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Mesenchimal Stem Cells in Inflammatory Bowel Diseases: Clinical Evidences And Potential Insights for The Clinicians

Review Article

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Abstract

Mesenchymal stem cells (MSCs) have been used experimentally and clinically in the treatment of a wide variety of pathologies. MSCs can be safely transplanted in autologous and allogeneic ways as they are non-immunogenic, and consequently represent a therapeutic option for refractory connective tissue diseases, fibrosing diseases like scleroderma and fistulizing colitis like in Crohn's disease (CD). The immunomodulatory properties of MSCs have already shown promise when used as therapy for otherwise medically refractory CD. Accumulating evidence suggests that these properties may also be exploited in the treatment of several other conditions.
The currently available experimental and clinical data indicate that, similar to previously obtained data in the setting of HSCT, MSC treatment for IBD is feasible and safe. Aim of this review is to analyze the pathophysiological insights for the use of MSCs in inflammatory bowel diseases, and to summarize the clinical evidences about the efficacy and safety of stem cell therapy in such disorders.

Key Words: Stem Cell; Inflammatory Bowel Disease; Safety.

Introduction

Mesenchymal stem cells (MSCs) have been used experimentally and clinically in the treatment of a wide variety of pathologies. It is now clear that a number of different mechanisms contribute to the therapeutic effects exerted by these cells. The ability of MSCs to interact with and modulate the functions of a wide variety of immune cells has been recognized as one such mechanism (1).

MSCs can be safely transplanted in autologous and allogeneic ways as they are non-immunogenic, and consequently represent a therapeutic option for refractory connective tissue diseases, fibrosing diseases like scleroderma and fistulizing colitis like in Crohn's disease. Actually, there are more than 200 registered clinical trial sites for evaluating MSC therapy, and 22 are on autoimmune diseases (2).

When entering the clinical arena, a few potential risks of MSC therapy have to be taken into account: (i) immunogenicity of the cells, (ii) biosafety of medium components, (iii) risk of ectopic tissue formation, and (iv) potential in vitro transformation of the cells during expansion (3)

Aim of this review is to analyze the pathophysiological insights for the use of MSCs in inflammatory bowel diseases, and to summarize the clinical evidences about the efficacy and safety of stem cell therapy in such disorders.

Immunomodulatory Properties of Mesenchimal Stem Cells

MSCs are easily obtained and can be maintained and expanded in culture for later use. In recent years, much enthusiasm has been generated concerning the use of these previously harvested and expanded cells as a therapeutic modality for a wide range of conditions. MSCs, for example, have been seeded on biologic mesh in an attempt to generate new bone or cartilage and infused directly into the myocardium in an attempt to improve myocardial healing and function following myocardial infarction (4-8). Interestingly, despite the wealth of information that has been learned about MSCs following ex vivo expansion and subsequent transplantation, their true in vivo function remains somewhat poorly understood.

Although readily obtained from such sources as bone marrow and adipose tissue, these cells comprise only minute proportions of the total population of cells in these tissues and their numbers appear to decline substantially with age (9-10). Because of their relative scarcity, determining what specific role these cells play under homeostatic and/or pathologic conditions has been difficult.

MSC act as potent modulators of immune responses by their (in vitro) ability to suppress T cell proliferation (11). Immune modulation requires their previous activation by immune cells through the proinflammatory cytokines interferon gamma (IFN-γ), tumour necrosis factor α (TNF-α) or interleukin- 1β (IL-1β) (12). Moreover, indoleamine-2,3-dioxygenase, heme oxidase as well as human leukocyte antigen G5 (HLA-G5) have been involved in MSC-mediated immune modulation. A distinct subpopulation of MSC, characterized by the expression of CXCL12 and vascular cell adhesion molecule-1, might provide a survival niche for memory plasma cells (13). Besides immune modulation, MSC exhibit healing capacities, improve angiogenesis and prevent fibrosis (14). Prostaglandin E2 (PGE2) is involved in the immunosuppressive activity of MSCs. PGE2 acts as a powerful immune suppressant representing inhibiting T cell mitogenesis and interleukin-2 production, and is a cofactor for the induction of T helper (Th) type 2 lymphocyte activity. Production of PGE2 by MSC is enhanced following TNF-α or IFN-γ stimulation. The use of specific inhibitors resulted in restoration of T lymphocyte proliferation (15). MSC-derived PGE2 was shown to act on macrophages by stimulating the production of interleukin-10 (IL-10) and on monocytes by blocking their differentiation toward dendritic cells (DCs) (16,17).

The interleukin IL-6, a major MSC-secreted factor, has been reported to be involved in the inhibition of monocyte differentiation toward DCs , decreasing their stimulation ability on T cells (18,19). In parallel, the secretion of IL-6 by MSC was reported to delay apoptosis of lymphocytes and neutrophils (20,21). HLA-G5 by MSC was recently shown to suppress T cell proliferation, as well as NK cell cytotoxicity and T cell cytotoxicity, and to promote the generation of regulatory T (TREG) cells. Cell contact between MSC and activated T cells induced IL-10 production, which was essential to stimulate the release of soluble HLA-G5 (22,23).

Both in vitro and in vivo, MSC promote the generation of CD4+CD25+ or CD8+ TREG cells with functional properties (24). In vivo data, however, are contradictory (25,26). Recent studies suggest that MSC may

induce a cytokine profile shift in the Th1/Th2 balance toward the antiinflammatory phenotype Th2 that is accompanied by an increase of T regulatory lymphocytes and in consequence an increase of IL10 (27,28). MSC inhibit the proliferation of B lymphocytes that are activated with antiimmunoglobulin antibodies, soluble CD40 ligand or cytokines (29). The use of human adipose tissue-derived MSC led to decreased antigen-specific Th1/Th17 cell expansion, enhanced secretion of IL-10 and generation of CD4+CD25+FoxP3+ TREG cells with the capacity to suppress selfreactive T effector responses in a xenogeneic collageninduced arthritis model (30)

Mechanism of Msc Action

The study of human MSCs derived from subcutaneous adipose tissue (hASCs) in the 5% dextran sulfate sodium (DSS) mouse model has shown that hASCs inhibit T-cell activation with the superantigen staphylococcal enterotoxin E (SEB), as measured by cytokine secretion and T-cell proliferation. The inhibitory effect was partially reversed when peripheral blood mononuclear cells (PBMCs) and hASCs were separated by a semipermeable Transwell membrane suggesting partial cell–cell contact dependence (31). Moreover, the coculture of allogeneic PBMCs and hASCs in the same chamber of the Transwell system fully restored their inhibitory activity on SEB-activated PBMCs situated in the other chamber, suggesting that PBMC– hASC contact induces the secretion of an immunosuppressive factor(s) for T cells. IL-10 production increased in a cell– cell contact-dependent manner in cocultures of hASC with PBMCs or monocytes but not with T cells. IL-10 blockade partially reversed the inhibitory activity of hASC on T cells.

Modifications in MSCs decrease or enhance their immunomodulatory effects (32-34). Duijvestein et al. recently showed that IFN-γ-stimulated MSCs (IMSCs), but not nonstimulated MSCs, showed a significantly attenuated DSS-induced colitis and trinitro-benzene sulfunate–induced colitis. Human MSCs significantly inhibited PBMC proliferation at lower PBMC:IMSC ratios compared with untreated MSCs, indicating that IMSCs have higher immunomodulatory capacities. Treatment with mouse IMSCs resulted in significantly lower serum amyloid A levels, confirming the decreased inflammatory responses observed in these animals. IMSC migration to the intestine was significantly increased during colitis induction, whereas MSC distribution was unaffected, suggesting that IMSCs gain homing potential to sites of inflammation (32). Ko et al. recently showed that when cells were coated with antiaddressin antibody and injected into C57BL6 mice with DSS-induced colitis, the mice showed dramatically improved survival rates, higher IBD therapeutic scores, and significantly improved body weight gain compared with mice injected with MSC-only, isotype Ab, free Ab plus MSCs, or vehicle-only controls (33). hASC-treated colitic mice had significantly higher numbers of T regulatory cells (Tregs) in mesenteric lymph nodes than untreated colitic mice, and they persisted for a long period of time. In vivo depletion of IL-10 or CD25+ T cells partially reversed the beneficial action of hASCs on colitis, demonstrating involvement of a therapeutic effect. When cells were coated with antibody against vascular cell adhesion molecule-1 (Ab VCAM-1-MSC), mice exhibited a percentage of Tregs nearly five times greater than with PBS-only-injected mice (analysis of variance, $P < 0.0001$), whereas MSC-onlyinjected mice showed nearly a tripling of the Treg percentage compared with PBS-injected mice ($P < 0.05$) (34).

Colons of hASC-treated mice contained reduced levels of inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-1β, and IL-12), chemokines (RANTES), and macrophage inflammatory protein-2 and increased levels of the anti-inflammatory/ regulatory cytokine IL-10 in comparison with untreated DSS colitic mice. This effect was not just a consequence of a diminished inflammatory infiltration in the mucosa because mononuclear cells isolated from the lamina propria of hASCtreated mice produced less TNF-α, IL-12, and IFN-γ on ex vivo culture, suggesting that hASC deactivated the colonic inflammatory response (31). Both syngeneic and allogeneic murine ASCs were as efficient as hASCs in ameliorating the colitis suggesting that the immunosuppressive action of ASCs is non–major histocompatibility complex (MHC)–restricted and that the infused ASCs are immune-tolerated by the host, which is convenient for future clinical application of these cells in CD (31).

Msc Therapy

Systemic Infusion of Human MSCs

In the first human trial of systemic MSCs in CD, Onken et al. , from Duke University, treated 10 patients who had failed previous treatment with steroids and immunosuppressants and had active disease; patients were randomized to receive either low- (2 million cells/kg) or high-dose (8 million cells/kg) i.v., allogenic, third-party, healthy, human bone marrow– derived MSCs as i.v. Infusions in two doses 7 d apart. All nine evaluable

patients had a decrease in CDAI score by day 28. Mean CDAI scores decreased significantly from baseline to day 28 (341 vs. 236, P = 0.004, Wilcoxon signed rank). The primary end point was clinical response defined as a ≥100-point reduction in CDAI. This response was achieved in 3 patients (33%) by day 14, 2 of whom met the end point within 7 d of the first infusion. All clinical responders had previously failed infliximab therapy. Mean IBD quotient scores increased significantly from baseline to day 28 (113 vs. 146, P = 0.008, Wilcoxon signed rank). IBD quotient scores increased to ≥170 in 22% of patients by day 14 and 38% by day 28. There appeared to be an association between mean change in IBD quotient and clinical response at day 28 (P = 0.07, Wilcoxon rank sum). Although not statistically significant, the mean reduction in the CDAI score at day 28 was greater in the high-dose than in the low-dose group (−137 vs. −65, P = 0.39, Wilcoxon rank sum). All infusions were well tolerated, and there were no treatmentrelated serious adverse events (35).

Another phase I study of autologous bone marrow–derived MSCs for luminal refractory CD was published in the Netherlands. Enough MSCs were expanded in 9 of 10 patients to administer two doses of 1–2 Å~ 106 cells/kg body weight, intravenously, 7 d apart. All patients had previously failed corticosteroids, at least two anti-TNF drugs, and 9 of 10 patients also had failed two immunosuppressants (thiopurine and methotrexate). In this study, no clear signal of efficacy was observed; remission was not achieved in any patient, and 3 patients had a reduction of at least 70 points in CDAI, but the disease worsened significantly in 4 patients requiring surgery (three cases) or rescue medication (one case) within 14 wk after cell treatment. Endoscopy improved in two cases, but no significant changes in C-reactive protein levels were seen (36). The currently ongoing largest, randomized, placebo-controlled, double-blind phase III study of prochymal (allogenic marrow–derived MSCs) in CD was initiated in 2007 by Osiris (http://clinicaltrials.gov/ct2/show/NCT00294112). The study plan is to enroll 270 patients with active CD (CDAI 250–450) who have a history of treatment failure with or intolerance to steroids, immunosuppressants, and biologics. Patients are randomized to receive four infusions over 2 wk of either 600 million cells (low dose: two infusions of 200 Å~ 106 hMSCs in week 1, then two infusions of 100 Å~ 106 hMSCs in week 2), 1,200 million cells (high dose: two infusions of 400 Å~ 106 hMSCs in week 1, then two infusions of 200 Å~ 106 hMSCs in week 2) or placebo. The primary end point of the study is remission at day 28 with secondary end points being clinical response, improved quality of life (increased IBD quotient score), and decreased number of draining fistulae.

Local Injection of MSCs in CD

The first trial of cell therapy using autologous MSCs (ASCs) obtained from a lipoaspirate for local treatment of fistulae for CD in 5 patients was published in 2005 (37). The same group published a phase II, multicenter, randomized controlled trial describing the effectiveness and safety of ASCs in the treatment of complex perianal fistulas in 2009. Patients with complex perianal fistulas (cryptoglandular origin, n = 35; associated with CD, n = 14) were randomly assigned to intralesional treatment with fibrin glue or fibrin glue plus 20 million ASCs.

Fistula healing and quality of life (SF-12 questionnaire) were evaluated at 8 wk and 1 y. If healing was not seen at 8 wk, a second dose of fibrin glue or fibrin glue plus 40 million ASCs was administered. Fistula healing was observed in 17 (71%) of 24 patients who received ASCs in addition to fibrin glue, compared with 4 (16%) of 25 patients who received fibrin glue alone (relative risk for healing, 4.43; confidence interval, 1.74–11.27; P < 0.001). The proportion of patients with healing was similar in CD and non-CD subgroups. ASCs were also more effective than fibrin glue alone in patients with a suprasphincteric fistulous tract (P = 0.001). Quality-of-life scores were higher in patients who received ASCs than in those who received fibrin glue alone. At 1-y follow-up, the recurrence rate in patients treated with ASCs was 17.6%. Both treatments were well tolerated (38). In a second study, published recently, a local injection of autologous marrow–derived MSCs was given to nine patients with perianal and one patient with enterocutaneous fistulas. Injections of a median of 20 Å~ 106 cells (range 15–30) were given every 4 wk until a response was obtained or no more cells were available. Complete fistula closure sustained for 1 y was obtained in 7 patients and a response (reduction of at least 50% of fistula tracts) in 3. Furthermore, all 9 patients with perianal fistulas had active disease in the rectum at baseline, and healing of rectal lesions was observed in the 7 patients who underwent endoscopy at month 12 of follow-up. Thus, the latter study suggests a considerable therapeutic benefit of local injection of MSCs in fistulizing lesions (39). At this stage, reasons for the apparent discrepancies between efficacy of local injection of MSCs for treatment of fistulas compared with systemic administration for treatment of luminal CD are not completely clear. MSCs have been reported to

home to sites of injury and disease following i.v. infusion and contribute to the repair process. The expression of adhesion molecules and chemokine receptors on MSCs may be responsible for their ability to migrate selectively to sites of inflammation through a ICAM1- and VCAM1-dependent interaction with endothelial cells (40). In an experimental model of colitis, systemically injected human adipose tissue–derived MSCs were detected in the mesenteric lymph nodes and spleen of the recipient colitic mice 1–3 d post injection (31). Interestingly, labeled MSCs were recruited by the inflamed colon but not by the noninflamed intestine. However, the proportion of cells recruited to inflamed or damaged organs and the survival of cells at sites of inflammatory lesions remain to be clarified, a necessary prerequisite for optimizing a potential systemic treatment. In studies showing efficacy of local injections, of human autologous MSCs, 30–60 Å~ 106 are injected in a single fistulous tract, and these injections are generally repeated (38,39). In a study using systemic injection for treatment of luminal disease, a higher number of 100–400 Å~ 106 allogenic or autologous MSCs were injected (32). Considering the extension of the inflamed intestine, and the fact that only a portion of the MSCs will reach the inflamed organ, the cell density at sites of luminal inflammation would be considerably lower than that achieved in fistula tracts by local injection. To circumvent this, Ghosh et al., from the United Kingdom, injected haploidentical MSCs after catheterization of the mesenteric artery via the femoral route into a 35-y-old patient with severe refractory fistulizing CD failing all conventional therapies, biological therapies, and surgical defunctioning ileostomy. The patient received 105/kg MSCs, and 4 wk later a second dose of 106/kg (41). The pretreatment CDAI was 384; it dropped to 258 2 weeks after the first infusion and remained as such at the time of the second infusion administered after 4 wk. Magnetic resonance imaging of the abdomen/pelvis post–MSC treatment showed slight changes in the transphincteric fistulae on the right and an unchanged horseshoe intersphincteric extension on the left side. There was no adverse effect. All the above studies point to a difference in cell density achieved at inflammatory sites, with systemic and local injections. Ongoing trials are testing fourfold higher systemic doses (42).

Safety Issues

When considering the use of ex vivo expanded MSCs for clinical application, several potential risks should be considered: (i) the immunogenicity of the cells, (ii) the biosafety of medium components, (iii) the risk of ectopic tissue formation, and (iv) the potential in vitro transformation of the cells during expansion (3)

Immunogenicity

Concerning the immunogenicity of MSCs, it has been demonstrated that MSCs are not intrinsically immunoprivileged. Infusion of allogeneic MSCs into immunocompetent and major histocompatibility complex (MHC)-mismatched mice may induce an immune response, resulting in their rejection (43). On the contrary, the infusion of syngeneic host–derived MSCs resulted, in the same mouse model, in enhanced engraftment of allogeneic stem cells (43). Moreover, it has been demonstrated that IL-2–activated autologous and allogeneic natural killer (NK) cells are capable of effectively lysing MSCs in vitro (44) . Although MSCs express normal levels ofMHC class I, which should protect against NK-mediated killing, they display ligands that are recognized by activating NK receptors that, in turn, trigger NK alloreactivity (44). The majority of the clinical reports on MSC therapeutic application have suggested low immunogenicity of MSCs in humans. However, when gene-markedMSCs were employed to treat children with osteogenesis imperfecta, the gene-marked cells were not detected in the treated patients, indicating their potential recognition and rejection by the host immune system (45).

On the basis of these experimental and clinical findings, some fundamental issues should be taken into consideration when determining the clinical application of MSCs, including whether autologous or allogeneic MSCs should be employed, the state of immune competence of the patient at time of infusion, and the number of infusions needed to treat the patient.

The Safety of The Culture Medium

Although current standard conditions for ex vivo expansion of MSCs are based on the presence of fetal calf serum (FCS), the use of bovine proteins might be associated with the risk of transmission of zoonoses and potential immune reactions in the host, resulting in rejection of the cells especially after repeated treatments (46,47). For these reasons, various animal-free additives have been considered for clinical-grade expansion ofMSCs: autologous and allogeneic human-serum (48), cytokines and growth factors (49), and platelet lysate (PL)/platelet rich plasma (PRP) (50). In particular, PL/PRP has been proposed as a suitable and efficacious candidate substitute for FCS in the near future (50-52). Several research groups have

demonstrated that the growth factors contained in PL/PRP are able to promote MSC expansion, and that a concentration of PL/PRP of 5% is sufficient to guarantee the optimal growth of MSCs while substantially preserving their biological and functional properties, including those relating to modulation of immune responses (50-52).

However, clinical trials conducted so far have mainly employed FCS-expanded cells, and available data in vivo on PL/PRP-cultured MSCs are still scarce. Therefore, these latter cells need to be extensively tested in vivo before being considered as a safe and effective substitute for MSCs generated in the presence of FCS-based media.

Ectopic Tissue Formation

One of the potential risks of MSC treatment involves the formation of mesenchymal tissues at ectopic sites. In a rat myocardial infarction model, it has been reported that MSCs may form bone following local injection into the myocardium (53). Similarly, formation of adipose tissue in kidneys has been observed in a rat model of experimental glomerulonephritis (54). Moreover, it has been recently reported in amurine model of GvHD that local implantation of MSCs resulted in ectopic bone formation in syngeneic recipients, whereas it lead to transplant rejection in allogeneic mice (55). These studies underline the potential danger of ectopic tissue formation in patients treated with MSCs for myocardial infarction and other diseases; however, in clinical trials so far, no ectopic tissue or tumor formation in vivo has been observed. Few studies have attempted to specifically address this concern, and factors governing the postinfusion fate ofMSCs and the influence of the local environment on MSC behavior are still largely unknown and need further investigation. Therefore, a strict and long-term follow-up of patients treated with MSCs is recommended to monitor the potential formation of mesenchymal tissues at ectopic sites.

Malignant Transformation

It has been shown by a few groups that longterm manipulation in vitro of both adipose tissue (AT) and bone marrow (BM)–derived MSCs may alter their functional and biological properties, leading to the accumulation of genetic alterations and malignant transformation (56-58). By contrast, other researchers have suggested that human MSCs of various tissue origin can be cultured in vitro long term without losing their usual phenotypical/functional characteristics and without developing chromosomal aberrations (59-61). In particular, genetic studies performed through both conventional karyotyping and molecular techniques, such as array-comparative genomic hybridization (array-CGH), have been employed to document the absence of chromosomal abnormalities in cultured MSCs (59-61)

Telomere length and telomerase activity analyses, together with the study of several proteins and genes involved in the regulation of cell cycle, senescence, and tumorigenesis have been also tested, confirming the absence of transformation (59,61) Moreover, French researchers have demonstrated that the occasional presence of aneuploidy in some MSC preparations may be related to the occurrence of senescence, but not to the development of transformation (61).

More importantly, the reports on MSC malignant transformation have been recently retracted because it was found that the tumor cells in MSC cultures were unrelated to the original MSCs; rather, they derived from contaminating tumor cell lines (62,63). Together, these data indicate that malignant transformation in ex vivo expanded humanMSCs is likely to be an extremelyuncommonevent, estimated to be in the frequency of< 10−9 (64). As a general recommendation, phenotypic, functional and genetic assays, although known to have limited sensitivity, should be routinely performed on MSCs before in vivo use; in particular, a genetic characterization of MSCs through conventional/molecular karyotyping may be consideredbefore release of MSCs for clinical application, in particular for patient-derived MSCs.

Conclusions

Adult-tissue stem cells have a property that makes them attractive to research¬ers in the emerging field of regenerative . In particular, the immunomodulatory properties of MSCs have already shown promise when used as therapy for otherwise medically refractory CD. Accumulating evidence suggests that these properties may also be exploited in the treatment of several other conditions.

The currently available experimental and clinical data indicate that, similar to previously obtained data in the setting of HSCT, MSC treatment for IBD is feasible and safe. Neither early toxicity nor later side effects have been registered in treated patients, although a longer follow-up is necessary to drawdefinitive conclusions on potential long-term adverse events.

Ongoing efforts focused on evaluating in vivo effectiveness, shortcomings, and adverse effects of MSCs are needed to determine if their immunomodulatory properties will evolve from theoretical interest to clinical benefit.

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