Immunological characteristics of mesenchymal stem cells

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Although bone marrow is the main source, mesenchymal stem cells have already been isolated from various other tissues, such as the liver, pancreas, adipose tissue, peripheral blood and dental pulp. These plastic adherent cells are morphologically similar to fibroblasts and have a high proliferative potential. This special group of cells possesses two essential characteristics: self-renewal and differentiation, with appropriate stimuli, into various cell types. Mesenchymal stem cells are considered immunologically privileged, since they do not express costimulatory molecules, required for complete T cell activation, on their surface. Several studies have shown that these cells exert an immunosuppressive effect on cells from both innate and acquired immunity systems. Mesenchymal stem cells can regulate the immune response in vitro by inhibiting the maturation of dendritic cells, as well as by suppressing the proliferation and function of T and B lymphocytes and natural killer cells. These special properties of mesenchymal stem cells make them a promising strategy in the treatment of immune mediated disorders, such as graft-versus-host disease and autoimmune diseases, as well as in regenerative medicine. The understanding of immune regulation mechanisms of mesenchymal stem cells, and also those involved in the differentiation of these cells in various lineages is primordial for their successful and safe application in different areas of medicine.

Keywords: *Stem cells; Mesenchymal stem cells/immunology; Autoimmune diseases/therapy; Immunomodulation; Immune system; Cell differentiation*

Introduction

Mesenchymal stem cells (MSCs) are plastic adherent cells that are morphologically similar to fibroblasts and are capable of self-renewal and multilineage differentiation under appropriate conditions[1]. This capacity to generate mature cells of a particular tissue has been shown in several studies where MSCs were able to differentiate into osteoblasts, chondrocytes, adipocytes and even neurocytes[2-5]. Due to these special characteristics, in addition to the availability of these cells in several tissues, their high proliferation rate, and the lack of ethical and legal problems with their use in research, MSCs are considered one of the most promising types of stem cells for tissue engineering and the application of stem cell therapies in the prevention and treatment of different conditions[6-8].

MSCs were first isolated from the bone marrow and are found in the stroma of this tissue. In bone marrow, MSCs are an important component of the hematopoietic stem cell (HSC) niche, the best characterized adult stem cell until now[1,9]. The search for MSC-like cells in specific tissues has led to the discovery of a variety of stem cells in different tissues and organs, including in the pancreas, liver, cornea, retina, adipose tissue, umbilical cord, peripheral blood, intestine and dental pulp[2,5,10-12]. The presence of stem cells in different structures of the organism suggests that these cells are involved in the repair and/or regeneration of tissues throughout the life of an individual[13].

Although there is a wide range of positive markers described for MSCs, until now no single specific marker has been identified. For this reason, biological characteristics such as plastic adherence, expression of certain surface markers, self-renewal and capacity of differentiation to other cell types are important requirements for a cell to be considered a MSC[1]. Some of the most common surface markers expressed by MSCs include STRO-1, CD29, CD44, CD73, CD90, CD105, CD106, CD146, SSEA-1 and SSEA-4, while these cells are negative for CD31, CD34, CD45, CD80, CD86 and HLA-DR[9,14-22].

Several studies have demonstrated the superior immunomodulatory capacity of MSCs over different cells from the immune system[18,22-32]. This unique feature of MSCs has great importance in the field of transplantation, treatment of autoimmune diseases, as well as in the modulation of inflammatory responses.

Immunological profile of mesenchymal stem cells

By flow cytometry, human MSCs isolated from bone marrow present positive expression for major histocompatibility complex (MHC) class I molecules, minimal expression for

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MHC class II and do not express the co-stimulatory molecules CD40, CD40 ligand, CD80 and CD86[22,23,27,33]. MSCs stimulated with the pro-inflammatory cytokine interferon γ (IFN-γ) can upregulate the expression of MHC class I molecules and induce the expression of MHC class II[22,27,34], but are not able to modify the expression of co-stimulatory molecules[22,27]. Besides attempting to increase the stimulatory capacity of MCSs, treatment of these cells with IFN-γ aims to reproduce an inflammatory condition in the organism, as MSCs can be used at sites of inflammation[27].

When MSCs are induced to differentiate along the adipogenic, osteogenic and chondrogenic lineages, they express MHC class I, but not MHC class II molecules on their surface. A slight increase in the expression of MHC class II on cells differentiated in the chondrogenic lineage, no expression of these molecules in the adipogenic lineage and even a decreased expression of MHC class II molecules on cells differentiated in the osteogenic lineage were observed after cell treatment with IFN-γ for 48 hours when compared with undifferentiated MSCs[34].

This phenotype observed in undifferentiated and differentiated MSCs (along the adipogenic, osteogenic and chondrogenic lineages) is considered non-immunogenic and suggests that these cells can induce tolerance. MHC class II molecules can activate alloreactive T cells, but in the absence of co-stimulatory molecules, the secondary signal would not be activated, leading to the anergy of T cells[3].

Immunomodulatory capacity of mesenchymal stem cells

Normally, allogeneic cells are deleted by the host immune system. A major surprise to immunologists was that MSCs do not follow these 'rules' of immune rejection. Apart from not being recognized as alloantigens, MSCs are able to suppress the activation and proliferation of different cells of the host immune system[22,28,29,31,35].

Dendritic cells

It has been observed that MSCs are capable of interfering in the differentiation, maturation and function of dendritic cells (DCs)[25,30,35], which are considered the most efficient antigen-presenting cells (APCs), playing a crucial role in orchestrating cellular and humoral immune responses against self and foreign antigens[36]. MSCs and their supernatants can interfere in the endocytosis of DCs, and are able to affect the capacity of these cells to secrete interleukin-12 and activate alloreactive T cells[25]. The inhibition of molecules associated with antigen presentation, such as CD40, CD83, CD80, CD86 and HLA-DR, was observed during DC differentiation when these cells were in contact with MSCs[25]. These results agree with those observed in other studies, where MSCs prevented the differentiation of monocytes from peripheral blood into DCs[30,37]. The DC cytokine secretion profile can be altered by MSCs, stimulating the production of anti-inflammatory molecules, such as interleukin-10, and inhibiting the release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-12[18].

T cells

Results of different studies have shown that when MSCs are co-cultivated with mixed lymphocyte cultures, T cell proliferation is suppressed[22,27,34,38,39]. This immunosuppressive ability of MSCs was demonstrated in different species, such as in humans, mice[40] and baboons[41]. It seems that the inhibitory effect of MSCs is dose-dependent, since when higher concentrations of stem cells are added to the cell culture, the inhibition is more pronounced[38,39]. Likewise, this unique ability of MSCs to inhibit T cell alloresponses appears to be independent of the major histocompatibility complex, as similar results were obtained regardless of whether autologous or allogeneic MSCs were used[39].

Allogeneic MSCs were not capable of inducing T cell proliferation even when the culture medium was supplemented with the potent pro-inflammatory cytokine IFN-γ, or when MSCs were transfected with co-stimulatory molecules. Conversely, T cells responded vigorously to allogeneic peripheral blood mononuclear cells (PBMCs). Cytokines with APC function, such as interleukin-1α, interleukin-1β and TNF, were ineffective in generating T cell proliferative responses to allogeneic MSCs. This may be due to the active suppressive mechanisms of MSCs over T cell proliferation, rather than the lack of immunogenicity or tolerance induction[27].

MSCs can interfere in the differentiation of naive $CD4^+$ T cells into T helper 1 (Th1) effector cells. When MSCs were present during T cell differentiation, there was a significant decrease in the amount of IFN-γ production (about 50%). In contrast, when MSCs cells were present during Th2 cell differentiation, there was a significant increase in the amount in interleukin-4 production. These results indicate that MSCs may play an anti-inflammatory and regulatory role, directing the profile of cytokines produced by these immune cells[18].

Cytotoxic T lymphocytes (CTLs) can lyse allogeneic cells after antigen recognition via MHC class I molecules[36]. When MSCs were added early in a mixed lymphocyte culture (MLC) they inhibited the cytotoxicity, presumably by preventing the formation of active CTLs. However, little effect on cytotoxicity was noted when these cells were added to MLC in the cytotoxic phase of CTLs. MSCs were not lysed by allogeneic CTLs, suggesting that these cells can escape recognition by cytotoxic lymphocytes[42].

MSCs differentiated into adipocytes, osteoblasts and chondrocytes retain the ability of inhibit the proliferation of T cells in mixed lymphocyte cultures. Also, the suppressive effect on T cells was increased as was seen in cultures of MSCs differentiated along the osteogenic lineage[34]. A proliferative response of T cells was not observed when these two cell types were co-cultured even after treatment of osteogenic-differentiated MSCs with IFN-γ [27].

B cells

MSCs exert an inhibitory effect on the proliferation of B cells[28,31,43] and are able to suppress terminal differentiation of these cells into plasmocytes[28,31]. In addition, MSCs can inhibit the chemotactic properties of B cells, since the B cells chemokine receptors CXCR4, CXCR5, CXCL12 and CXCR4 ligand are downregulated when B cells are co-cultured with MSCs[28].

This suppressive effect of MSCs on the B-cell function was also evaluated *in vivo*. The humoral factor(s) of MSCs released in culture medium were capable to suppress antigen-specific IgM and IgG1 secretion in mice immunized with T-cell-dependent and T-cell-independent antigens[31]. These results confirm previous *in vitro* studies, where the addition of MSCs to a mixed lymphocyte culture[44] or the co-culture of MSCs and purified B cells from peripheral blood[28] suppressed the production of IgM, IgG and IgA.

Natural killer cells

Natural killer (NK) cells, which are considered the major effector cells of innate immunity, are also influenced by the immunomodulatory potential of MSCs. When MCSs were co-cultured with NK cells (stimulated by interleukin-2), there was a decreased secretion of IFN-γ from these cells[18]. Furthermore, activating receptors such as NKp30 and NKp44 were downregulated on these innate immune cells. Likewise, a marked decrease in the cytolytic capacity of NK cells was observed[29].

The expression of MHC class I molecules by MSCs protects them against certain detection mechanisms by NK cells. Tumor cells, as well as virus infected cells, which have little or no expression of MHC class I, are usually killed by NK cells[33].

Immunosuppressive mechanisms of mesenchymal stem cells

Although data from several studies suggest that the use of MSCs in regenerative therapies could be successful, the mechanisms responsible for the tolerance of the host immune system to MSCs are not fully understood[33]. Some possible mechanisms have been identified, such as the induction of a local immunosuppressive microenvironment by MSCs, the ability of immunomodulation of the immune cell phenotype by MSCs and the lack of immunogenicity of MSCs. Possibly, all these mechanisms are inter-related and involve both direct contact between cells and indirect mechanisms, through the production and release of soluble factors, such as cytokines[33].

The involvement of soluble molecules in the suppressive activity of MSCs on T cells was demonstrated in a study where $CD4^+$ T and $CD8^+$ T cells were co-cultured with MSCs in transwell chambers. Although the cells were not in direct contact, a reduction in T cell proliferation was noted[23]. Similar results were found by other studies, evidencing the immunosuppressive effect of MSCs over T[27] and B cells[31], even in the presence of the membrane, indicating that cell-cell contact is not essential. Interleukin-10, transforming growth factor β (TGF-β), hematopoietic growth factor (HGF), prostaglandin E2 (PGE_2), indoleamine 2,3-dioxygenase (IDO) and nitric oxide (NO) were some of the soluble molecules associated with the immunosuppressive effect of MSCs[18,23,33,45-47].

Another important soluble molecule involved in the immunoregulation of MSCs is HLA-G5. This is a non-classical human leukocyte antigen (HLA) class I protein that protects the fetus against rejection from the maternal immune system[48]. The HLA-G5 isoform released by MSCs after contact with allostimulated T cells contributes to the immunomodulatory properties of MSCs, as this molecule can suppress allogeneic T cell proliferation and can also induce the expansion of $CD4^+CD25^{high}FOXP3^+$ regulatory T cells (Tregs). With regard to innate immunity, HLA-G5 is able to inhibit the lysis of MSCs mediated by NK cells, as well as the secretion of IFN-γ by these cells[49].

For Krampera et al., the inhibitory effect of MSCs on T cells is due exclusively to direct contact between the two cell types, where MSCs physically hinder T cells from entering in contact with APCs, impairing antigen presentation and, consequently, preventing the activation and proliferation of T cells[38].

Although several studies have identified different soluble factors as possible mechanisms of immune regulation by MSCs, blocking any of these molecules does not result in a complete loss of the immunosuppressive capacity of MSCs, suggesting that immune regulation by MSCs is a complex phenomenon, where distinct mechanisms act synergistically[50].

Tregs play an important role in the induction of peripheral tolerance and inhibition of pro-inflammatory immune responses[32]. This special cell population suppresses the activation of the immune system, maintaining organism homeostasis and tolerance to autoantigens[18]. It was suggested that MSCs exert their immunosuppressive activity inducing an increase in the proportion of $CD4^+CD25^+$ Tregs[18]. Besides providing the significant expansion of Tregs, MSCs are able to increase the inhibitory capacity of these cells[32].

Therapeutic application of mesenchymal stem cells

The immunosuppressive nature of MSCs is of great relevance in the field of allogeneic transplantations, since this 'immunologically privileged' cell population may be used to reduce the incidence and severity of graft-versus-host disease (GVHD)[39]. Severe acute GVHD is a serious complication associated with the transplantation of allogeneic hematopoietic grafts. The observation that MSCs induce the inhibition of mixed lymphocyte cultures and T cells, regardless of the MHC, may have important clinical implications.

Le Blanc et al. reported a surprising improvement in the clinical response of a 9-year-old boy with severe acute GVHD of the gut and liver, resistant to conventional therapy, after an infusion of allogeneic MSCs. The patient had developed diarrhea of up to 20 times daily and a high concentration of bilirubin. Four days after an intravenous infusion of MSCs (2×10^6 cells per kg), the frequency of diarrhea fell to twice daily and there was a decline in total bilirubin[6].

In a phase II experimental study, which included 55 patients with severe steroid-resistant acute GVHD (grades III and IV), a complete response to an infusion of allogeneic MSCs isolated from bone marrow (1.4×10^6 cells per kg) was observed in 30 patients and nine showed improvements. Survival of patients with complete response was significantly higher than those with partial response or no response to treatment with MSCs[51].

Several studies have shown that MSCs contribute to tissue repair and that these cells might be useful in the regeneration of different tissues of the organism. Allogeneic murine MSCs injected directly into the infarcted heart, or administered intravenously in animals were able to home to the site of the

injury and prevent deleterious remodeling of heart tissue[7]. In an experimental model of Duchenne muscular dystrophy in mice, human MSCs isolated from the synovial membrane, promoted the regeneration of skeletal muscle tissue[52]. Likewise, the transplantation of stem cells from human exfoliated deciduous teeth (SHEDs) with hydroxyapatite and tricalcium phosphate was able to repair bone defects produced in the calvaria of mice with substantial bone formation[53].

Osteogenesis imperfecta (OI) is a genetic disorder caused by a deficiency in the production of type I collagen, the major structural protein in bone, resulting in bone fragility and growth deficiency[54]. Horwitz et al. reported a significant increase in body length and in the bone mineralization in five children with severe OI after allogeneic bone marrow transplantation from HLA-compatible donors[55]. In another study, an acceleration of growth in six children with OI was observed after infusions of purified bone marrow MSCs[56]. These studies indicate that the use of MSCs to treat severe OI may be a promising approach for this condition.

The known immunomodulatory characteristics of MSCs has also been used to develop therapies to treat autoimmune diseases[50]. In a murine model of experimental arthritis treatment, the administration of human adipose-derived MSCs significantly reduced the incidence and severity of the disease. This therapeutic strategy decreased the production of various inflammatory cytokines and chemokines, decreased antigen-specific Th1/Th17 cell expansion and induced the production of interleukin-10 in lymph nodes and joints. Additionally, the generation of antigen-specificTregs with the capacity to suppress self-reactive T effector responses was observed[57]. In relation to autoimmune type I diabetes, the administration of allogeneic murine MSCs delayed the onset of disease in pre-diabetic non-obese diabetic (NOD) mice, promoting a shift toward Th2 immune response[58]. Similarly, murine MSCs were able to prevent beta cell destruction in NOD mice through the induction of Tregs[59]. In the search for more effective and safe therapies to treat systemic lupus erythematosus (SLE), Sun et al. used an infusion of allogeneic bone marrow MSCs (1 x 10^6 cells per kg) in four patients with acute disease and nephritis caused by SLE, unresponsive to intravenous cyclophosphamide and oral prednisone. Promising results were found, such as the recovery of the levels of Tregs ($CD4^+Foxp3^+$) and a significant improvement in renal function. Moreover, a stable 12-18 months disease remission was observed in all treated patients[60].

Conclusions

The evident immunomodulatory properties of MSCs over immune system cells, as well as the capacity of multi-differentiation and application of this special group of cells in the regeneration of damaged tissue, suggests that MSCs are a great potential for use in immunotherapy and tissue engineering.

However, well delineated *in vivo* studies regarding immunogenicity, mechanisms of immunomodulation and differentiation of MSCs, as well as the safe use of these cells in the long term are necessary for their application in different clinical conditions.

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