesodermal lineage, but also into cells of endodermal and ectodermal lineages, including cardiomyocytes (14–16), endothelial cells (17), lung epithelial cells (18–21), hepatocytes (22–24), neurons (25–28), and pancreatic islets (29–32). Liechty et al. showed that intraperitoneal injection of adult human BM derived MSCs using an in utero sheep transplantation model results in engraftment in multiple organs with site-specific differentiation (33). However, this study did not show regeneration of different cell types by transplantation of MSCs at a single stem cell level. The presence of MSCs at injury sites and their presumed transdifferentiation potential provided a rationale for testing their use in a variety of injury models (12,34,35). Nevertheless, the degree of contribution to different tissues through trans-differentiation is now a matter of strong debate since many later studies could not duplicate the results of original reports on transdifferentiation potential of these cells (36–39). However, MSCs remain a very promising modality in cell therapy and continue to be tested in many different applications as new functional mechanisms for these cells are discovered.

While in the normal undisturbed host BM may be the preferred homing site for intravenously administered MSCs (40,41), MSCs can be detected in low levels in numerous tissues following intravenous infusion (42,43). Furthermore, in the presence of inflammation or injury MSCs may preferentially home to the inflammatory site (44,45), probably via the SDF1/CXCR4 pathway (45–48). The combined ability of these cells to migrate to the site of injury/inflammation, stimulate proliferation and differentiation of resident progenitor cells, and promote recovery of injured cells through growth factor secretion and matrix remodeling keeps these cells in the forefront of regenerative medicine (49). Finally, exploitation of these cells in

different clinical settings has been greatly facilitated by the emerging body of evidence that MSCs are immunoprivileged and, more importantly, possess immunosuppressive and other favorable immunomodulatory properties (50,51).

Pluripotent MSCs have also been derived from BM using modifications in the culturing process and have been labeled with a variety of names such as multipotent adult progenitor cells (MAPCs) (52), marrow-isolated adult multilineage inducible cells (MIAMIs) (53), very small embryonic-like cells (VSEL) (54) and pre-mesenchymal stem cells (pre-MSC) (55) among others. These potentially more primitive types of MSCs require specific and stringent culture conditions such as prolonged culture duration at low cell density on matrix coated culture dishes, and use of certain types of medium incorporating pre-tested fetal calf serum and growth factors. Due to the complex nature of these isolation methodologies it is not known if such cells have any in vivo counterparts, especially in humans, or if they merely represent artefactual byproducts of the culture system. Thus, it is not clear if these MSC variants will reach the clinic anytime soon.

Although MSCs were originally isolated from BM, similar populations have been isolated from a wide variety of other adult tissues including, but not limited to, adipose tissue (56), skeletal muscle (57) synovium (58), and dental pulp (59); from neonatal tissues such as placenta (60), amniotic fluid (61), and umbilical cord blood (62); and from fetal lung, liver, and blood (63). It appears that MSCs essentially reside within connective tissue of most organs (64).

Furthermore, many studies suggest that MSCs isolated from these diverse tissues possess similar biological characteristics, differentiation potential, and immunological properties (65–67). Although adipose tissue derived MSCs are reported to be an easily obtainable source of MSCs, and even have been used in a few small clinical trials (68–70), the bulk of the published literature concerns BM-derived MSCs as they are the best characterized and most advanced in clinical use. Additionally, their ease of generation in both autologous and allogeneic settings, extensive expansion capacity, and stable phenotype and function over many passages allows enough clinically usable MSCs to be generated from a small amount of BM aspirate over a period of several weeks. Thus, in this article we will focus primarily on the immunologic properties of BM-derived MSCs and their potentially beneficial effect in solid organ transplantation. However, other favorable characteristics of these cells including tissue remodeling/repair properties, growth factor release, angiogenesis, and nonspecific anti-inflammatory properties could all potentially play a role (Figure-1).

2. Immunomodulatory properties of MSCs

One of the most intriguing properties of ex vivo expanded MSCs is their ability to affect the immune response in vitro and in vivo. The emerging body of data indicates that MSCs derived from BM and other tissues modulate the immune system through interaction with a broad range of immune cells including T-lymphocytes, B-lymphocytes, natural killer cells, and dendritic cells (71–74). These immunomodulatory properties of MSCs have been the basis for their use in treating conditions characterized by immunological dysregulation such as Crohn's disease and graft versus-host-disease (GVHD) after allogeneic hematopoietic stem cell (HSC) transplantation. By extrapolation, the same immunomodulatory properties might be potentially useful for prevention or treatment for solid organ transplant rejection. However, there are many discrepancies and contradictions between reported immunological aspects of MSCs. These differences may be due to biological differences between MSCs isolated from different species or tissues, variables involved in culture conditions, or experimental methods.

Human MSCs express human leukocyte antigen (HLA) class I on their cell surface but not HLA class II; however, Western blotting of cell lysates demonstrates intracellular HLA class II molecule expression (75). Treatment with interferon-gamma (IFN- γ), a pro-inflammatory

cytokine known to increase cell surface expression of HLA molecules, increases expression of both cell surface HLA class I and class II molecules. Under normal conditions, the immune system will reject HLA-expressing allogeneic cells also expressing appropriate co-stimulatory molecules. Human MSCs do not express co-stimulatory molecules CD80, CD86, or CD40, even after IFN- γ stimulation. However, even transfection with CD80 and CD86 costimulatory molecules (76) or engagement of CD28 by anti-CD28 antibody (77) fail to allow human MSCs to induce proliferation of allogeneic lymphocytes in vitro in co-culture experiments.

2.1. MSCs and T-lymphocytes

T-lymphocytes are major executors of the adaptive immune response. A variety of in vitro studies on MSCs of human (77–79), nonhuman primate (80), and murine (81) origin have shown that MSCs possess the ability to suppress activation and proliferation of T-lymphocytes induced by both cellular and nonspecific mitogenic stimuli and have no immunological restriction (82). Similar suppression of T cell proliferation by MSCs is observed with cells that are either autologous or allogeneic to the responder cells in mixed lymphocyte reaction (MLR) assays. However, it has been suggested that at lower ratios MSCs might enhance proliferation of T-lymphocytes in MLR assays (75). Some studies have shown nonresponsiveness of T-lymphocytes in the presence of MSCs could be restored by removal of MSCs (78), while other studies variously indicated these cells induce anergy due to divisional arrest in T cells (83), induce T-cell tolerance (84), or induce regulatory T-lymphocytes to modulate immune responses. An increase in the population of CD4⁺CD25⁺ regulatory T-lymphocytes was demonstrated in mitogen-stimulated peripheral blood mononuclear cell cultures in the presence of MSC (85). Despite this, Krampere et al. demonstrated presence of CD4⁺/CD25⁺ regulatory T-lymphocytes was not required for MSCs to inhibit T-lymphocyte proliferation (81).

Evidence for involvement of soluble factors in inhibition of proliferation of T-lymphocytes comes from transwell experiments in which MSCs and effector cells are separated by semi permeable membranes. Transforming Growth Factor-beta (TGF- β), hepatocyte growth factor (HGF), prostaglandin E2 (PGE2), and tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) have been reported to mediate suppression of T-lymphocyte proliferation; although occasionally these findings are contradictory to each other (86–88). It has also been suggested that suppressive factor(s) are not constitutively secreted by MSCs, but are secreted during MSCs co-culture with lymphocytes (89).

Although many have interpreted these in vitro data as indicating immunosuppression by MSCs, others have suggested that this presumed “immunosuppressive” property of MSCs might be a nonspecific result of a more general anti-proliferative effect of MSCs (73) and may be a property shared by all stromal cells (90). The relevance of these interactions observed in vitro to actual physiologic or pathologic conditions in vivo remains to be determined.

2.2. MSCs and B-lymphocytes

Although the mechanisms involved are not yet fully understood, both murine (91) and human studies (92) have shown that MSCs inhibit proliferation of B-lymphocytes stimulated by various methods. Intriguingly, based on Corcione et al. study, this inhibition is dose dependent; more MSCs lead to less inhibition. This contrasts with inhibition of T-cell proliferation, where more MSCs lead to greater inhibition of T-cell proliferation. Additionally, MSCs affect differentiation, antibody production, and chemotactic behavior of B cells (92). Transwell experiments again indicate that soluble factors released by MSCs play a major role in inhibiting proliferation of B cells (92).

2.3. MSCs and dendritic cells

Dendritic cells (DCs) constitute a heterogeneous population of professional, multiple lineage-derived antigen-presenting cells (APCs) from BM (93). Depending on their stages of cell development, activation and maturation, DCs have potential to induce both immunity and tolerance (94). MSCs interact with DCs at different levels of differentiation, maturation and function. MSCs inhibit differentiation of both CD14⁺ monocytes and CD34⁺ hematopoietic progenitors into DCs in a dose dependent fashion; and the differentiated DCs are less mature and exhibit less capability to stimulate allogeneic T-lymphocyte proliferation (95,96). Studies have also shown that in the presence of MSCs, monocyte-derived DCs change from proinflammatory cytokine production to anti-inflammatory cytokine production (95,97). Some transwell studies indicated MSCs suppression of DC differentiation might be partially mediated by soluble factors such as interleukin-6 (IL-6) and Macrophage colony-stimulating factor (MCSF) (96), or even PGE2. Taken together, allogeneic MSCs may potentially induce a state of tolerance by modulating the generation, activation, and function of DCs.

2.4. MSCs and natural killer cells

Natural killer (NK) cells are a subset of lymphocytes participating in innate immunity, but having potential to coordinate sophisticated immune responses, and determine the outcome of adaptive immune responses (98,99). Sotiropoulou demonstrated that at low NK:MSC ratios, MSCs alter NK cell phenotype and suppress proliferation, cytokine secretion, and cytotoxicity against HLA class I expressing targets (100). They concluded that some of these functions require cell-to-cell contact, whereas others are mediated by soluble factors, including TGF- β 1 and PGE2. Still, although MSCs express HLA class I and should be protected against NK mediated killing, they were susceptible to lysis by activated NK cells, but not by freshly isolated NK cells. Spaggiari et al. showed that MSCs sharply inhibit IL-2-induced proliferation of resting NK cells; and they showed that IL-2-activated NK cells (but not by freshly isolated NK cells) efficiently lyse autologous and allogeneic MSCs. (101). In a more recent study, the same authors also showed that MSCs inhibit cytokine-induced proliferation of freshly isolated NK cells, and prevent their cytotoxic activity and cytokine production. They concluded that IDO and PGE2 represent key mediators of MSC-induced inhibition of NK cells(102).

2.5. MSCs as antigen presenting cells

Since many studies have shown that human MSCs are unable to induce proliferation of allogeneic lymphocytes in MLR assays, it is thought that MSCs are poor antigen presenting cells (APCs). However, Stagg et al. reported that mouse or human MSCs behave as a novel subset of nonhematopoietic conditional APCs, and provided experimental evidence that IFN- γ -treated MSCs process exogenous antigens and efficiently activate in vitro and in vivo antigen-specific immune responses (103). Chan et al. also demonstrated APC functions of MSCs to recall antigens *Candida albicans* and Tetanus toxoid (104). Furthermore, they showed major histocompatibility complex class II (MHC-II) expression on MSCs requires autocrine stimulation by endogenous low level IFN- γ . In contrast to other studies, as levels of IFN- γ increased, MHC-II expression gradually decreased. Although the significance of reduced MHC-II expression was not addressed, the authors concluded MSCs may be able to change their functions from APCs to immune suppressor cells depending on their surrounding environment.

2.6. Immunological properties of MSCs in vivo

One of the first studies evaluating MSC function in vivo demonstrated systemic infusion of allogeneic BM-derived MSCs in baboons prolongs survival of skin allografts to 11 days compared to 7 days in control animals (80). Subsequently, the immunomodulatory effects of MSCs have been examined in a variety of animal models of organ and stem cell transplantation,

autoimmunity, or tumor immunity. Nauta et al. studied the *in vivo* immunomodulatory properties of MSCs in a murine model of allogeneic BM transplantation in which sublethally irradiated recipients received allogeneic BM with or without host or donor MSCs (105). They showed addition of host MSCs significantly enhances long-term engraftment associated with tolerance to host and donor antigens, however, the infusion of donor MSCs is also associated with significantly increased rejection of allogeneic donor BM cells. They also showed injection of allogeneic donor MSCs alone in naive mice is sufficient to induce a memory T-cell response. Based on these results, the authors concluded allogeneic MSCs are not intrinsically immunoprivileged and under appropriate conditions allogeneic MSCs induce a memory T-cell response resulting in rejection of an allogeneic stem cell graft. Sudres et al. verified MSCs suppress alloantigen-induced T cell proliferation *in vitro* in a dose-dependent manner. However, addition of MSCs to a BM transplant at a MSC:T-lymphocyte ratio providing strong inhibition of allogeneic responses *in vitro*, did not result in clinical improvement in incidence or severity of GVHD. This absence of clinical effect is not due to MSC rejection, because cells are detected in grafted animals (106). On the other hand, Yanez et al. showed infusion of *ex vivo* expanded adipose tissue derived MSCs could control lethal GVHD in mice transplanted with haploidentical HSC (107). This study demonstrated only early and repeated infusions of MSCs are effective in controlling GVHD suggesting variations in dosage and timing of MSC infusion may explain contradictory results reported in other studies (106).

Another potential application for MSCs is amelioration of autoimmunity. Two studies showed MSCs could potentially ameliorate experimental autoimmune encephalomyelitis (EAE) in mice, a model of human multiple sclerosis, if given at the onset of disease but not if given after disease stabilization (84,108). In contrast, infusion of MSCs did not have any beneficial effect in a murine model of rheumatoid arthritis (109).

In summary, although mechanisms underlying immunological properties of MSCs are still poorly understood, numerous studies have demonstrated MSCs are able to modulate function of different immune cells *in vitro*. However, some of these results are contradictory with animal model studies. This might be due to species differences, sources of MSCs or culture conditions used. Importantly, immunological characteristics of MSCs *in vitro* is usually examined in the context of interaction of *ex vivo* expanded MSCs with one or two populations of immune cells, a situation very different from *in vivo* in which cells interact with a much larger number of different cells in a dynamic and highly intricate manner. The true biological relevance of these cells and their interactions with immune system *in vivo* has yet to be shown.

3. Clinical experience with MSCs

Lazarus et al. were first to report a phase I trial to determine feasibility of collection, *ex vivo* culture-expansion, and intravenous infusion of human BM-derived MSCs (110). BM aspirates obtained from 23 patients with hematological malignancies in complete remission and autologous MSCs were culture expanded *in vitro* for 4–7 weeks. Fifteen of 23 patients underwent MSC infusion after *ex vivo* expansion. A BM examination two weeks later assessed histology and collected hematopoietic cells for *in vitro* culture. No adverse reactions were observed with infusion of these *ex vivo* expanded BM-derived MSCs. The same group subsequently conducted a phase I–II clinical trial to determine feasibility, safety, and hematopoietic effects of BM-derived, culture-expanded autologous MSCs infused into breast cancer patients in the course of high-dose chemotherapy and HSC rescue. Again, autologous MSCs were infused without any toxicity and hematopoietic recovery was rapid. However, since patients were also supported with large numbers of autologous peripheral blood CD34⁺ hematopoietic cells, MSCs' role in enhancing hematopoietic recovery was uncertain (111). In a multicenter clinical trial, culture-expanded allogeneic MSCs derived from BM of HLA-identical sibling donors were infused 4 hours before infusion of HSCs in 46 patients

undergoing myeloablative HSC transplantation for various hematological malignancies (112). There were no infusion-related toxicities, ectopic tissue formation, or increase in incidence or severity of GVHD. However, in comparison with historical controls, no acceleration of hematopoietic engraftment was observed. Nevertheless, these studies provided evidence that culture expansion of MSCs under good manufacturing practices (GMP) conditions is feasible and these cells are safe to infuse.

Recently, clinical interest has arisen in using the immunosuppressive capacities of MSCs to prevent/control GVHD after HSC transplantation. Le blanc et al. were the first to report the potential of MSC infusions for treatment of GVHD in a 9-year-old boy who received a matched unrelated donor HSC transplant for leukemia. The patient developed severe acute GVHD of the gut and liver that was unresponsive to all types of immunosuppression. Haploidentical MSCs were generated from the patient's mother, not the original donor. After the infusion of one dose of MSCs, the GVHD disappeared. Infusion of a second dose of MSCs was also effective in treating GVHD when it recurred later. Importantly, after MSC infusion, lymphocytes from the patient continued to proliferate when co-cultured with maternally-derived lymphocytes, suggesting an immunosuppressive effect *in vivo*, but not development of tolerance. This was followed by a larger study in which MSCs were given to eight patients with steroid-refractory grades III–IV GVHD and one with extensive chronic GVHD. Two patients received MSC from HLA-identical siblings, six from haploidentical family donors, and four from unrelated mismatched donors. There were no acute side-effects after MSC infusions. Acute GVHD disappeared completely in six of eight patients, but two died soon after MSC treatment with no obvious response. Five patients were alive between 2 months and 3 years after transplantation (113). More recently, MSCs from haploidentical donors were co-transplanted with CD34+ cells from the same donors into 14 children. Compared to a graft failure rate of 15% in 47 historic controls, all patients given MSCs showed sustained hematopoietic engraftment without any adverse reaction; suggesting MSCs, possibly due to their potent immunosuppressive effect on alloreactive host T lymphocytes, could reduce risk of graft failure in haploidentical HSC transplant recipients (114). Currently, prospective randomized phase III studies in Europe and USA are in progress to further define the therapeutic potential of MSCs for promotion of HSC engraftment, and/or treatment/prevention of acute GVHD following allogeneic HSC transplantation (115). Despite all the encouraging results so far, clinical use of MSCs is still not a standardized and accepted form of cell therapy for treatment or prevention of GVHD.

Finally, culture expanded BM-derived MSCs have been used in several small phase I–II trials for a variety of non hematological indications including treatment of patients with metachromatic leukodystrophy and Hurler's disease (116), osteogenesis imperfecta (117), myocardial infarction (118), amyotrophic lateral sclerosis (119), and Crohn's disease (120), among others (115). Amazingly, MSCs derived from other sources including fetal-derived MSCs have already reached the clinic (121).

4. Potential role of MSCs in solid organ transplantation

As early as 2000, it was suggested that immunomodulatory properties of MSCs could be exploited in solid organ transplantation for prevention and/or treatment of organ rejection (122). In contrast to most current pharmacological agents that target only a single pathophysiological pathway, MSCs potentially work through multiple mechanisms and have the potential to effect immunological, inflammatory, vascular, and regenerative pathways (123). Thus, harnessing both the immunosuppressive capabilities of MSC as a potential treatment for acute rejection following solid organ transplantation, and their ability for tissue repair provides an exciting opportunity for further research. Their ease of production combined with their apparent lack of need for HLA matching could also have significant implications

for the therapeutic application of MSCs since previously expanded and cryopreserved MSCs derived from unrelated healthy donors can potentially be available for acutely ill patients in a timely manner. However, to date results reported with pre-clinical animal models have been conflicting and further research is urgently needed to clarify the utility of MSCs in solid organ transplantation.

4.1. MSCs and kidney

Acute and chronic kidney injuries post transplantation have a complex pathophysiology involving ischemic, inflammatory, and immunological mechanisms. Since in vitro and in vivo studies indicate MSCs might interfere with any of these arms, beneficial effects may arise through multiple mechanisms. In animal models, intravenously or locally injected syngeneic and/or allogeneic MSCs localize to the kidney and potentially improve organ function although the mechanism for improvement remains highly controversial. For example, Morigi et al suggested MSCs engraft in damaged kidneys and differentiate into tubular epithelial cells, thereby restoring renal structure and function in a cisplatin-induced renal injury model (124). In contrast, other investigators suggested MSC treatment is associated with improvement of renal function independent of differentiation into target cells, but rather through complex paracrine effects (125,126). Although the ultimate role of BM-derived MSCs in regeneration of kidney tissue in different pathological conditions needs to be determined, theoretically, harnessing immunomodulatory capabilities of MSCs in prevention and/or treatment of acute rejection coupled with their potential to repair damaged kidney is an attractive possibility. So far there is no report on the effect of MSCs in an animal kidney transplant model. Given the highly successful nature of anti-rejection medications especially for short term survival of transplanted kidneys, demonstrating potential benefits of MSCs would require a randomized study involving thousands of patients. It is highly unlikely that such randomized clinical trials would be implemented any time soon. The complex nature of chronic renal allograft nephropathy makes it much less likely that MSCs could be harnessed in prevention/treatment of this complication.

4.2. MSCs and pancreas

MSCs derived from BM and other tissues have been shown to differentiate into cells with a pancreatic phenotype by several investigators (29–32). Although this issue remains highly controversial, several attempts have been made to use these cells therapeutically in animal diabetes models. Lee et al. observed that in human MSC-treated diabetic mice, there was an increase in pancreatic islets and beta cells producing mouse insulin, thus raising the possibility that human MSCs may be useful in enhancing insulin secretion by mechanisms other than trans-differentiation of MSCs into pancreatic islets (127). Whatever the mechanism, pancreas supporting activity of MSCs combined with their immunomodulatory properties might provide a boost to immunosuppressive regimens post islet transplantation. It should be noted that many studies reporting effectiveness of BM stem cells for diabetes control used BM HSCs, not MSCs (128–130).

Itakura et al. tested the potential of MSCs to induce hematopoietic chimerism and thus immune tolerance through BM transplantation in a rat allogeneic islet transplantation model (131). They showed co-infusion of MSCs with BM cells and pancreatic islets facilitates induction of stable mixed hematopoietic chimerism under a non-myeloablative conditioning regimen. Although the primary islet grafts were rejected, half of the animals developed stable mixed hematopoietic chimerism and donor specific immune tolerance as evidenced by engraftment of donor skin and second set of islet transplants, and rejection of third party skin grafts. Interestingly, they also showed that the intravenous route of administration of MSCs and BM cells did not result in chimerism, but the intraportal route did and that infusion of islets was necessary for induction of chimerism. Thus, promoting hematopoietic chimerism and tolerance toward transplanted

solid organs might be potentially another role for MSCs in promoting solid organ transplantation.

4.3. MSCs and heart

Zhou et al. reported transplantation of Fisher344 rats with hearts from inbred Wistar rats followed by intravenous MSCs infusion at specific intervals modestly prolongs survival compared with controls.(132). However, Inoue et al. showed that while MSCs retain their immunosuppressive effect on MLR in vitro, their administration in a rat allogeneic heart transplant model not only does not prolong allograft heart survival, but tends to promote rejection (133). Also, Wu et al. demonstrated intravenously administered MSCs vigorously migrate to the allograft rejection site using a rat cardiac model; and these animals exhibit a significantly shortened graft survival time compared to animals receiving lactated Ringer's solution without cells (134). Such discrepancies in the literature might be due to subtle differences in methodologies used. Further research in appropriate animal models especially large animal models is warranted before moving into clinical trials.

4.4. MSCs and lung

MSCs have been also tried for treatment of various lung injuries. Mei et al. used MSCs with and without pANGPT1-transfection in LPS-induced acute pulmonary inflammation in mice and showed improvement in alveolar inflammation (135). Ortiz et al. showed that murine MSCs home to the lung in response to bleomycin-induced injury, and reduce inflammation and collagen deposition in lung tissue (136,137). Gupta et al. studied the direct intrapulmonary injection of MSCs 4 h after intrapulmonary administration of Escherichia coli endotoxin and showed MSCs increased survival compared with PBS-treated control mice. This beneficial effect of MSCs was independent of the ability of cells to engraft in the lung and was not related to clearance of endotoxin by MSCs (138). The potential of MSCs in reducing inflammation and fibrosis in the injured lung combined with their immunomodulatory properties might provide a rationale for testing in transplantation models.

4.5. MSCs and liver

Parekkadan recently reported that administration of MSC-derived molecules in two clinically relevant forms-intravenous bolus of conditioned medium (MSC-CM) or extracorporeal perfusion with a bioreactor containing MSCs (MSC-EB)-can provide a significant survival benefit in rats undergoing fulminant hepatic failure. Histopathological analysis of liver tissue after MSC-CM treatment showed dramatic reduction of panlobular leukocytic infiltrates, hepatocellular death and bile duct duplication. Furthermore, using computed tomography of adoptively transferred leukocytes they showed that MSC-CM functionally diverts immune cells from the injured organ indicating altered leukocyte migration by MSC-CM therapy may account for absence of immune cells in liver tissue (139). Such anti-inflammatory properties of MSCs might prove useful after liver transplantation, although again there is no preclinical data to support this hypothesis.

5. Safety concerns

Infusion of ex vivo expanded MSCs is considered relatively safe based on the general assumption these cells are hypoimmunogenic and elicit a suppressive effect on allogeneic lymphocyte responses. However, there is some evidence MSCs can potentially function as APCs and activate immune responses under appropriate conditions (103,104). Indeed, some xenotransplantation studies with human MSCs in non-immunocompromised animals suggest MSCs are not intrinsically immunoprivileged (140). Recent studies also suggest that MSCs could become neoplastic after long-term culture (141–144). Furthermore, Djouad et al. demonstrated MSCs prevent rejection of allogeneic tumor cells in immunocompetent mice and

promote tumor formation when injected locally or systemically (89). Karnoub et al. showed in a murine xenograft model that BM-derived human MSCs, when mixed with otherwise weakly metastatic human breast carcinoma cells and injected into a subcutaneous site, cause cancer cells to greatly increase their metastatic potency (145). Thus, although not seen in clinical trials, it is theoretically possible MSCs could promote tumor growth either through a direct effect on tumor growth or via immunosuppression of anti-tumor responses. It can be also argued that potential lack of immunoprivilege by allogeneic MSCs might be desirable. Administration of MSCs to exert an immunomodulatory/anti-inflammatory function might only temporarily suppress the immune system before their disappearance, reducing risks associated with prolonged immune suppression.

6. Future directions

Much of our knowledge of MSCs is based on in vitro experiments and much more research is needed to understand both the physiological role of these cells in vivo, and the mechanisms through which they mediate their apparent beneficial effects in regeneration, repair, and immunomodulation. Many of the perceived characteristics of MSCs might be artificially induced by in vitro culture and not have any in vivo counterpart. However, this deficit should not deter their use if larger clinical trials validate their applicability, as therapeutic modalities are routinely used in clinical medicine without knowing the exact mechanism of action.

Nevertheless, there are several clinically important questions to be addressed: What are candidate indications for use of MSCs? What are the optimal dosing, timing, frequency, and routes of administration for different indications? How can production of MSCs be standardized? What is the role of additional immunosuppressive modalities combined with MSCs? Is it preferable to use autologous MSCs or allogeneic MSCs? What safety assays should be used to verify genetic stability of transplanted cells? Despite lack of apparent adverse effects seen in clinical trials to date, longer-term follow-up is also required given the immunosuppressive properties and tumor-growth promoting effects of MSCs, and potential for malignant transformation of transplanted cells.

Finally, but not least important: What should be the role of MSCs in the field of solid organ transplantation since there is still no report on their use in solid organ transplant recipients? Since the currently available in vitro and in vivo data does not yet support use of MSCs in solid organ transplantation, these cells should first be screened in relevant pre-clinical and large animal models before testing their potential in repairing solid organ damage or preventing/ameliorating rejection.

6.1. Embryonic stem cell derived MSCs

We and others have reported generation of cells from BM with characteristics very similar to MSCs (146–149). Furthermore, ESC-derived MSCs also possess immunological properties very similar to BM-derived MSCs including expression of HLA-I but not HLA-II molecules, lack of immunogenicity when co-cultured with third party lymphocytes, and immunosuppression in MLR assays (in press). We propose co-transplantation of ESC-derived MSCs could provide protective immunomodulatory functions toward other cells/tissues derived from the same human ESC lines.

7. Conclusions

Today still, we know little about the exact anatomical location, tissue distribution, and functional role(s) of MSCs in vivo in health and disease conditions. However, these questions have not deterred clinicians from testing MSCs in several clinical applications. The original use of MSCs to accelerate hematopoietic engraftment after transplantation has now expanded

to testing their potential to differentiate into and/or participate in tissue regeneration. More recently, their immunomodulatory properties are also being tested. However, mechanisms underlying possible in vivo immunomodulatory effects remain a critical and unresolved question. The potential role of MSCs to promote engraftment of tissues/organs and prevent/treat rejection may be multifactorial and might be dependent on secretion of soluble growth factors, increasing angiogenesis, suppressing alloreactive T cells, interacting with several arms of the immune system, and potentially cell fusion or even direct transdifferentiation. Preliminary clinical results are encouraging but there is no report yet on their potential in the setting of solid organ transplantation. It is important not to overestimate the potential therapeutic effects of MSCs given the nonrandomized nature of almost all the clinical studies reported so far.

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Figure-1.

Some of the proposed mechanisms for the potential beneficial effects of mesenchymal stromal cells in solid organ transplantation.