REVIEW

Immunomodulatory effect of mesenchymal stem cells

O efeito imunomodulatório de células-tronco mesenquimais

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ABSTRACT

Mesenchymal stem cells represent an adult population of nonhematopoietic cells, which can differentiate into a variety of cell types such as osteocytes, chondrocytes, adipocytes, and myocytes. They display immunomodulatory properties that have led to the consideration of their use for the inhibition of immune responses. In this context, mesenchymal stem cells efficiently inhibit maturation, cytokine production, and the T cell stimulatory capacity of dendritic cells. They also can impair proliferation, cytokine secretion, and cytotoxic potential of T lymphocytes. Moreover, mesenchymal stem cells are able to inhibit the differentiation of B cells to plasma cells by inhibiting their capacity to produce antibodies. A variety of animal models confirm the immunomodulatory properties of mesenchymal stem cells. Clinical studies including patients with severe acute graft-*versus*-host disease have revealed that the administration of mesenchymal stem cells results in significant clinical responses. Therefore, mesenchymal stem cells improve acute graft-*versus*-host disease and represent a promising candidate for the prevention and treatment of immune-mediated diseases, due to their immunomodulatory capability and their low immunogenicity.

Keywords: Mesenchymal stem cells; Immunomodulation

RESUMO

As células-tronco mesenquimais são uma população adulta de células não hematopoiéticas, que podem se diferenciar em uma variedade de tipos celulares, como osteócitos, condrócitos, adipócitos e miócitos. Apresentam propriedades imunomoduladoras, que levaram a considerar seu uso para inibir as respostas imunes. Nesse contexto, as células-tronco mesenquimais inibem com eficiência a maturação, a produção de citocinas, e a capacidade de estimular as células T das células dendríticas. Podem também impedir a proliferação, secreção de citocina e o potencial citotóxico dos linfócitos T. Além disso, as células-tronco mesenquimais são capazes de inibir a diferenciação de células B em plasmócitos ao inibir sua capacidade de produzir anticorpos. Uma variedade de modelos animais confirma as propriedades imunomoduladoras das células-tronco mesenquimais. Alguns estudos clínicos que incluíram pacientes com doença do enxerto contra hospedeiro mostraram que a administração de células-tronco mesenquimais resultou em respostas clínicas significativas. Portanto, as células-tronco mesenquimais parecem melhorar a doença do enxerto contra hospedeiro e são candidatas promissoras na prevenção e tratamento de doenças imunomediadas devido à sua capacidade imunomoduladora à baixa imunogenicidade.

Descritores: Células-tronco mesenquimais; Imunomodulação

INTRODUCTION

The best characterized source for adult stem cells is still adult bone marrow, which contains a heterogeneous population of cells, including hematopoietic stem cells, macrophages, erythrocytes, fibroblasts, adipocytes, and endothelial cells. In addition to these cell types, bone marrow also contains a subset of nonhematopoietic stem cells with multilineage potential[1,2]. These stem cells are called marrow stromal stem cells or mesenchymal stem cells, and more commonly now, mesenchymal stromal cells (MSCs). MSCs are primitive cells originating from the mesodermal germ layer, and classically have been described as giving rise to connective tissues, skeletal muscle cells, and cells of the vascular system.

More than 30 years ago, Friedenstein et al.[3] first reported evidence of fibroblast-like cells that could be isolated from bone marrow via their adherence to plastic in cultures. They described a population of multipotential stromal precursor cells that were spindle-shaped and clonogenic in culture conditions, defining them as colony-forming unit fibroblasts (CFU-F).

Lately, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed minimal criteria to characterize human MSCs[4].

Thus, MSCs must be plastic-adherent when maintained in standard culture conditions. In

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addition, they must express CD29, CD73, CD90, and CD105, and lack expression of CD34, CD45, CD14, and human leukocyte antigen (HLA)-DR on the surface. Furthermore, they must have the potential to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*. These observations formed the basis for most of the current studies of bone marrow-derived stromal cells. However, many unanswered questions still remain as to the true nature and identity of MSCs, including location, origin, and multipotential capacity. Isolation of MSCs was reported from several tissues, such as bone marrow, adipose tissue, liver, muscle, amniotic fluid, placenta, umbilical cord blood, and dental pulp[5-8].

Immunomodulatory effect of MSC

Human MSCs (hMSC) are characterized by the low expression of the major histocompatibility complex (MHC) class I and by the absence of co-stimulatory molecules, such as CD80, CD86, or CD40, and are described by their immunomodulatory effect on immune system cells. Additionally, hMSC fail to induce proliferation of allogeneic or xenogeneic lymphocytes, causing effects on B lymphocyte function, inhibiting its differentiation into plasma cells, and showed to interfere with dendritic cell differentiation, maturation, and function[9-11].

Impact of hMSC on T cells

Some studies demonstrated that MSCs affect several properties of T cells and definitely suppress the proliferation of CD4+ and CD8+ T cells[12]. T cell proliferation reduction was evident even when the cells were co-cultivated with MSC separated by a Transwell membrane, suggesting that besides cell-cell interaction, there are soluble factors involved in this suppressive mechanism[12].

In addition to their ability to impair the proliferation of activated T cells, MSCs prolonged the survival of T cells in a quiescent state. MSCs rescued T cells from activation-induced cell death by down-regulation of the Fas receptor and Fas ligand on T cell surface and by the inhibition of endogenous proteases involved in cell death. MSCs also reduced Fas receptor-mediated apoptosis of CD95-expressing Jurkat leukemic T cells. In contrast, rescue from activation-induced cell death was not associated with a significant change in Bcl-2 expression, an inhibitor of apoptosis induced by cell stress[13].

MSCs inhibit the formation of cytotoxic T cells, but they do not interfere with CTLs and NK cell lysis[14]. When focusing on the specificity of the T cells inhibited by MSCs, studies demonstrated that MSCs exhibit different effects on alloantigen- and virus-specific T cell responses[15]. Thus, virus-induced T cell proliferation and IFN-γ secretion were less affected by MSCs than was the response to alloantigens[15].

Recently, several studies investigated the impact of MSCs on regulatory T cells, which play an important role in induction of peripheral tolerance and inhibition of proinflammatory immune responses[16]. Interestingly, MSCs markedly promote the expansion and the inhibitory capacity of regulatory T cells[16].

Several cell membrane-associated and soluble molecules were identified as contributing to MSC-mediated inhibition of T-cell proliferation and activity. They comprise prostaglandin E2 (PGE2), transforming growth factor-β (TGF-β), indoleamine 2,3-dioxygenase (IDO), and nitric oxide (NO)[17-20].

Effect of hMSC on B cells

Culture-expanded MSCs consistently suppressed the terminal differentiation of B cells into plasma cells. Previous investigations measured MSC effects by 3H-thymidine uptake of B cells stimulated by various other antigens[21-23], and all of these studies, with one exception[22], showed the MSC inhibitory effect on B cell proliferation.

Recent studies, however, have reported two opposite effects on B cell differentiation. Two studies showed "suppression of B cell differentiation"[22, 24] and the other two showed "augmentation"[25, 26].

On the other hand, even in latter studies, Rasmusson et al. identified suppression of LPS-stimulated B cell differentiation by MSC-secreted humoral factors[25]. These discrepancies may be due to various factors and conditions, including different signaling pathways initiated by stimuli through the BCR, TLR, or CD40 molecules, via cell-cell contact or humoral factors, strength of the stimuli, species of the MSC origin, purity of B cells, and/or MSC. Asari et al., showed that the suppression of LPS-stimulated B cell proliferation *in vitro* requires a higher B/MSC cell ratio than that required for suppressing B cell differentiation. Moreover, this study suggested that the decreased numbers of differentiated plasma cells in B/MSC co-cultures is not mediated by apoptosis[27].

Effect of MSCs on dendritic cells

Dendritic cells (DC) display a large capacity to induce T cell responses and to produce proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-1β, and IL-6[28]. Due to these properties, DCs play a

critical role in the initiation and maintenance of GVHD and several autoimmune diseases[28].

Recent studies have investigated if MSCs are able to modulate the phenotype and function of DCs. It was demonstrated that MSCs inhibit the immunostimulatory capacity of human DCs, which are differentiated from blood monocytes after several days in the presence of various cytokines[29]. Therefore, MSCs markedly impaired the differentiation of human blood monocytes into immature DCs as well as DC maturation by cell-cell interaction, since the supernatant of MSC cultures does not impair DCs maturation. In addition, MSCs inhibited endocytosis and the production of IL-12 by DCs. MSCs also efficiently suppressed the capacity of DCs to stimulate T-cell proliferation, reduced DC-mediated polarization of CD4+ T cells into pro-inflammatory Th1 cells, and drove the induction of Th2 cell responses[29].

All these findings reveal that MSCs direct human DCs toward a tolerogenic phenotype. When centering on the mechanisms for the effects of MSCs on the immunomodulatory properties of DCs, some studies demonstrated that prostaglandin E2 contributes to diminished cytokine release by DCs[30]. Furthermore, it was reported that other soluble factors, such as IL-6 and macrophage-colony stimulating factor (M-CSF), as well as cell-to-cell contact, play a role in MSC-mediated inhibition of differentiation, cytokine production, and the T cell stimulatory capacity of DCs[31].

MSC use for treating graft-*versus*-host disease

MSC immunomodulatory properties were demonstrated in various animal models related to graft-*versus*-host disease (GVHD) and graft rejection after cell or organ transplantation. Systemic administration of allogeneic bone marrow-derived MSCs of baboons led to prolonged skin graft survival[32]. Moreover some studies documented that the co-administration of autologous bone marrow-derived MSCs and donor bone marrow improved skin graft survival and reversed GVHD in rats [33].

Additional studies analyzed the potential of MSCs for the treatment of GVHD. Yañez et al. demonstrated that the infusion of adipose tissue-derived MSCs in mice transplanted with haploidentical hematopoietic grafts was able to control GVHD[34]. They showed that only early infusions of MSCs after transplantation are effective in controlling GVHD, and repeated MSC applications are required to improvement of GVHD[34].

In addition, Nauta et al. demonstrated that the infusion of donor bone marrow cells in combination with host murine MSCs enhances engraftment in a murine allogeneic bone marrow transplantation model[35]. However, they also observed that co-transplantation of donor bone marrow cells and donor murine MSCs results in an increased rejection of bone marrow cells and that infused donor MSCs are able to induce a memory T cell response[35].

Based on the immunomodulatory capacities of MSCs *in vitro* and *in vivo* using animal models, several clinical studies were performed to evaluate potential GVHD treatment with MSCs. Bone marrow-derived MSCs were co-infused with HLA-identical sibling-matched bone marrow or peripheral blood hematopoietic stem cells in 46 patients after a myeloablative conditioning regimen[36]. On day zero, MSCs were administered intravenously ($1.0–5.0 \times 10^6$ per kg of bodyweight) four hours before hematopoietic stem cell infusion. Treatment was well-tolerated and no ectopic bone or cartilage formation was observed. Grade II to IV acute GVHD was found in 28% of patients and chronic GVHD was observed in 61% of patients who survived at least 90 days[36].

Le Blanc et al. reported the administration of MSCs to a 9-year-old boy with severe treatment-resistant acute GVHD after allogeneic stem cell transplantation [37]. By day 70, the patient developed grade IV acute GVHD, including diarrhea up to 20 times daily and a high bilirubin concentration. MSCs were given intravenously (2.0×10^6 per kg of bodyweight) on day 73, and within several days after MSC infusion, the frequency of diarrhea fell to twice daily and a decline of the bilirubin concentration was observed. No treatment-related toxicity was noted after the infusion[37].

In another clinical trial, 8 patients with steroid refractory grades III and IV acute GVHD were treated with MSCs[38]. Patients received bone marrow-derived MSCs at a median dose of 1.4×10^6 per kg of bodyweight. Complete clinical responses were observed in 6 of the 8 patients. Acute GVHD of the gut disappeared completely in 6 patients, and of the liver and skin in one patient. No side effects or ectopic tissue formation were seen[38].

More recently, Le Blanc et al. conducted a phase II study enrolling 55 patients with severe steroid-resistant grade II to IV acute GVHD[39]. MSCs were infused at a median dose of 1.4×10^6 per kg of bodyweight. Thirty patients displayed a complete response and 9 patients a partial response. Sixteen patients had stable or progressive disease. Survival of patients with complete response was significantly higher than the patients with partial or no response. No side effects were observed[39].

In one more clinical trial, 13 patients with steroid-refractory acute GVHD were treated with bone marrow-derived MSCs expanded without bovine serum, in platelet lysate-containing medium with the mean

dose of 0.9×10^6 per kg of bodyweight[40]. Two patients showed clinical responses.

Further, a clinical study enrolling 32 patients with acute GVHD was performed. Patients with grade II to IV GVHD were randomized to receive 2 treatments of MSCs at a dose of either 2 or 8×10^6 per kg of bodyweight in combination with corticosteroids. Seventy-seven percent complete responses and 16% partial responses were reported. No MSC infusion-related toxicities or ectopic tissue formation was found. Comparing the low and high MSC dose, there was no difference between safety and efficacy results[41].

CONCLUSIONS

Current data indicates that MSCs represent an attractive tool for clinical applications. MSCs efficiently inhibit the expansion and activation of different cellular components of innate and adaptive immunity. They have also emerged as a promising tool for the treatment of immune-mediated disorders including GVHD, graft rejection, and autoimmune diseases.

Some preliminary studies using MSCs for treatment of GVHD patients showed encouraging clinical responses in the absence of severe side effects or ectopic tissue formation. However, additional studies concerning MSC preparation techniques, immunogenicity, tumorigenic potential, their migration, homing, and survival *in vivo*, as well as the optimal dose, frequency, and route of administration should improve the efficacy and safety of MSC-based therapeutic strategies.

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