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Review Article

Immune modulation by mesenchymal stem cells

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ARTICLE INFORMATION

Article Chronology:
Received 8 February 2006
Revised version received 22 March 2006
Accepted 23 March 2006
Available online 24 April 2006

Keywords:
Multipotent stem cells
Immune System
Prostaglandin
T cells
Graft versus host disease
Cytotoxicity
Proliferation

ABSTRACT

Mesenchymal stem cells (MSCs) have been shown to suppress activation of T cells both in vivo and in vitro. In vivo, this may be a way for the body to maintain homeostasis and inhibit immune activation in distinct compartments, such as the bone marrow and the interface between mother and fetus. MSCs modulate the immune function of the major cell populations involved in alloantigen recognition and elimination, including antigen presenting cells, T cells, and natural killer cells. The molecular mechanism that mediates the immunosuppressive effect of MSCs is not completely understood.
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0014-4827/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2006.03.019

Introduction

Mesenchymal stem cells (MSCs) can be isolated from postnatal bone marrow (BM), adipose tissue [1], placenta [2], and scalp tissue [3], as well as from various fetal tissues [4,5]. MSCs are tridifferential, having the capacity to form bone, cartilage, and fat [6,7]. In vitro studies have also shown that MSCs can form muscle [8–11], hepatocytes [12], endothelium [13], and cells of the central nervous system [14–16]. The cell plasticity potential of MSCs is under investigation. So far, the defined MSC has not shown the capacity to engraft and form defined tissues after transplantation of a single clone. Thus, the term multipotent mesenchymal stromal cell has been proposed as a new definition for these cells [17]. MSCs have shown promise as cellular therapy in cardiac repair, bone disorders, and metabolic diseases. One of the most intriguing features of MSCs is that they escape immune recognition and can inhibit immune responses. These phenomena are described in detail in this review.

MSCs and allogeneic recognition

There is an emerging body of data to suggest that MSCs escape recognition of alloreactive cells, or at least possess a hypo-immunogenic character [7,18–20], even when co-stimulatory CD28 signals are delivered [21]. Human and xenogenic rat MSCs have been found not to elicit interferon-gamma (IFN-γ) production by human peripheral blood mononuclear cells (PBMCs), in contrast to human and murine fibroblasts [20]. This allogeneic escape mechanism may be of therapeutic value because transplantation of allogeneic human MSCs in stock would be readily available, as opposed to culture of autologous MSCs or MSCs from donors related to each patient. An in vitro study suggested a stronger immunosuppressive effect of allogeneic MSCs than of autologous MSCs [22]. In addition, Klyushnenkova et al. [23] saw a significant proliferation in response to allogeneic MSCs that peaked on day 8, as compared to the alloreactions against PBMCs that peaked on day 6. Still, the response was never greater than 40% of the response against PBMCs. No proliferative response was left after removal of cells expressing HLA class II, CD14 and CD19. MSCs are generally believed to express HLA class I and can be induced by interferon-gamma (IFN-γ) to upregulate HLA class II. Nevertheless, Krampera et al. [24] used murine MSCs devoid of both HLA class I and II, whereas Potian et al. [19] used human MSCs that expressed both class I and II. Neither population showed immunogenic potential and both could inhibit immune responses. This may indicate that HLA expression on MSCs is not the major reason for immune escape and immune suppression. Furthermore, upregulation of HLA class II by IFN-γ still did not elicit a proliferative response [5,19,21,23]. This may indicate that MSCs could exert suppressive functions without inducing and immune reaction at sites of inflammation where inflammatory cytokines can upregulate expression of HLA.

In vitro suppression by MSCs

MSCs suppress lymphocyte proliferation induced by alloantigens in mixed lymphocyte cultures (MLCs) [18,19,21,25], mitogens such as phytohemagglutinin (PHA) [25–27], concanavalin A (ConA) [25,28], and tuberculin [20], as well as activation of T cells by CD3 and CD28 antibodies [21,24,29]. The inhibition increases with increased concentration of MSCs. Interestingly, a low concentration of human MSCs or MSC-derived culture supernatants have been shown to stimulate rather than inhibit MLCs [19,20,25]. The suppression by MSCs was greatest when added at the start of the MLC, but the MSCs also had some effect when added later [23,26]. Murine MSCs have been reported to inhibit the activation of T cells by strong inhibition of cyclin D2 and also by induced upregulation of the cyclin-dependent kinase inhibitor p27kip1 [30]. Without activation of cyclin D, the T cells remain in the G_0 phase of the cell cycle.

Suppression of immune responses by MSCs can be mediated by soluble factors because separation of MSCs and the activated PBMCs by a semipermeable membrane (Transwell) – which allows exchange of soluble factors without cell contact – still inhibits proliferation [21,23]. On the other hand, supernatants from human or murine MSC cultures show no suppressive capacity [19,20,27,31] unless the MSCs have been co-cultured with lymphocytes [20,28]. Groh et al. [32] cultured human MSCs with different enriched immune cell populations and found $CD14^+$ cells to induce the immunosuppressive feature of MSCs. Addition of interleukin 1β (IL-1β) to MSCs also endowed culture supernatants with suppressive capacity, suggesting that this is the factor secreted by monocytes to induce immune suppression by MSCs. In some systems, cell-to-cell contact may be required. Augello et al. [31] showed that cell-to-cell contact via the inhibitory molecule programmed death 1 (PD-1) and its ligands PD-L1 and PD-L2 was necessary for murine MSCs to inhibit activation of murine splenocytes, T and B cells. Fibroblasts have not shown suppressive capacity in MLCs when run in parallel with suppressive MSCs [19,20], thereby excluding crowding of cells as the suppressive mechanism. The possibility of a bulk effect has also been evaluated by the addition of irradiated T cells autologous with the responder cells in MLCs. This did not alter proliferation [26].

The effect of MSCs on lymphocytic subpopulations has been evaluated in several studies. Mitogen- and alloantigen-activated human $CD3^+$, $CD4^+$, and $CD8^+$ T cells were all inhibited by human MSCs [22,26,27]. Murine BM-derived MSCs were shown to inhibit mitogenic stimulation of murine T cells and B cells [31]. In these experiments, MSC culture supernatant showed no effect on T cell activation, whereas significant suppression of stimulated B cells was observed. The inhibitory effect of MSCs on B cells was confirmed after stimulation of murine splenic B cells with anti-CD40 and IL-4 in the presence or absence of murine MSCs [30].

Several studies have shown similar suppressive effects, both when using MSCs autologous or allogeneic to the responder cells, indicating a genetically unrestricted suppression [18,22,23,25]. Djouad et al. [28] showed that both human and mouse-derived MSCs could suppress xenogeneic MLCs. Similar xenogeneic suppression has been reported for minipig-derived MSCs, which inhibited proliferative responses of human PBMCs to mismatched allogeneic and porcine PBMCs [33]. Together, these results suggest general inhibitory mechanisms that may cross species barriers.

MSCs and antigen presenting cells

MSCs modulate dendritic cell (DC) and T cell function and promote the induction of suppressor or regulatory cells. MSCs have been found to inhibit upregulation of antigen-presenting cell (APC)-related molecules, such as CD1a, CD40, CD80 (B7-1), CD86 (B7-2), and HLA-DR during DC maturation [22,34,35]. Jiang et al. [36] showed that MSCs inhibited the in vitro generation of DCs from monocytes, both when in contact and when the MSCs were present in transwell inserts [36]. This inhibition was abrogated by removal of the MSCs and continuous culture of the monocytes in DC-promoting medium. The cells isolated from cultures that had been co-cultured with MSCs showed a reduced potential to activate CD4$^+$ T cells to proliferation, measured in MLCs (DCs$^+$ CD4$^+$ cells), as well as by pulsing DCs with keyhole-limpet hemocyanin and culturing them with CD4$^+$ cells [36]. Maccario et al. [22] also confirmed a reduction in DC formation with MSCs present. Reduced pro-inflammatory cytokines, such as IFN-γ, IL-12, and tumor necrosis factor-alpha (TNF-α) in MSC/monocyte co-cultures, have also been reported, together with increased production of IL-10 [29,35,36]. Taken together, these results suggest that a key mechanism of MSC-mediated inhibition of lymphocyte proliferation is to direct maturing APCs toward a suppressor phenotype that results in an attenuated T cell response. Nevertheless, MSCs inhibit T cell proliferation by mechanisms that do not require APCs, using direct stimulation with CD3 and CD28 antibodies, enriched T cell populations or T cell clones [21,24–26].

MSCs and anergy

Naive T cells circulate in the blood and lymphatic system in the quiescent G_0 phase of the cell cycle. On encountering the appropriate antigen, they proceed through the cell cycle and form effector or memory cells. Interactions occur between the T cell receptor/CD3 complex and HLA/peptide, together with co-stimulatory signals provided by CD28 (T cell) and B7 (APC). CD28 is expressed on both resting and activated T cells, but activated T cells also express cytotoxic T-lymphocyte antigen-4 (CTLA-4), an inhibitory ligand to B7, that downregulates the activation of the cell. Without co-stimulation, the naive T cell becomes unresponsive. An anergic cell does not proliferate or secrete IL-2 in response to appropriate antigenic stimulation. It does, however, express the IL-2 receptor (IL-2R), and the anergy can be abrogated by the addition of exogenous IL-2 [37].

MSCs lack surface expression of the co-stimulatory molecules B7-1 (CD80), B7-2 (CD86), and CD40 [21,23,38]. Thus, MSCs can render T cells anergic. Several studies have shown that the proliferation of suppressed T cells to allogeneic cells, mitogens, or IL-2 is restored after removal of MSCs [23,24,26]. Klyushnenkova et al. [23] showed that the lack of response to MSCs was not due to a deficiency in co-stimulation because retroviral transduction of MSCs with B7 did not result in increased T cell proliferation.

MSCs have been demonstrated to induce a split anergy phenotype in T cells. Glennie et al. [30] showed that removal of MSCs from inhibited cultures only restored IFN-γ production, and not proliferation of murine PBMCs, despite addition of exogenous IL-2 [30]. Whereas others have shown a resumed proliferative capacity upon secondary stimulation [23,24,26], Maccario et al. [22] demonstrated resumed proliferation of CD4$^+$ but not CD8$^+$ cells.

MSCs and regulatory T cells

Regulatory T cells are thought to have a critical role in the suppression of immune responses. This naturally occurring subset of CD4$^+$ cells that express CD25 (the α-chain of the IL-2R) was first described by Sakaguchi et al. [39] in mice. Regulatory T cells have proven to be important in protection against autoimmune diseases [39,40]. This subgroup of naturally occurring suppressor cells has also been described in humans and constitutes about 5–10% of peripheral CD4$^+$ T cells [41].

MSCs increased the proportion of the regulatory subsets CD4$^+$ CD25^{bright}, CD4$^+$ CTLA-4$^+$, and CD4$^+$ CD25$^+$ CTLA-4$^+$ in MLCs [22]. When PBMCs were cultured with MSCs in the absence of stimulatory PBMCs, less than 10% of CD4$^+$ T cells expressed CD25 and/or CTLA-4 molecules, indicating that lymphocyte stimulation other than the presence of MSCs was needed to increase the number of regulatory T cells. Aggarwal and Pittenger [29] also demonstrated an increase in the proportion of CD4$^+$ CD25$^+$ in IL-2 stimulated PBMCs co-cultured with MSCs. In contrast, Beyth et al. [35] showed that removing CD25$^+$ cells from the CD4$^+$ subpopulation before stimulation with monocytes had no effect on inhibition by MSCs. This may indicate that MSCs potentiate the expansion of regulatory T cells but do not stimulate the T cells to formation of new regulatory cells from naive T cells. Other studies have evaluated CD25 as an activation molecule, where MSC-induced inhibition of mitogen-stimulated T cells reduced the expression of CD25, as well as CD38 and CD69 [27,32].

MSCs and cell-mediated cytotoxic responses

NK cells and cytotoxic T cells (CTLs) are important cytotoxic effector cells for elimination of transformed or infected cells. CTLs are generated from CD8$^+$ precursors that, upon activation, are reactive against peptides expressed on HLA class I. In contrast to CTLs, NK cells are constitutively cytotoxic cells that mainly target cells that lack expression of HLA class I [42]. NK cells express several different inhibitory and activating receptors, where the inhibitory killer immunoglobulin-like receptors (KIR) recognize HLA class I alleles. Thus, HLA class I expressing cells can be lysed by NK cells if the targets are KIR-ligand mismatched and the target cells do not express inhibitory HLA class I alleles [43,44].

Several studies have shown a suppressive effect of MSCs on cytotoxicity. We and others have shown that MSCs are capable of inhibiting alloantigen-specific cytotoxic activity in a dose-dependent fashion when present during the priming of cytotoxic cells in MLCs [22,45]. Cytotoxic activity against human MSCs was not detected, and addition of MSCs to the lysis assay showed no effect on lysis. Angoulvant [46] showed that human MSCs suppressed the induction of cytotoxic

responses to alloantigens. PBMCs and MSCs derived from the same donor were used as stimulators to trigger CTLs. PBMCs induced formation of active CTLs that could lyse various targets, including MSCs, whereas MSCs did not stimulate to lysis of target cells. Addition of MSCs to cultures stimulated with PBMCs led to a lower frequency of active CTLs in a dose-dependent manner. There was partial recovery of target cell lysis by addition of IL-2. Potian et al. [19] proposed that MSCs could blunt the cytotoxic effects of alloreactive CTLs to stimulator target PBMCs, whereas fibroblasts derived from the same donor as the MSCs had no effect [19]. Human MSCs were also reported to inhibit IFN-γ production by IL-2-stimulated NK cells [29]. MSCs themselves did not induce production of IFN-γ by NK cells, indicating that they do not activate NK cells, nor are KIR-ligand mismatched MSCs lysed by NK cells [45]. Kang et al. [47] published that human MSCs reduced the lytic response of CTLs against viral infected PBMCs. The MSCs did not completely suppress the lysis, unless the production of IFN-γ by MSCs was inhibited by short interfering RNA. IFN-γ knockdown MSCs yielded an even stronger suppression of CTL lysis compared to untreated MSCs present, indicating that MSCs secrete IFN-γ and thereby adjust the response of the CTLs.

It has been proposed that MSCs can function as "veto cells". Veto-mediated suppression is based on infusion of a low dose of cells that delete $CD8^+$ T cells reactive against the infused cells, thereby inducing a transient state of tolerance in the host [48]. In 2003, Potian and co-workers reported that MSCs inhibited lysis when added to the lysis assay, and suggested that this was a "veto-effect" [19]. However, others have not seen an inhibitory effect of MSCs in the lytic phase of CTLs or NK cells [22,45]. In the same year, Djouad et al. [28] reported that murine MSCs induced formation of $CD8^+$ regulatory cells that were responsible for the inhibition of allogeneic lymphocyte proliferation. After depletion of $CD8^+$ cells from the responder population, MSCs showed no effect on proliferation. Splenocytes that were depleted of $CD8^+$ cells after primary MLCs showed no inhibitory effect in secondary MLCs, whereas the portion that contained $CD8^+$ cells transferred suppression to the culture [28]. Still, MSCs can suppress proliferation in enriched $CD4^+$ cell cultures, thereby excluding an effect on $CD8^+$ cells as the sole inhibitory mechanism. Independent of the mechanism, MSCs can inhibit cytotoxic effector responses, which make MSCs an interesting cellular therapy against diseases where damage is induced by $CD8^+$ cells.

Soluble versus contact-dependent inhibition by MSCs

Several studies have shown that the inhibition elicited by MSCs is mediated by soluble factors. Transforming growth factor-β (TGF-β) is the potential candidate that has been most studied. Di Nicola et al. [26] showed that the suppression induced by human MSCs of responder T cells against stimulator PBMCs could be abrogated by high concentrations of neutralizing antibodies against TGF-β1 and hepatocyte growth factor (HGF). Blocking of each factor separately resulted in a minimal effect on inhibition, whereas neutralization of the cytokines simultaneously restored all proliferation of T cells. Simultaneous addition of recombinant TGF-β1 and HGF to MLCs induced a similar degree of suppression to that when using MSCs. Le Blanc et al. [27] failed to reproduce this. These reports may not be comparable because Di Nicola and co-workers used enriched T cells and allogeneic stimulation, whereas Le Blanc analyzed proliferation in unseparated mitogen-stimulated PBMCs. We have shown that suppression by MSCs may be caused by different mechanisms depending on whether the T cells are stimulated with mitogens or allogeneic cells [49], which could explain these different findings (see Table 1). Angoulvant et al. [46] showed that neutralization of these factors partially restored CTL formation after suppression by MSCs. Enriched T cells were stimulated with allogeneic PBMCs to yield active CTLs, strengthening the evidence that TGF-β1 and HGF could mediate the suppression by MSCs in alloantigen-stimulated enriched T cells. Similar to the results of Di Nicola and co-workers, several studies have excluded a single role for TGF-β in MSC-induced suppression [21,24,35].

The characterization of cytokines produced by MSCs is still rudimentary and has been hampered by the diversity of cells and culture systems used. Studies have not detected IL-2, IL-4, or IL-10 in unstimulated MSC cultures [23,49,50]. However, IL-1, IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, IL-27, GM-CSF, G-CSF, and M-CSF can be detected [19,29,51,52]. Di Nicola et al. [26] tried to restore proliferation by neutralizing IL-6 and IL-11, but neither of these factors appeared to be of importance in MSC-mediated suppression of alloresponses. Even though IL-10 is not secreted constitutively by MSCs, increased IL-10 levels have been reported in MLCs when MSCs are present [23,35,49]. The inhibitory effect of MSCs on cytokine release and proliferation of T cells has been partially restored by blocking IL-10 signaling [35]. Enriched $CD4^+$ cells were co-cultured with monocytes and staphylococcal enterotoxin B (SEB) in the presence of MSCs. The addition of neutralizing antibodies against IL-10R partially restored proliferation as well as IFN-γ and TNF-α production. Meanwhile, neutralization of IL-10 in MLCs potentiated the inhibition, showing the complexity of IL-10 [49].

Several possible mechanisms concerning MSC-mediated suppression have been evaluated. Stromal cell-derived factor 1 (SDF-1) was investigated as a potential candidate. SDF-1 exerts chemotactic activities at low doses, whereas high concentrations can repel T cells [53]. Moreover, BM and thymic stroma, which produce SDF-1 in abundance, lack extensive infiltration of mature T cells [54]. SDF-1 was not detected on the cell surface of MSCs even after treatment with inflammatory cytokines [27]. However, a low level of soluble SDF-1 was detected in culture supernatants, but the addition of neutralizing antibodies against SDF-1 to MLCs co-cultured with MSCs showed no effect on inhibition.

Another mechanism that has been investigated is the possible role of receptor activator of NF-κB ligand (RANK-L) and osteoprotegerin (OPG). RANK-L is expressed by activated lymphocytes and promotes the survival and function of dendritic cells, T cell activation, and communication between T cells and DCs in vitro [55]. RANK-L signaling is blocked by OPG, a soluble decoy receptor produced by stromal cells [56]. Human MSCs express OPG mRNA [57] and secrete low levels of OPG. However, neutralization of OPG appeared to have no effect on the inhibition of PHA-stimulated lymphocytes [27].

Table 1 – Potential candidates responsible for MSC-induced immune suppression

Responder	Stimulatory agent	MSC	Neutralizing factor	Reduced inhibition	Response measured	Ref.
TGF-β and/or HGF						
PBMCs	Anti-CD3+ Anti-CD28	+	Anti-TGF-β1	NO	Proliferation	[21]
PBMCs	Superantigen SEB	+	Anti-TGF-β1, 2	NO	IFN-γ production	[35]
$CD2^+$ T cells	PBMCs	+	Anti-TGF- β1	NO	Proliferation	[26]
$CD2^+$ T cells	PBMCs	+	Anti-HGF	NO	Proliferation	[26]
$CD2^+$ T cells	PBMCs	+	Anti-TGF-β1 + Anti-HGF	YES	Proliferation	[26]
PBMCs	Mitogen PHA	+	Anti-TGF-β1 + Anti-HGF	NO	Proliferation	[27]
T cells	PBMCs	+	Anti-TGF-β1 + Anti-HGF	YES	CTL formation	[46]
PGE_2						
PBMCs	Anti-CD3+ Anti-CD28	+	Indomethacin	NO	Proliferation	[21]
PBMCs	Mitogen PHA	+	Indomethacin	YES	Proliferation	[29]
DC1 [a]	LPS	+	NS-398	YES	TNF-α production	[29]
T_{H1} [b]	Mitogen PHA	+	NS-398	YES	IFN-γ production	[29]
PBMCs	Mitogen PHA	+	Indomethacin	YES	Proliferation	[49]
PBMCs	PBMCs	+	Indomethacin	NO	Proliferation	[49]
IDO						
T cells	PBMCs	+	Tryptophan	YES	Proliferation	[65]
PBMCs	Anti-CD3+ Anti-CD28	+	Tryptophan	NO	Proliferation	[21]
PBMCs	Anti-CD3+ Anti-CD28	+	IDO-inhibitor	NO	Proliferation	[21]
Various factors						
$CD2^+$ T cells	PBMCs	+	Anti-IL-6	NO	Proliferation	[26]
$CD2^+$ T cells	PBMCs	+	Anti-IL-11	NO	Proliferation	[26]
PBMCs	PBMCs	+	Anti-SDF-1	NO	Proliferation	[27]
PBMCs	Mitogen PHA	+	Anti-OPG	NO	Proliferation	[27]
PBMCs	PBMCs	+	Anti-IL-10	NO	Proliferation	[49]
$CD4^+$ T cells +monocytes	Superantigen SEB	+	Anti-IL-10R	YES	IFN-γ production	[35]
				YES	TNF-α production	
				YES	Proliferation	

Addition of MSCs to stimulated cells inhibited the various responses. This table lists the different factors neutralized in the various assays and indicates whether the inhibition induced by MSCs was effected. Where the inhibition was reduced (indicated by YES), only a partial restoration of the effector response was seen.
[a] DC1 cells refer to $CD1a^+$ cells of the myeloid lineage, cultured in GM-CSF and IL-4.
[b] T_{H1} cells refer to culture of $CD45RA^+$ T cells stimulated with IL-2, IL-12, anti-IL-4.

Prostaglandin E2 (PGE_2) is presently one of the more intriguing candidates for MSC-induced immune suppression. PGE_2 influences numerous immune functions, including B cell activation [58] and induction of regulatory T cells [59]. Cyclooxygenase (COX) enzymes are involved in the synthesis of PGE_2. The COX-1 isoform is constitutively expressed and COX-2 is upregulated during inflammation. MSCs constitutively express both COX-1 and COX-2, resulting in a constitutive production of PGE_2 [29,60]. Both COX-2 and PGE_2 production have been found to be increased upon co-culture of MSCs with PBMCs [21,29]. However, the role of PGE_2 in MSC-mediated suppression is contradictory. Inhibition of COX activity and subsequently PGE_2 synthesis by indomethacin reduced PGE_2 expression in co-cultures of MSCs and PBMCs stimulated with anti-CD3/CD28 antibodies, but without restored proliferation [21]. Aggarwal and Pittenger recently showed that inhibition of PGE_2 synthesis by either indomethacin or NS-398 could restore the greatest part of proliferation of mitogen-activated PBMCs co-cultured with MSCs [29]. Even the obstructed TNF-α and IFN-γ secretion by activated DCs and T cells was restored when PGE_2 synthesis was blocked. The different roles of PGE_2 might be explained by the nature of the T cell stimulus that is used. Blocking of PGE_2 partially restored proliferation in mitogen-stimulated cultures but had no effect in MLCs [49].

Expression of indoleamine 2,3-dioxygenase (IDO) is induced by IFN-γ and it catalyzes the conversion of tryptophan to kynurenine. Active IDO thus causes depletion of tryptophan, resulting in reduced lymphocyte proliferation [61–63]. Regulatory suppressor T cells were found to be induced by depletion of tryptophan by IDO-expressing plasmacytoid DCs [64]. Human MSCs do not express IDO constitutively, but IDO protein and functional IDO activity were detected upon stimulation of MSCs with IFN-γ [65]. Reduced tryptophan levels were also detected in MLCs suppressed by MSCs, and the addition of tryptophan significantly restored T cell proliferation. Besides tryptophan depletion, the conversion of tryptophan to kynurenine can result in kynurenine breakdown products that also mediate inhibition of T cell proliferation [66]. Tse et al. [21] excluded a role for IDO in MSC-induced suppression because the addition of tryptophan or an IDO inhibitor (1-methyltryptophan) showed no effect on suppression. IDO-mediated suppression of T cells was reported to induce apoptosis of thymocytes and T_{H1} cells,

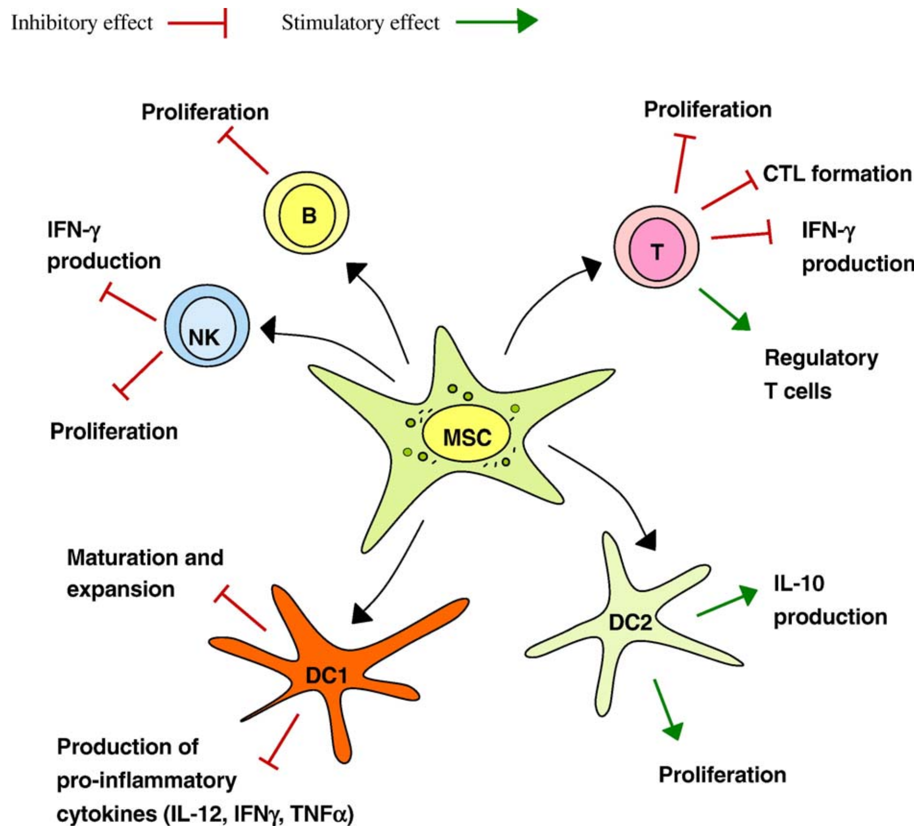

Fig. 1 – Schematic illustration of the effects of MSCs on the immune system. NK, B, and T refer to NK, B, and T cells, respectively. DC1: mature monocytic dendritic cells; DC2: mature plasmacytoid dendritic cells.

but not of T_{H2} cells [67]. Several studies have demonstrated that MSCs do not increase apoptosis in the suppressed cultures [21,26,68]. However, a recent report has proposed that MSCs inhibit proliferation by inducing apoptosis of activated T cells [69]. This apoptosis was related to the conversion of tryptophan to kynurenine by IDO.

The results concerning MSC-induced suppression of cells of the immune system are complex and may include different inhibitory and stimulatory mechanisms (Fig. 1). Different studies may appear contradictory; however, this may depend on which model systems are used: whether enriched cell populations or unfractionated PBMCs, the species and source of MSCs, the isolation protocol, and the timing of measurement. Variation in these parameters could probably lead to different results. The lack of a clear-cut definition of what constitutes MSCs also makes the analyses difficult to compare. Most studies on immune regulation by MSCs have used human or mouse bone marrow-derived MSCs. A recent report showed that minipig-derived MSCs did not induce proliferation of human PBMCs, while minipig-derived PBMCs did [33]. Minipig MSCs inhibited mitogenic stimulation as well as allo- and xenogeneic proliferation of human PBMCs. Neutralizing antibodies against FasL (CD95L) and TGF-β1 were found (separately) to restore all proliferation of ConA-stimulated PBMCs. Neutralization of IL-10 slightly increased inhibition. This study by Liu and co-workers has partly corroborated studies using human and murine MSCs. However, in several reports it has been excluded that TGF-β1 alone has a significant role and may be a feature specific for porcine-derived MSCs.

Immune modulation by MSCs in vivo

The immunosuppressive capacity of MSCs has also been evaluated in vivo. Bartholomew et al. [18] demonstrated that intravenous administration of MSCs derived from BM of baboons prolonged the survival of allogeneic skin grafts. The magnitude of suppression obtained with a single dose of MSCs injected intravenously was similar to that of potent immunosuppressive drugs currently used in the clinic [70,71]. A second infusion of MSCs did not extend skin graft survival; neutrophils eventually infiltrated the graft and rejection occurred. Grinnemo et al. [72] studied whether human MSCs could survive and engraft in experimentally induced ischemic rat myocardium. Rat PBMCs were analyzed for xenogeneic responses against human MSCs in vitro 1 week after injection of MSCs. MSCs induced significant lymphocyte proliferation in PBMC cultures of immunized rats, but no proliferation was seen in PBMCs from rats not injected with MSCs. There was significant infiltration of (primarily) macrophages in the area of injection in immunocompetent rats. Although MSCs have been shown to be transplantable across allogeneic barriers, this study suggests that xenogeneic transplant rejection may occur.

In 2003, Djouad et al. [28] demonstrated two aspects of in vivo suppression of MSCs. Allogeneic murine MSCs could engraft and form bone in immunocompetent mice. However, lymphocytic infiltrates were seen at the periphery of the newly formed bone, possibly indicating that MSCs awoke an

immune response. Still, the allogeneic bone was not rejected. Suppression of the immune system is a vital therapeutic tool, but Djouad et al. also showed that the MSCs facilitated tumor development. MSCs infused systemically or adjacent to subcutaneously placed melanoma cells increased tumor formation in immunocompetent allogeneic mice. When injected subcutaneously, the MSCs were seen in the stroma surrounding the tumor, whereas systemically infused MSCs could not be detected. Melanoma cells or MSCs injected alone did not give rise to tumors [28].

Murine MSCs have been shown to prevent experimental autoimmune encephalomyelitis (EAE) in mice [68]. EAE is a mouse inflammatory disease model of human multiple sclerosis. Intravenous administration of MSCs before onset of disease ameliorated development of EAE. The therapeutic scheme was effective when MSCs were administered at onset and at the peak of disease, but not after disease stabilization. CNS pathology showed decreased inflammatory infiltrates and reduced demyelination in mice transplanted with MSCs.

MSCs and graft-versus-host disease

Acute graft-versus-host disease (GVHD) is a complication following allogeneic hematopoietic stem cell transplantation (HSCT), where the immunocompetent cells in the graft react against host-derived antigens [73–75]. The HSC graft contains a mixture of cells, including mature T cells. At the HSCT, cells are infused into a host that has been profoundly damaged by underlying disease and by conditioning, which results in activation of host cells with secretion of proinflammatory cytokines such as TNF-α and IL-1 [75–77]. A mild form of GVHD is beneficial to avoid relapse of the underlying disease, especially leukemia, but can in its more severe forms be lethal [78–80]. Acute GVHD occurs when donor T cells react to host APCs with sequential activation of donor T cells [81,82]. Human MSCs suppress the formation of CTLs and also alter the cytokine profile and maturation of APCs. MSCs may therefore be a potential cellular therapy for GVHD. Murine MSCs have been found to significantly increase the survival rate after HLA-mismatched murine allogeneic HSCT [83]. Co-transplantation of MSCs with hematopoietic cells resulted in both a lower GVHD score and reduced serum levels of IFN-γ. Lazarus et al. [84] performed two phase I studies to evaluate the feasibility of transplanting MSCs to improve engraftment of HSCs, as well as to reduce GVHD. In 1995, they isolated and culture-expanded BM-derived MSCs from 23 patients with hematological malignancies in complete remission. Autologous MSCs were infused intravenously and no adverse reactions were observed. In 2005, a second phase I trial was reported, where culture-expanded MSCs were co-infused with HLA-identical HSCs in 46 hematological malignancy patients. MSCs were administered 4 h before infusion of HSCs without any infusion-related adverse events, ectopic tissue formation, or increased GVHD in response to the allogeneic MSCs [85]. These studies focused on evaluating the safety of MSC infusions, but any clinical benefit of MSC infusions still remained to be established. One case report presented a 20-year-old woman with acute myeloid leukemia treated with HSCT combined with MSCs from her haplo-identical father [86]. The patient engrafted rapidly without acute or chronic GVHD, the reported follow-up being 31 months. Perhaps the most striking effect of MSCs to combat acute GVHD was presented by Le Blanc and co-workers in 2004. A leukemia patient treated with allogeneic HSCT presented with grade IV acute GVHD of the gut and liver [87]. He was unresponsive to all types of immunosuppression and was given an infusion of MSCs from his HLA haplo-identical mother. Recovery, in terms of improved liver values and intestinal recovery was prompt. Upon discontinuation of cyclosporin A, the patient's acute GVHD recurred but was still responsive to a second MSC infusion. In vitro data showed no proliferation against the MSCs, indicating that he was not immunized after the first or second infusion. The ability of MSCs to inhibit the development of – or reverse – acute GVHD may be due to soluble factors secreted by the MSCs. There are at least two possibilities: (1) the inhibition of alloreactive T cells by some immunosuppressive factor and (2) the release of factors that could increase the healing rate of wounded tissues. A combination of both possibilities may be the most likely scenario. Le Blanc et al. [87] found female epithelial cells in the intestine of the transplanted male leukemia patient described above, which may indicate that the MSCs had been incorporated into the healed intestinal tissue.

Maternal–fetal immune suppression

The body desires to maintain homeostasis and only induce immune reactions when beneficiary. The regulatory effects of MSCs may be vital to inhibit immune activation and potentiate a well-functioning immune system. Mesenchymal stromal cells are important for the positive selection of thymocytes in the thymus [88]. Bacigalupo et al. [89] investigated whether the lack of immunoprotection by MSCs may be important in patients with marrow failure. The ability of MSCs to down-regulate T cell priming, proliferation, and cytokine release was deficient in patients with severe aplastic anemia. This raises the question whether the suppressive feature of MSCs has to be activated at some state by functioning immune cells.

A recent review by Barry et al. [90] focused on the striking similarity between immune suppression by MSCs and the maternal acceptance of the fetal allograft. Careful immune regulation is needed to keep the fetus alive and well in the uterus because the mother is not immunologically ignorant and can delete circulating fetal cells without rejection of the fetus [91]. These immunomodulatory changes are linked to suppression of inflammatory cytokines and to the induction of T cells with regulatory or suppressive phenotypes. Foremost among the mediators of such effects are IL-10 [92,93], TGF-β [94], HGF [95], PGE_2 [96], and IDO [97]. The tryptophan concentration in maternal circulation falls steadily during pregnancy [98,99]. The fact that human MSCs can be isolated from several fetal tissues, as well as the placenta, amniotic fluid [100], fetal blood [4], and term umbilical cord blood [101] strengthen the theory that MSCs may have a role in fetal

acceptance. Fetal MSCs show expression of surface markers that is similar to that of adult BM-derived MSCs [4,5,102]. Fetal MSCs express HLA class I, but not HLA class II [103]. Mitogen-stimulated proliferation of PBMCs was inhibited by human fetal MSCs. However, unlike adult MSCs, fetal MSCs were not found to inhibit MLCs [5]. Additional studies by the same group showed that fetal MSCs could suppress proliferation in MLCs after treatment of the MSCs with IFN-γ [103]. Further, comparisons between adult and fetal MSCs may provide key clues to understanding the mechanisms of inhibition.

Acknowledgments

Work associated with this article was funded by the Swedish Cancer Society (0070-B04-18XCC, 0070-B03-17XBC, and 4562-B03-XAC), the Children's Cancer Foundation (03/039 and 03/007), the Swedish Research Council (K2003-32X-05971-23A and K2003-32XD-14716-01A), the Tobias Foundation, the Stockholm Cancer Society, the Swedish Society of Medicine, the Sven and Ebba-Christina Hagberg Foundation, and the Karolinska Institutet.

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