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Interactions Between Mesenchymal Stem Cells and Dendritic Cells

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Abstract Mesenchymal stem or stromal cells (MSC) are considered a promising new therapeutic strategy for the treatment of several pathological conditions. Due to their immunomodulatory properties, they are currently employed in clinical trials aimed at preventing or treating steroid-resistant acute graft-versus-host disease (GvHD), a frequent complication of allogeneic hematopoietic stem cell transplantation (HSCT). In addition, the use of MSC has been proposed for the treatment of autoimmune diseases. A number of recent studies have focused on the influence of MSC on dendritic cell (DC) function. DCs play a critical role in initiating and regulating immune responses by promoting antigen-specific T cell activation. Moreover, they are involved in efficient cross-talk with different cells of the innate immune system. DC are the most effective antigen-presenting cells and prime na T cells to initiate adaptive immune responses including those against allogeneic cells or self-antigens. Thus, alteration of DC generation or function may greatly contribute to the inhibition of T cell responses. In this context, MSC were shown to interfere with DC maturation from monocytes or $CD34^+$ hemopoietic precursors thus further confirming their role in immune regulation and their usefulness in cell-based therapies.

Keywords DC cytoskeleton rearrangement · DC maturation · DC phenotype · Dendritic cell differentiation · Dendritic cells · Hematopoietic stem cell transplantation · Immune synapse · MSC licensing · MSC-mediated immunosuppression · Notch-2 · PGE2 · Tolerogenic DC · Treg cells · Unrestricted somatic stem cells

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List of abbreviations

Ag	Antigen
DC	Dendritic cells
GM-CSF	Granulocyte macrophage-colony stimulating factor
GvHD	Graft-versus-Host Disease
HGF	Hepatocyte growth factor
HO-1	Haemoxygenase-1
HSCT	Hematopoietic stem cell transplantation
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon-γ
IL	Interleukin
LPS	Lipopolysaccharide
MHC	Major histocompatibility complex
MLR	Mixed lymphocyte reactions
MSC	Mesenchymal stem cells
NK cells	Natural killer cells
NO	Nitric oxide
PGE2	Prostaglandin E2
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α

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1 Introduction

Mesenchymal stem cells (MSC) are known for their characteristic of being multipotent stem cells, capable of forming bone, cartilage, and other mesenchymal tissues [20]. In particular, in vitro experiments demonstrated that clonal MSC can differentiate into different lineages including not only osteoblasts, chondrocytes, and adipocytes but also muscle cells, cardiomyocytes, and neural precursors. Moreover, MSC are a component of the bone marrow stroma that have been shown

to support hematopoiesis by providing suitable cytokines and growth factors [15]. More recently, another function has been ascribed to MSC: a strong immunosuppressive effect on cells of both innate and adaptive immunity including T and B cells, natural killer (NK) cells, and dendritic cells (DC; [18], [24]). The mechanisms underlying the MSC-mediated inhibitory effect are only in part understood. Both contact-dependent mechanisms and soluble factors are thought to be involved in the induction of MSC-mediated immunosuppression. The first step in these interactions usually involves cell-to-cell contact mediated by adhesion molecules. Subsequently, several soluble factors appear to be involved in MSC-mediated immunoregulation, produced constitutively by MSC or released as a result of the interaction with other cell types. Examples of these molecules are indoleamine 2,3-dioxygenase (IDO) and nitric oxide (NO), which are released by MSC only after triggering by interferon-γ (IFN-γ). Other soluble factors, including transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), prostaglandin E2 (PGE2), hemoxygenase-1(HO-1), interleukin (IL)-6, and soluble HLA-G5, are constitutively produced by MSC. In addition, the production of some of these molecules can be increased by cytokines, such as IFN-γ, released by cells interacting with MSC [6, 12].

In the setting of allogeneic hematopoietic stem cell transplantation (HSCT), MSC have been brought to the clinic mainly to promote hematopoietic engraftment and for prevention/treatment of graft-versus-host disease (GvHD) [7]. The use of MSC for clinical purposes takes advantage of their poor immunogenicity in vitro, in pre-clinical studies, and in human studies, which supported the possible infusion of MSC from allogeneic donors in adoptive immunotherapy. The therapeutic potential of MSC is currently being explored in a number of phase I/II and II clinical trials [23]. So far, most of the data reported in these studies have accounted for the safety of infusion of culture-expanded allogeneic MSC, together with sustained hematopoietic engraftment after HSCT, reduced incidence of GvHD [3], and, in the case of steroid-resistant acute GvHD, markedly improved survival rate of MSC-infused patients [13].

DC play a critical role in initiating and regulating immune responses by promoting antigen (Ag)-specific T cell activation [4, 5]. In addition, as revealed by recent studies, they can efficiently interact with and trigger or modulate cells of the innate immune system [9, 16, 17]. DC are the most effective antigen-presenting cells and prime naïve T cells to initiate adaptive immune responses including proliferative responses to allogeneic cells, that can be tested in vitro in mixed lymphocyte reactions (MLR). Some DC reside in an immature state in peripheral tissues and are highly specialized in Ag uptake. Immature DC (iDC) display low levels of major histocompatibility complex (MHC) and co-stimulatory molecules (CD80, CD86) at their surface. Remarkably, in mature DC, these molecules become highly upregulated. Immature DC can be rapidly recruited at the site of inflammation where Ag capture and processing primarily occur. After Ag cleavage into peptides, peptide loading on MHC molecules and migration to T cell areas of the draining lymph nodes, DC undergo complete maturation. Mature DC (mDC) lose their uptake capability while they acquire the ability to stimulate T cells

including unpolarized Th0 or polarized Th1 or Th2 responses and also tolerogenic T cells.

In 2005 [1], Aggarwal and Pittenger first reported that bone marrow-derived MSC could influence the outcome of an ongoing inflammatory immune response by altering the cytokine secretion profile of peripheral blood DC resulting in a shift from a pro-inflammatory immune response towards an anti-inflammatory or tolerant cell environment. In particular, they showed that MSC could decrease tumor necrosis factor-α (TNF-α) secretion by CD1c+ myeloid DC subset while inducing increases of IL-10 production by BDCA-4$^+$ plasmacytoid DC [1]. In addition, Beyth et al. [8] showed that MSC could inhibit T cells indirectly, by contact-dependent induction of regulatory or aberrant antigen-presenting cells, including dendritic cells, characterized by T cell suppressive properties.

Since then, a series of studies has been reported investigating the MSC-mediated inhibition of DC differentiation and function. However, in spite of the general evidence that MSC can inhibit the generation of functional DC, data on specific aspects of such inhibitory effects are contradictory, possibly reflecting differences in experimental protocols. In this chapter, we offer an overview of the most relevant data regarding DC-MSC interactions in humans.

2 MSC Inhibit Dendritic Cell Differentiation

A large body of evidence accounts for the ability of MSC to strongly inhibit DC generation from both monocytes and CD34$^+$ cell precursors. Indeed, MSC have been shown to affect the acquisition of DC-specific markers when added to monocytes induced to differentiate towards DC with granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-4 [10, 19, 22]. In particular, monocyte-derived cells obtained in the presence of MSC failed to express CD1a while still maintaining the monocyte marker CD14. Moreover, upon stimulation with lipopolysaccharide (LPS), which normally induces full DC maturation, cells expressed lower levels of CD80 and CD86 co-stimulatory molecules and of the DC maturation marker CD83 as compared to control cells. In agreement with an altered phenotype, cells did not display typical DC morphology (i.e., a veiled appearance and an abundant cytoplasm), but rather developed macrophage morphology [10]. On the other hand, different results were obtained by van den Berk et al. [25], who showed that cord blood-derived MSC (also called unrestricted somatic stem cells, USSC) did not interfere with DC differentiation from monocytes. Thus, immature DC generated in the presence of MSC lost CD14 and acquired normal levels of CD40, CD86, CD209, and HLA-DR. The different origin of MSC (cord blood vs. bone marrow) may explain these different results suggesting that MSC of different origin/site of isolation may display different functional properties.

It is of note that MSC-mediated inhibition of DC differentiation was not accompanied by cell loss. Indeed, Jiang et al. [10] reported that cell viability was not affected by co-culture with MSC, and the cell recovery of these co-cultures

was comparable to that of control cultures. However, although no evidence existed of a pro-apoptotic effect induced by MSC on monocytes, MSC prevented monocytes from entering the G1 phase of the cell cycle with a progressive number of cells accumulating in the G0 phase [21]. Although monocytes do not require DNA synthesis and cell division to become functional DC, they must enter the cell cycle. MSC could arrest monocytes in G0 rendering them unable to stimulate allogeneic T cells. Downregulation of cyclin D2 expression was shown to be primarily responsible for cell cycle arrest.

An important aspect of the inhibition exerted by MSC is the reversibility of the effect. Jiang et al. [10] reported that inhibition of DC differentiation from monocytes was reversible. Thus, upon removal of MSC and addition of fresh cytokines, monocyte-derived cells acquired the DC phenotype, that is, loss of CD14 and acquisition of CD1a and CD83. Nauta et al. [19] showed that when MSC were removed after 2 days from monocyte cultures, cells downregulated CD14 but did not express CD1a, suggesting that, in this experimental setting, inhibition was only partially reversible and that an early conditioning with MSC was essential for the inhibitory effect. The discrepancy of the results may indeed reflect differences in the experimental settings. Jiang et al. performed their experiments under transwell culture conditions, however, Nauta et al. performed co-cultures in which cells were in direct contact.

Other groups investigated the effect of MSC on DC differentiation by using $CD34^+$ cells as DC precursors. Nauta et al. [19] demonstrated that MSC could prevent DC generation from $CD34^+$ cells derived from umbilical cord blood. In these studies, they also showed that MSC inhibited the differentiation of dermal/interstitial DC, by blocking the transition of $CD14^+CD1a^-$ intermediate prescursors to the $CD14^-CD1a^+$ differentiation stage, whereas they did not prevent the generation of $CD14^-CD1a^+$ Langerhans cells. Moreover, the $CD14^+CD1a^-$ subset expressed low levels of CD80, CD86, CD83, and CD40 after cell stimulation with LPS. In contrast, Li et al. [14] reported that MSC could inhibit the differentiation of both dermal/interstitial and Langerhans cells. In addition, they showed that MSC could also inhibit the proliferation of DC precursors by inducing a threefold decrease of their proliferation rate. Also in this case, variability in results may be explained in part by the fact that different DC precursors were used in these experiments (i.e., cord blood- vs. adult bone marrow-derived $CD34^+$ cells).

Regarding the functional capabilities of the phenotypically abnormal DC obtained in the presence of MSC, all studies reported that their ability to stimulate allogeneic T cell proliferation in MLR was strongly impaired as compared to control DC. In addition, these abnormal DC, generated either from monocytes or $CD34^+$ cells, produced very low levels of IL-12 upon stimulation with LPS [10, 22] or CD40L [19]. Moreover, it was shown that LPS-induced phosphorylation of p38, a kinase involved in an intracellular signaling pathway positively regulating IL-12 secretion, was greatly reduced in the presence of MSC [10]. However, so far, it is still poorly defined whether cells generated in the presence of MSC are simply DC with an impaired function or rather "educated" DC with regulatory activity. In the study by Li et al. [14], secondary allostimulation of T cells by DC generated in the

presence of MSC induced the generation of FoxP3-expressing alloantigen-specific T cells. This finding implies that MSC may promote differentiation of tolerogenic DC, capable of stimulating expansion of Treg cells. In this context, future studies aimed at further characterizing MSC-conditioned DC will help to clarify this point. In this context, it is possible that, depending on the DC/MSC source or experimental settings, different types of "nonclassical" DC may be generated capable of finally exerting immunomodulatory/anti-inflammatory effects.

3 Effects of MSC on the Final Maturation of DC

Another relevant question concerning DC-MSC interactions is whether MSC can interfere not only with the early generation of DC from their precursors but also with later stages of differentiation, such as the progression from immature to mature DC. Several groups investigated this point in their studies and obtained contradictory results. In some cases, MSC were shown to moderately suppress LPS-induced maturation of monocyte-derived DC. Thus, the resulting cells displayed decreased ability to stimulate allogeneic T cell proliferation in MLR, associated with lower levels of IL-12 production and IFN-γ induction compared to control mDC [10]. On the other hand, Spaggiari et al. [22] demonstrated that MSC failed to interfere with LPS-induced maturation of DC. Indeed, DC induced to mature in the presence of MSC displayed normal phenotype, with adequate levels of surface CD80, CD86, and CD83 molecules and were even more efficient stimulators in MLR than mDC obtained under standard conditions. In an interesting study by Aldinucci et al. [2], a new pathway of MSC-mediated regulation of DC function was proposed. Immature DC, stimulated with LPS in the presence of MSC, became unable to form active immune synapses with lymphocytes, despite their expression of a mature phenotype and a normal IL-12/IL-10 production profile. In addition, MSC-treated DC retained endocytic activity and podosome-like structures, typical of immature DC. The inability of DC to establish synapses was associated with alteration of the cytoskeleton rearrangement, consisting of absence of actin redistribution, which normally occurs in iDC upon stimulation by LPS. As a consequence, DC while undergoing some sort of differentiation retained features of immaturity, thus becoming unable to activate alloreactive T cells efficiently.

An opposite effect, promoting rather than inhibitory on LPS-induced maturation of iDC, seems to be exerted by USSC [25]. Immature DC stimulated with LPS in the presence of USSC displayed higher migratory capacity in response to CCL21 chemokine than control DC. Accordingly they expressed significantly higher levels of its specific receptor CCR7. Also IL-12 production was increased in cells that had undergone maturation in the presence of USSC. Interestingly, even in the absence of LPS, USSC could positively contribute to DC maturation by significantly increasing expression of CD80 and CD83 markers. However, in this study, DC were not analyzed for their capability of stimulating T cell response, thus it was not demonstrated whether these MSC-treated DC would be efficient antigen-presenting cells.

Few studies investigated the possible effects of the presence of MSC on fully mature DC. Zhao et al. [26] demonstrated that DC with regulatory activity could be generated by culturing fully differentiated, LPS-stimulated mDC with MSC. After conditioning with MSC, these cells acquired the ability to suppress T cell proliferation in MLR by producing TGF-β. Moreover, they could promote the generation of $CD4^+CD25^+FoxP3^+$ Treg cells from $CD4^+CD25^-$ T cells. Jiang et al. [10] showed that culture of mDC with MSC reverted DC to the phenotypic profile of an immature state, characterized by decreased surface expression of HLA-DR, CD80, and CD86 molecules.

4 Mechanisms Involved in the MSC-Mediated Inhibitory Effect: Cell Contact Versus Soluble Factors

Various molecular pathways appear to be involved in MSC-mediated immune regulation, including IFN-γ, IL-1β, TGF-β, IDO, IL-6, PGE2, HGF, TNF-α, NO, HO-1, and HLA-G5, most of which are strictly related and reciprocally activating. It is now largely accepted that the immunosuppressive activity is not a constitutive property of MSC, but depends on a process of activation or "licensing" to be acquired [11]. Activation of MSC is mostly consequent to cell exposure to inflammatory cytokines, such as IFN-γ, TNF-α, and IL-1α/β, which are produced by different cell types following induction of an inflammatory or immune response. These soluble activators can induce changes in both MSC phenotype and gene expression, thus allowing cells to act as immune regulators. As for other cells of the innate immune system, the inhibitory effect can be mediated by the activity of soluble factors produced by activated MSC and/or by cell contact involving specific receptor/ligand interactions not completely elucidated so far.

In the case of dendritic cells, most studies support the idea of a major involvement of soluble factors. Indeed, blocking of their activity or their production by using specific inhibitors could significantly, if not completely, restore DC differentiation and function. In this context, IL-6 and M-CSF were shown to be partially involved in the MSC-mediated inhibition of DC differentiation from monocytes, although only partial restoration of the DC phenotype (i.e., loss of CD14 but lack of expression of CD1a marker) could be obtained by using anti-IL-6 and anti-M-CSF neutralizing antibodies.

Another important MSC product, PGE2, was demonstrated as playing a major role in the inhibitory effect [22]. Indeed, PGE2 levels were strongly increased in the supernatants of monocyte-MSC co-cultures as compared to those of monocytes alone. Moreover, the selective inhibition of cyclooxigenase-2 activity and thereby of PGE2 synthesis almost completely reverted the inhibitory effect as confirmed by the restoration of both DC phenotype and function. Notably, this effect was achieved in spite of the presence of high levels of IL-6 in co-culture supernatants, thus suggesting that PGE2 and not IL-6 was predominantly involved in the

inhibitory effect. Other authors reported a substantial role played by cell-to-cell interactions. Li et al. [14] showed that, in co-culture experiments performed using the transwell chamber system to separate MSC and $CD34^+$ DC precursors physically, inhibition of DC differentiation was significantly prevented. Accordingly, the inhibitory mechanism proposed was an MSC-induced expression and subsequent signaling through the Notch-2 receptor in $CD34^+$-derived DC. The inhibition of Notch-2 signaling resulted in complete restoration of DC phenotype and function. It should be noted that, in this case, cell targets of the MSC-mediated inhibition were bone marrow-derived $CD34^+$ precursors and not peripheral blood monocytes. Thus, it is conceivable that different mechanisms may be responsible for the interference with distinct differentiation pathways.

The modality of the inhibitory effect may also depend on the differentiation stage of immune cells. Indeed, Aldinucci et al. [2] demonstrated that the alteration of LPS-induced cytoskeleton rearrangement in differentiated immature DC was contact-dependent and partially mediated by V-CAM and N-cadherin molecules expressed on the MSC cell surface.

5 Concluding Remarks

The regulation of DC function represents an important strategy in the design of innovative therapeutic protocols aimed at suppressing pathological immune responses, such as GVHD and autoimmune disorders. In this context, the suppressive effect that MSC can exert on immune cells, including DC, reveals a promising therapeutic strategy. Most studies addressing the interaction between MSC and DC have demonstrated that MSC are capable of inhibiting DC at multiple levels. Indeed, cells generated in the presence of MSC from DC progenitors, either monocytes or $CD34^+$ cells, do not display the proper DC phenotype and have impaired function as compared to control DC. However, there are no converging conclusions on the immunoregulatory phenomenon, possibly as a result of different experimental settings (including DC progenitor or MSC sources employed, cell-to-cell ratios used, and the consideration of only one single MSC-derived mediator for the inhibitory effect with no comparison with others). Moreover, so far, it has not been clarified what kind of cells are generated upon interaction with MSC, whether functionally impaired DC, characterized by lower capacity of efficiently stimulating T cell proliferation and by altered cytokine profile, or regulatory DC, capable of generating $CD4^+CD25^+FoxP3^+$ Treg cells. Future studies aimed at clarifying these points will contribute to better knowledge of MSC biology and, it is hoped, to the optimal use of MSC in clinical practice.

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