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Research paper

Immunomodulatory mechanisms of mesenchymal stem cells and their therapeutic applications

Don K. Lee[b], Sun U. Song[a,b,*]

[a] Dept. of Integrated Biomedical Sciences, Inha University School of Medicine, Incheon 22332 Republic of Korea
[b] SCM Lifesciences Co. Ltd., Incheon 22332 Republic of Korea

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ABSTRACT

In the recent years, many studies have shown that MSCs must be stimulated by pro-inflammatory cytokines or other immune mediators before they can modulate immune cells in inflamed and damaged tissues. MSCs appear to be involved in inducing several regulatory immune cells, such as Tregs, Bregs, and regulatory NK cells. This new immune milieu created by MSCs may establish a tolerogenic environment that leads to an optimal condition for the treatment of immune diseases. The mechanisms of MSC action to treat immune disorders need to be further investigated in more detail. Since there have been some contradictory outcomes of clinical trials, it is necessary to perform large-scale and randomized clinical studies, such as a phase 3 placebo-controlled double-blind study of a third party MSCs to optimize MSC administration and to prove safety and efficacy of MSC treatment. MSCs offer great therapeutic promise, especially for the treatment of difficult-to-treat immune diseases.

1. Introduction

Mesenchymal stem cells (MSCs) were initially isolated according to their clonogenic potential represented by the ability to appear as colony-forming units-fibroblasts (CFU-F). The frequency of CFU-F in the bone marrow is ~ one cell in 10^4–10^5 mononuclear cells [1]. MSCs are characterized by the expression of various cell surface antigens [2], but no cell surface antigens are known to be expressed exclusively by MSCs. Therefore, minimal criteria for defining MSCs became necessary, and thus, the International Society for Cellular Therapy issued guidelines based on the following criteria [3]: MSCs should be plastic adherent, have fibroblast-like morphology, express stromal markers CD73, CD90, and CD105, and be negative for the hematopoietic markers CD14, CD34, and CD45. In addition, tri-lineage potential for osteogenic, adipogenic and chondrogenic differentiation should be demonstrable. However, cell populations satisfying these criteria from different tissues are likely to still be heterogeneous. There are currently no unique markers that identify MSCs or characterize their subpopulations, and standardization has thus been difficult. Furthermore, there is a discrepancy between the behavior of *ex vivo* culture-expanded MSCs and fresh, non-manipulated MSCs [4]. The most popular conventional method for isolating MSCs relies on fractionation of mononuclear cells from various tissues, with or without protease treatment, by gradient centrifugation; fibroblast-like cells adhering to the culture plate surface are selected after non-adherent floating cells are removed. Due to the heterogeneity, MSCs isolated by the conventional method are suggested to be termed mesenchymal stromal cells, not mesenchymal stem cells, although both have the same acronym, MSC. Recently, a new isolation protocol, the subfractionation culturing method (SCM), was developed to generate single cell-derived clonal MSCs from whole bone marrow aspirates and adipose tissues without employing centrifugation and enzyme treatment processes [5,6]. Using the SCM, bone marrow- or adipose tissue- and single cell-derived colony-forming fibroblastic cells can be identified as MSCs from relatively small amounts of bone marrow or adipose tissue aspirate. This method allows for rapid establishment of single cell-derived human clonal MSC (hcMSC) lines from raw bone marrow or adipose tissue aspirates, and the establishment of a library of clonal MSC lines. Furthermore, manufacture of clinical-grade hcMSC products from single colony forming unit-derived colonies based on the SCM was reported [7]. As various phenotype and function studies revealed, MSCs isolated from different sources are not exactly equivalent and represent highly heterogeneous populations of cells that are dramatically affected by various extrinsic and intrinsic factors [8]. Therefore, standard protocols for the safe and efficacious production of homogeneous population of MSCs, and compliance with Good Manufacturing Practices (GMP) must be developed for the rapid commercialization of MSC therapeutics. The most important factors include donor eligibility and screening, various types of isolation and

* Corresponding author at: Dept. of Integrated Biomedical Sciences, Inha University School of Medicine, 366 Seohae-daero, Jung-gu, Incheon 400-712, Republic of Korea.
E-mail address: sunuksong@inha.ac.kr (S.U. Song).

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expansion protocols, and media and supplements with/without animal or human origin [9,10].

MSCs secrete a wide variety of different growth factors cytokines and adhesion molecules by which they affect the immune cells in inflamed and damaged tissues and thus regulate cells and repair tissues via positive paracrine effects [11-14]. Since MSCs exhibit significant immunomodulatory properties and are poorly recognized by the host immune system they are able to escape the immune system recognition mechanisms and modulate the defense mechanisms of the host [11,15]. MSCs can modulate many functions of activated T cells [16], B cells [17], NK cells [18], dendritic cells (DCs) [19,20], and macrophages [21,22]. MSCs can mediate their immunomodulatory activity in numerous models such as graft versus host disease (GvHD) [23,24], Crohn's disease [25], type 1 diabetes mellitus [26,27], atopic dermatitis [28], experimental autoimmune encephalomyelitis (EAE) [29], acute pancreatitis [30], model of contusive spinal cord injury and its subsequent inflammation-related damage [31,32], and many others [33]. They may also act as primary matrices in tissue repair processes caused by the inflammation and injury [34,35]. In this review, the immunomodulatory mechanisms of MSCs on immune cells and their therapeutic applications are discussed.

2. Immunomodulatory mechanisms of MSCs

2.1. Stimulation of immunomodulation of MSCs

Recently, accumulating data indicate that MSCs are not spontaneously immunosuppressive but that they require stimulation for the manifestation of their immunomodulatory properties. In particular, the most important priming factors of MSCs are IFN-γ, TNF-α, and IL-1β [36–39]. The release and binding of IFN-γ on its receptor expressed by MSCs are key steps for the induction of the immunomodulatory properties, not only for various T cell subtypes but also against B and NK cells, which are usually unresponsive to IFN-γ action in the absence of MSCs [16,17,20]. It has been shown that MSCs activated by IFN-γ can not only function as cells with immunomodulating properties, but also act as effective antigen-presenting cells (APCs) [40,41]. After either TNF-α or IL-1β stimulation, there is a significant change in the MSC phenotype. This includes an induction of MHC class I expression and increase of ICAM-1 and VCAM-1 expression [42]. There is also a de novo expression of MHC class II molecules that could theoretically support the APC function of MSCs. Additionally, the expression of programmed death ligand 1 (PD-L1) has been detected [38,43,44]. During the synergistic action of IFN-γ and TNF-α, increased expression of IL-6, IL-8, HGF, PGE-2, and cyclooxygenase-2 (COX-2) can be observed [45,46]. On the contrary, the use of IFN-γ alone may result in the induction of IDO and PD-L1 expression [38,46]. In addition, the co-activation of MSCs by IFN-γ and TNF-α induces the production of chemokines, such as CCR5, CCR10, CXCR3, CXCL9, and CXCL10, which are involved in chemotaxis and can inhibit proliferation of the immune system effector cells [43,45]. Through the complex interactions between the pro-inflammatory factors, the production and activity of various immunomodulatory molecules produced by MSCs are established [11-14] (Fig. 1). Besides these pro-inflammatory cytokines, in relation to the activation of MSCs, signaling through the Toll-like receptors (TLRs) has been described [47]. Activation through TLR3 with polyinosinic:polycytidylic acid (poly I:C) induced anti-inflammatory phenotype of MSCs where production of IDO, PGE-2, IL-4, and IL-1RA was detected [48,49]. These findings suggest that it is necessary for MSCs to be stimulated before they show immunomodulatory effects.

2.2. Immunomodulatory effects of MSCs on immune cells

The most intriguing aspect of the biology of MSCs is their immunomodulatory effects on immune cells. These include suppression of T cell proliferation, induction of M2 macrophages and regulatory T and B cells, suppression of dendritic cell maturation and function, and modulation of NK cells [50]. These re-educated immune cells gather to create a tolerogenic environment suitable to modulate the immune response (Fig. 1).

2.2.1. Immunomodulatory effects on T cells

MSCs are able to suppress T cell activation proliferation and differentiation and they can induce cells that have regulatory properties [16]. MSCs inhibit the division of stimulated T cells by preventing their entry into the S phase of the cell cycle and by mediating irreversible G0/G1 phase arrest [51]. MSCs also induced arrest of T cell division in mixed lymphocyte reactions (MLRs) and this T cell inhibition does not appear to be antigen specific [52]. It is highly possible that T cell proliferation is inhibited in allogeneic and xenogeneic settings as well. Recently it was showed that in mild- and severe-acute pancreatitis rat models human clonal MSCs recovered pancreatic function by reducing infiltration of CD3+ T cells into the injured pancreatic tissue and by upregulating Foxp3+ regulatory T cells. In addition inflammatory cytokines were decreased and anti-inflammatory cytokines were increased in these tissues [30]. This group also reported that MSCs induced apoptosis of T cells *in vitro* [30]. Recently Lee et al. showed that the interaction of ICOSL on MSCs and ICOS on regulatory T cells was involved in inducing Foxp3+ regulatory T cells and subsequent IL-10 secretion [53].

2.2.2. Immunomodulatory effects on macrophages

Macrophages can be derived from circulating inflammatory monocytes, which are recruited to tissue sites of inflammation. In response to environmental signals, different populations of macrophages with distinct functions may arise, the pro-inflammatory M1 macrophages and the anti-inflammatory M2 macrophages [54]. It was reported that IDO activity in MSCs is involved in the generation of anti-inflammatory M2 macrophages. These M2 macrophages are involved in T cell inhibition in an IL-10–independent manner, thereby amplifying the immunosuppressive effects of MSCs [22]. Intriguingly, MSCs induce M2 macrophages via the constitutive secretion of IL-6 that are characterized by concomitant elevated production of IL-10 [55]. PGE2 regulates the production of a wide range of cytokines. IL-6 is involved in M2 macrophage generation and PGE2 up-regulates both IL-10 and IL-6 in activated macrophages [55,56]. IL-6 is a central player because it is induced by PGE2, and IL-6 positively regulates COX2 and EP2/EP4 [57,58]. These findings suggest that the interaction of IL-6 and PGE2 signaling pathways may play a role in the induction of macrophages from inflammatory monocytes by MSCs.

2.2.3. Immunomodulatory effects on DCs

MSCs also inhibit the differentiation of monocytes into immature DCs [59]. Expression by DCs of co-stimulatory molecules is down-regulated and DCs exhibit impaired cytokine production and a reduced ability to stimulate T cells. Aggarwal and Pittenger demonstrated that MSCs cause immature DCs to decrease TNF-α and mature DCs to increase IL-10 secretion [60]. Most studies that address the interaction between MSCs and APCs have demonstrated that MSCs modulate DCs at multiple levels. Overall MSCs alter the phenotype cytokine release differentiation and maturation of DCs and compromise their antigen presentation ability [20]. PGE2 is a potent inducer of IL-10 and both factors have a pivotal role in the cross-regulation of DCs. Thus PGE2-induced IL-10 is a key regulator of the bone marrow-DC (BMDC) pro-inflammatory response [61]. MSCs have the ability to induce regulatory DCs (MSC-DCs) with T cell-suppressing properties. Most important MSC-DCs are accompanied by elevated IL-10 secretion [62,63] and MSC-derived PGE2 plays a central role in such immunomodulation [19,64]

2.2.4. Immunomodulatory effects on B cells

B cell proliferation is inhibited by MSCs in a dose dependent manner