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Review

Immunomodulatory functions of mesenchymal stem cells and possible mechanisms

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Summary. In addition to their well-studied self-renewal capabilities and multipotent differentiation properties, mesenchymal stem cells (MSCs) have been reported to possess profound immunomodulatory functions both *in vitro* and *in vivo*. More and more studies have shown that MSCs are capable of interacting closely with almost all subsets of immune cells, such as T cells, B cells, dendritic cells, natural killer cells, macrophages, and neutrophils etc. The immunomodulatory property of MSCs may shed light on the treatment of a variety of autoimmune and inflammation-related diseases. In this article, we will review the studies on the immunomodulatory and anti-inflammatory functions of MSCs and the mechanisms responsible for the interaction between immune cells and MSCs, which could improve the development of promising approaches for cell-mediated immune therapies.

Key words: Mesenchymal stem cells, Immunomodulation, Cell therapy, Regenerative medicine

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Introduction

Mesenchymal stem cells (MSCs) were first described by Fridenstein and colleagues (Friedenstein et al., 1968, 1970, 1976), as clonogenic non-hematopoietic stem cells and plastic adherent fibroblast-like populations, possessing self-renewal and multipotent differentiation capabilities and were regarded as new therapeutic tools for tissue engineering and regenerative medicine (Caplan, 1991; Prockop, 1997; Pittenger et al., 1999). Although only a small percentage of the total number of mononuclear cells can be isolated by density gradient centrifugation, approximately 0.01-0.001% (Pittenger et al., 1999), the main source of MSCs is bone marrow, and thus bone marrow derived MSCs are intensely studied. In addition, it is demonstrated that MSCs can be obtained from virtually all adult human tissues (Covas et al., 2008) and MSCs have been successfully isolated and characterized in a wide spectrum of post-natal tissue types, including adipose tissue, placenta, amniotic fluid, umbilical cord blood, and orofacial tissues, etc (Zuk et al., 2001; In 't Anker et al., 2003a, 2003b, 2004; Bieback et al., 2014; Liu et al., 2014).

Characterization of MSCs

In the absence of an agreed standardized marker, MSCs are typically defined by a combination of phenotypic and functional characteristics. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed

minimal criteria to define human MSCs as following (Dominici et al., 2006): (1) MSCs must be plastic-adherent in standard culture conditions. (2) MSCs must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and human leucocyte antigen (HLA)-DR surface molecules. (3) MSCs must differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*. Recent studies indicated that MSCs not only differentiate into cells of the mesoderm lineage, but also into endoderm and neuroectoderm lineages, including neurons (Sanchez-Ramos et al., 2000), hepatocytes (Schwartz et al., 2002), and endothelia (Caplan and Bruder, 2001). In addition to the expression profiles of the above mentioned cell surface molecules, MSCs are also positive for STRO-1 and CD146 (Shi and Gronthos 2003), neural ganglioside GD2 (Martinez et al., 2007), and embryonic stem cells markers, such as Octamer-4 (Greco et al., 2007), stage-specific embryonic Ag 1 (SSEA-1) (Anjos-Afonso and Bonnet, 2007), and SSEA-4 (Gang et al., 2007).

Immunological properties of MSCs

Except for high self-renewal and multilineage differentiation capacities, MSCs have recently been shown to possess unique immunoregulatory properties, both *in vitro* and *in vivo*, and may play specific roles as immunomodulators in maintenance of peripheral tolerance, transplantation tolerance, and autoimmunity. Making use of multiple pathways, MSCs have been demonstrated to modulate the functions of a broad range of immune cells, including T cells, B cells, natural killer (NK) cells, and antigen presenting cells, macrophages, neutrophils, etc, which make them promising candidates in cell-based therapies of a variety of immune and inflammation-related diseases. Herein, we present an overview of MSC-mediated immune regulation.

Immune phenotype and immunogenecity of MSCs

MSCs express low level of major histocompatibility complex (MHC) class I molecules, and express minimal or no MHC class II molecules nor co-stimulatory molecules, such as CD40, CD80, CD86 on their surface, therefore they do not exhibit antigen presenting activities (Fibbe and Noort, 2003; Le Blanc et al., 2003a,b; Tse et al., 2003; Angoulvant et al., 2004; Klyushnenkova et al., 2005). The addition of interferon-γ (IFN-γ) to the cultures of MSCs increases the expression of MHC class I molecules and triggers the expression of MHC class II molecules, but not of the co-stimulatory molecules (Le Blanc et al., 2003a,b; Tse et al., 2003). In addition, MSCs differentiated into adipose, bone and cartilage cells also exhibit MHC class I molecules, but the expression of MHC class II molecules can no longer be induced (Le Blanc et al., 2003a,b). In co-culture experiments, MSCs fail to induce proliferation of allogenice lymphocytes *in vitro* (Bartholomew et al., 2002; Le Blanc et al., 2003a,b; Klyushnenkova et al., 2005), even after provision of a co-stimulatory signal by addition of CD28-stimulating antibodies or transfection of CD80 or CD86 co-stimulatory molecules (Tse et al., 2003), or MSCs were pre-treated with IFN-γ (Le Blanc et al., 2003a,b), thus indicating their low immunogenecity. However, recent studies involving bone marrow MSCs demonstrated that stimulation by IFN-γ at low levels induces MHC class II-mediated antigen presentation in MSCs both *in vitro* and *in vivo* (Chan et al., 2006; Stagg et al., 2006; Romieu-Mourez et al., 2007; Tang et al., 2008; François et al., 2009), and MSCs have the ability to capture and release antigens (Sánchez-Abarca et al., 2013). As for adipose tissue-derived MSCs, it was shown that early passage cells (P0/P1) induced a proliferative response of allogeneic T cells, whereas later passage cells did not (McIntosh et al., 2006), and in another study, adipose tissue-derived MSCs significantly increased the proliferation of allogeneic T cells after a 7-day-coculture (Crop et al., 2010).

MSCs-T cells interaction

MSCs are able to suppress T lymphocyte activation and proliferation *in vitro* irrespective of the sources of MSCs from human (Di Nicola et al., 2002; Le Blanc et al., 2003a,b; Potian et al., 2003; Tse et al., 2003), rodents (Djouad et al., 2003; Krampera et al., 2003), or baboon (Bartholomew et al., 2002). This inhibition affects the proliferation of T cells induced by alloantigens (Djouad et al., 2003; Krampera et al., 2003), mitogens (Di Nicola et al., 2002), mixed lymphocyte reaction (MLR) (Maitra et al., 2004) as well as activation of T cells by CD23 and CD28 antibodies (Krampera et al., 2003; Tse et al., 2003). Suppression is MHC independent and mostly marked if MSCs are added on the first day of the 3- or 5-day culture (Krampera et al., 2003; Le Blanc et al., 2003a,b).

MSCs could efficiently suppress the proliferation of CD4+ T lymphocytes (Di Nicola et al., 2002; Glennie et al., 2005; Krampera et al., 2006) as well as CD8+ T lymphocytes (Di Nicola et al., 2002; Djouad et al., 2003; Rasmusson et al., 2003; Aggarwal and Pittenger, 2005; Krampera et al., 2006). MSCs significantly inhibited CD4+ T lymphocytes proliferation triggered by both cellular and nonspecific mitogenic stimuli at the suppressive rate of 65±5%, which is independent of the expression of cell-activation markers and induction of cell apoptosis, and may require the presence of IFN-γ produced by activated T cells (Di Nicola et al., 2002; Krampera et al., 2006). In the presence of signals that favor T-helper (Th) cells 1 development, naïve T cells mature into IFN-γ-secreting cells. If MSCs are present in this process, MSCs cause Th1 cells to decrease IFN-γ and cause the Th2 cells to increase secretion of IL-4 (Aggarwal and Pittenger, 2005), thus inducing to Th2 differentiation and towards a more anti-inflammatory phenotype (Tang et al., 2008). Moreover, MSCs have been shown to be able to suppress differentiation of

cytolytic CD8+ T lymphocyte precursors into effectors (Angoulvant et al., 2004). MSCs inhibited cytolytic CD8+ T lymphocytes-mediated cell lysis if added at the beginning of MLR, whereas the lysis was not affected on day 3 or in the cytotoxic phase (Rasmusson et al., 2003). Once cytotoxic T cells are activated, MSCs are not effective (Martinez et al., 2007; Romieu-Mourez et al., 2007). Further, MSCs have been reported to induce formation of CD8+ T regulatory cells that were responsible for inhibition of allogeneic lymphocyte proliferation (Bartholomew et al., 2002).

Furthermore, the population of CD4+CD25+FoxP3+ regulatory T cells (Tregs), which display an immunosuppressive activity, has been generated in co-cultures of mitogen-stimulated T cells with MSCs (Aggarwal and Pittenger, 2005; Maccario et al., 2005; Prevosto et al., 2007). MSCs have been shown to be able to recruit, regulate, expand and maintain T-regulatory phenotype and function, thereby up-regulating the regulatory capacity of Tregs (Di Ianni et al., 2008; Selmani et al., 2008).

In addition to be implicated in graft-vs-host disease, gamma-delta T lymphocytes play an important role in cancer immunosurveillance and immunotherapy. MSCs effectively suppress the *in vitro* expansion of gamma-delta T cells without interfering with their cytotoxic activity (Petrini et al., 2009). In another study, MSCs have been demonstrated to inhibit the proliferation, cytokine production and cytolytic responses of gamma-delta T cells *in vitro* by the production of prostaglandin E2 (PGE2) (Martinet et al., 2009).

Th17, a newly described T-helper cell subset that produces IL-17, IL-17F and IL-22, is highly pathogenic during the inflammatory process and autoimmune diseases (Awasthi and Kuchroo, 2009). MSCs are able to reduce Th17 cells and their associated cytokines and decrease Th17 cell expansion *in vitro* and in animal models of experimental arthritis, encephalomyelitis, systemic lupus erythematosus, etc (Bai et al., 2009; González et al., 2009; Sun et al., 2009).

As mentioned above, MSCs markedly impaired proliferation, cytokine secretion, and cytotoxic potential of T lymphocytes, and studies recently showed that this effect is a fundamental property shared by all stromal cells, indicating that stromal cells represent promising candidates for the prevention and treatment of immune-mediated diseases (Haniffa et al., 2007; Jones et al., 2007; Zhao et al., 2010).

Apart from the effect of MSCs on T cells, T cells also influence the viability of MSCs and the benefical effect of MSC-based therapy. It was shown that activated T cells induce MSCs apoptosis via Fas/Fas ligand pathway, and proinflammatory T cells inhibit the ability of exogenously added bone marrow MSCs to mediate bone repair, which is governed by recipient T lymphocytes via IFN-γ and tumor necrosis factor (TNF)-α (Yamaza et al., 2008, 2011; Liu et al., 2011). MSCs can be recognized as targets by pre-activated alloreactive CD8+ cytotoxic T cells (Angoulvant et al., 2004), whereas another study showed that MSCs were not lysed by allogeneic CD8+ cytotoxic T cells (Rasmusson et al., 2003).

MSCs-B cells interaction

B lymphocytes produce antibodies and closely interact with T cells, thereby contributing to several autoimmune diseases (Wang et al., 2012). MSCs inhibit *in vitro* human B cell activation, proliferation, differentiation to plasma cells, antibody production and chemotaxis, while intriguingly, increase B cell viability significantly (Corcione et al., 2006; Comoli et al., 2008; Tabera et al., 2008; Asari et al., 2009; Liu et al., 2013). Corcione et al found that MSCs co-cultured with purified CD19+ B cells in the presence of a cocktail of stimuli significangly inhibited B cell proliferation (Corcione et al., 2006). In the study, maximum inhibition was observed at the B cell/ MSC ratio of 1:1 and disappeared at ratios of 1:5 and 1:10, in marked contrast with the inhibition of T cell proliferation induced by MSCs (Djouad et al., 2003; Corcione et al., 2006). MSCs co-cultured with B cells in transwell plates similarly inhibited B cell proliferation, suggesting that at least one soluble factor was implicated, and IL-6 and programmed death 1 pathway may be involved in the effect (Liu et al., 2013). Krampera et al found that MSC-mediated inhibition of B cell proliferation, differentiation and chemotaxis required the presence of IFN-γ, which stimulated the production of indoleamine 2, 3-dioxygenase (IDO) by MSCs, and ultimately led to suppression of B cells function (Krampera et al., 2006). However, some other studies obtained controversial results. MSCs at a 10-fold lower dose stimulated blood and splenic B cells to IgG production (Rasmusson et al., 2007). Bone marrow MSCs promoted proliferation and differentiation into immunoglobulin-secreting cells of transitional and naive B cells isolated from healthy donors and total B cells from pediatric systemic lupus erythematosus patients (Traggiai et al., 2008). IFN-γ was reported to be one of the factors responsible for these contradictory results. It was demonstrated that MSCs did not exhibit any suppressive effect on B cell proliferation without IFN-γ, while MSCs could inhibit the proliferation, differentiation and chemotaxis of B cells in the presence of exogenously added IFN-γ, which enhanced MSCs' production of indoleamine 2, 3-dioxygenase (IDO), and IDO contribute to catalyze the conversion from tryptophan to kynurenine and ultimately result in the inhibition of B cells functions (Krampera et al., 2006). This discrepancy may also result from the dose of MSCs, i.e. a higher dose of MSCs inhibts the functions of B cells, whereas a lower dose of MSCs stimulated B cells (Rasmusson et al., 2007). In addition, different experimental settings, including the origin of MSCs and B cells, the stimuli of B cells, and the co-culture patterns, may play an important role in examining the effect of MSCs on B cells function (Corcione et al., 2006; Rasmusson et al.,

2007; Comoli et al., 2008; Tabera et al., 2008; Traggiai et al., 2008; Liu et al., 2013).

MSC-NK cells interaction

As important effector cells of innate immunity, NK cells play a fundamental role in antiviral and antitumor responses by their cytotoxic potential and secretion of proinflammatory cytokines.

MSCs alter the phenotype of NK cells and suppress proliferation and cytokine secretion of resting and activated NK cells (Aggarwal and Pittenger, 2005; Sotiropoulou et al., 2006; Spaggiari et al., 2006, 2008). This inhibitory effect is related to a sharp down-regulation of the surface expression of the activating NK receptors NKp30, NKp44, and NKG2D. IDO and PGE2 represent key mediators of the MSC-induced inhibition of NK cells (Sotiropoulou et al., 2006; Spaggiari et al., 2008). As for the cytotoxic activity of NK cells, MSCs did not inhibit the lysis of freshly isolated NK cells (Rasmusson et al., 2003), whereas NK cells cultured for 4 to 5 days with IL-2 in the presence of MSCs had a reduced cytotoxic potential against K562 target cell (Krampera et al., 2006). Furthermore, Sotiropoulou et al demonstrated that short term culture with MSCs only affected NK cell cytotoxicity against HLA class I positive tumors cells but not against HLA class I negative targets (Sotiropoulou et al., 2006). Previously, MSCs were considered not to be lysed by freshly isolated NK cells (Rasmusson et al., 2003). However, recent studies have indicated that activated NK cells, but not freshly isolated NK cells, were capable of effectively lysing MSCs (Sotiropoulou et al., 2006; Spaggiari et al., 2006, 2008). NK cell-mediated lysis on IFN-γ-treated MSCs was inhibited as a result of the up-regulation of HLA class I molecules on MSC surface (Spaggiari et al., 2006).

MSC-dendritic cell interaction

Dendritic cells (DC) are the most potent type of antigen-presenting cells and play crucial roles in the initiation and control of the adaptive immune responses. MSCs were able to strongly inhibit the generation of both CD34+ cells-derived and monocyte-derived DCs (Jiang et al., 2005; Nauta et al., 2006; Djouad et al., 2007; Spaggiari et al., 2009), and this effect is through the secretion of IL-6 (Djouad et al., 2007) and may be reversible (Jiang et al., 2005). However, MSCs were able to induce the production of regulatory DC with T cell-suppressive properties partly via cell-cell contact, thereby inhibiting T cells indirectly (Beyth et al., 2005; Zhang et al., 2005). MSCs increased CD11b expression, while they reduced the expression of presentation molecules (MHC class II and CD1a), co-stimulatory molecules (CD40, CD80and CD86) on mature DCs, and more importantly, reduced the expression of CD83, suggesting their conversion to immature status (Zhang et al., 2004, 2005; Jiang et al., 2005; Nauta et al., 2006; Djouad et al., 2007). Because of MSC-induced down-regulation of DCs' antigen presentation, the allostimulatory ability of MSC-treated mature DCs on allogeneic T cells was impaired (Zhang et al., 2004; Jiang et al., 2005; Nauta et al., 2006; Djouad et al., 2007; English et al., 2008; Spaggiari et al., 2009). In terms of secretion alteration of DCs, it was shown that MSCs decreased their capacity to secret IL-12 (Zhang et al., 2004; Jiang et al., 2005), and another study demonstrated that MSCs caused mature DC type 1 to decrease TNF-α secretion and mature DC type 2 to increase IL-10 secretion (Aggarwal and Pittenger, 2005). Furthermore, MSCs also suppress the migration, maturation, and endocytosis of DCs (Zhang et al., 2004; English et al., 2008).

MSC-macrophage interaction

Macrophages co-cultured with MSCs acquired an anti-inflammatory M2 phenotype, i.e. turned into alternatively activated macrophages, characterized by an increased expression of mannose receptor (CD206) and a marked increase in their susceptibility to infection by intracellular pathogens (Kim and Hematti, 2009; Maggini et al., 2010; Zhang et al., 2010). As for the cytokine production of macrophages, MSCs markedly suppressed the production of inflammatory cytokines TNF-α, IL-6, IL-12p70 and IFN-γ while they increased the production of IL-10 and IL-12p40, whether or not macrophages were stimulated by lipopolysaccharide or un-treated (Kim and Hematti, 2009; Németh et al., 2009; Maggini et al., 2010; Zhang et al., 2010). Functionally, MSCs significantly enhanced phagocytic activity and migration of macrophages (Chen et al., 2008; Kim et al., 2009). In an aimal model of excisional wound healing, systemically infused MSCs were able to home to the wound site in a tight spatial interaction with host macrophages, promoted them toward M2 polarization, and when concentrated MSC-conditioned medium was applied, MSC-conditioned medium had increased proportions of macrophages, thereby significantly enhancing wound repair, showing that MSCs recruit macrophages into the wound to enhance wound healing (Chen et al., 2008; Zhang et al., 2010). In mice model of sepsis, MSCs were able to reprogram macrophages by releasing PGE2 and the beneficial effect of MSCs was eliminated by macrophage depletion (Németh et al., 2009).

MSC-neutrophil interaction

Neutrophils are phagocytic cells of the innate immune system that act as the first line of defense in the inflammatory response. Neutrophil is a kind of cell capable of spontaneous apoptosis. Un-treated MSCs, LPS-activated MSCs, and Toll-like receptor-3 (TLR3)- and TLR4-activated MSCs significantly inhibited apoptosis of resting and IL-8-activated neutrophils (Raffaghello et al., 2008; Brandau et al., 2010; Cassatella et al., 2011; Maqbool et al., 2011; Chen et al., 2014). The anti-apoptotic activity of un-treated MSCs

did not require cell-cell contact, and IL-6 was responsible for neutrophil protection from apoptosis (Raffaghello et al., 2008). The effects exerted on neutrophil by TLR3-activated MSCs are mediated by the combined action of IL-6, IFN-β, and granulocyte macrophage colony-stimulating factor (GM-CSF), while those exerted by TLR4-activated MSCs mostly depend on GM-CSF (Cassatella et al., 2011). In the presence of MSCs, the viability of neutrophils increases as measured in 24 h of incubation at various supplementation of serum concentration (1, 5 and 10%) (Maqbool et al., 2011). Apart from bone marrow tissues, MSCs isolated from thymus, spleen, and subcutaneous adipose tissue also behave similarly on the anti-apoptosis effect of neutrophils (Cassatella et al., 2011). CD11b expression of neutrophil could be promoted by MSCs (Cassatella et al., 2011) or MSC-conditioned medium (Chen et al., 2014). In addition, un-treated MSCs were shown to reduce N-formyl-L-methionin-L-leucyl-L-phenylalanine-induced respiratory burst (Raffaghello et al., 2008), TLR3- and TLR4-activated MSCs also could enhance respiratory burst ability (Cassatella et al., 2011). While neutrophils exhibited chemotaxis to MSC-conditioned medium, which was inhibited by IL-8 depletion (Chen et al., 2014), MSCs had no effect on neutrophil chemotaxis in response to N-formyl-L-methionin-L-leucyl-L-phenylalanine, or C5a (Raffaghello et al., 2008). Moreover, neutrophil phagocytosis was influenced by MSCs or MSC-conditioned medium (Raffaghello et al., 2008; Chen et al., 2014).

Possible mechanisms of immunosuppression by MSCs

Although the immunomodulatory functions of MSCs have been extensively studied, the mechanisms underlying MSC-mediated immunomodulation still remain largely unknown. It is generally accepted that T cells anergy, cell-cell contact, soluble factors, and extracellular vesicles are responsible for the effects of MSC-induced immunomodulation (Fig. 1).

Some studies have shown that cell-cell contact is crucial for MSCs to induce immunosuppression (Krampera et al., 2003; English et al., 2009; Aldinucci et al., 2010). When MSCs were added to the T cell cultures in a Transwell system or MSCs were replaced by MSC culture supernatant, the inhibitory activity was abrogated (Krampera et al., 2003). Aldinucci et al demonstrated that MSCs mediated inhibition of DC function only upon cell-cell contact (Aldinucci et al., 2010). English et al showed that MSCs display multiple potent capabilities on T cells, which required direct contact between MSCs and purified T cells (English et al., 2009).

Many soluble factors have been reported to be involved in immune modulation by MSCs.

Transforming growth factor-beta1 (TGF-β1)

TGF-β1 secreted by MSCs mediates MSC-induced T cell inhibition, and the concentration of TGF-β1 was elevated significantly in the co-culture of MSCs, T cells, and phytohemagglutinin (Groh et al., 2005). Neutra-

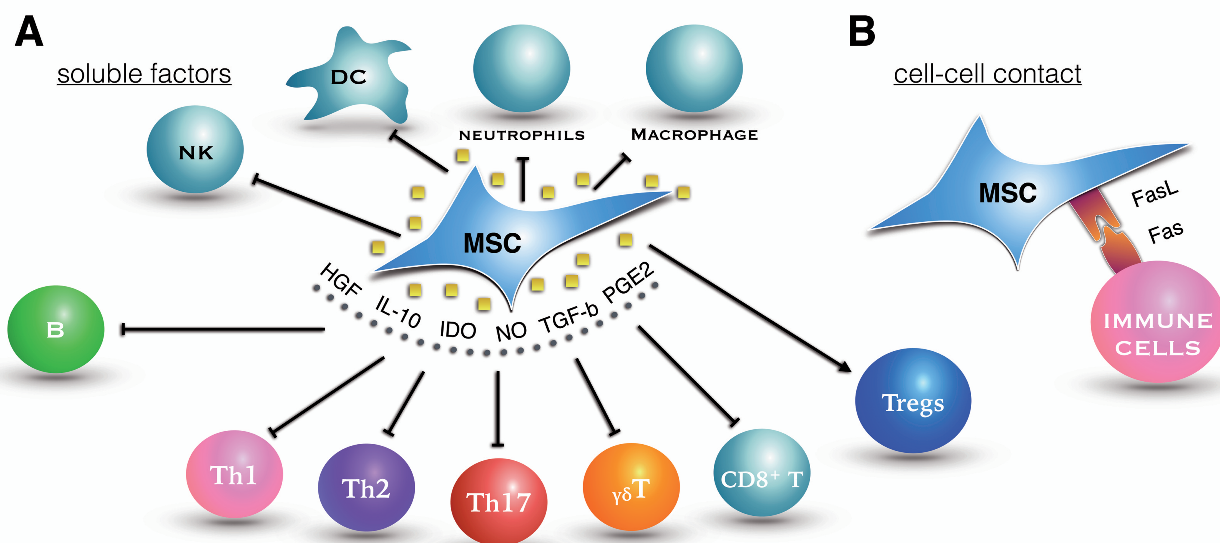

Fig. 1. Immunomodulatory characteristics of MSCs. **A.** Several soluble factors, including HGF, IL-10, IDO, NO, TGF-β and PGE2, are involved in the MSC-induced immunomodulation. **B.** Cell-cell contact or EVs play an important role in the immunoregulatory functions mediated by MSCs. MSCs: mesenchymal stem cells. HGF: hepatocyte growth factor. IL-10: interleutin-10. IDO: indoleamine 2,3-dioxygenase. NO: nitric oxide. TGF-β: transforming growth factor-β. PGE2: prostaglandin E2. Th cells: T-helper cell. Tregs: regulatory T cells. NK: natural killer cells. DC: dendritic cells. EVs: extracellular vesicles.

lizing monoclonal antibody against TGF-β1 restored the T cell proliferation suppressed by MSCs (Di Nicola et al., 2002; English et al., 2009).

Hepatocyte growth factor (HGF)

HGF was also identified as a possible factor of T cell proliferation suppression, manifested by the data that neutralizing monoclonal antibody against HGF counteracted MSC-mediated T cell suppression (Di Nicola et al., 2002).

IL-10

IL-10 has been implicated in MSC-mediated immunosuppression (Aggarwal and Pittenger, 2005; Yang et al., 2009). Co-culture of splenocytes and MSCs in the presence of alloantigen produced a significant amount of IL-10, and the blockade of IL-10 and IL-10 receptor interaction by anti-IL-10 or anti-IL-10-receptor antibodies abrogated the suppressive capacity of MSCs, indicating that IL-10 plays a major role in the suppression of T cell proliferation (Yang et al., 2009).

IDO

Human MSCs express IDO protein and exhibit functional IDO activity. IDO activity resulting in tryptophan depletion and kynurenine production is detected in MSC/ mixed lymphocyte reactions coculture supernatants, thus identifying IDO-mediated tryptophan catabolism as a novel T-cell inhibitory effector mechanism in human MSCs (Meisel et al., 2004). In addition, the suppressive activity of MSCs required the presence of IFN-γ produced by activated T cells and NK cells, which was related to its ability to stimulate the production of IDO by MSCs, therefore in turn inhibiting the proliferation of activated T or NK cells (Krampera et al., 2006; Di Spaggiari et al., 2008).

Nitric oxide (NO)

NO is also involved in the suppression of T cell proliferation. The induction of inducible NO synthase (iNOS) was detected in MSCs, and a specific inhibitor of iNOS reversed the suppression of T cell proliferation (Sato et al., 2007). At the same time, MSCs from iNOS(-/-) mice had a reduced ability to suppress T cell proliferation. In addition, administration of wild-type MSCs, but not iNOS(-/-) MSCs, prevented graft-versus-host disease in mice, an effect reversed by iNOS inhibitors. Wild-type MSCs also inhibited delayed-type hypersensitivity, while iNOS(-/-) MSCs aggravated it (Ren et al., 2008). These results suggest that NO produced by MSCs is one of the major mediators of T cell suppression by MSCs.

Investigators found that the mechanism of MSC-mediated immunosuppression varies among different species. Ren et al. showed that human MSCs expressed extremely high levels of IDO, and very low levels of iNOS, whereas mouse MSCs expressed high iNOS and very little IDO when human and mouse MSCs were stimulated by their inflammatory cytokines, respectively, suggesting that immunosuppression was achieved via IDO and iNOS by human and mouse, respectively (Ren et al., 2012). Later, this group also demonstrated that MSCs from monkey and pig use IDO to suppress immune responses, whereas MSCs from rat, rabbit, and hamster utilize iNOS, under the same culture conditions, which is crucial to select the origin of MSCs for preclinical studies of disease models (Su et al., 2014).

PGE2

MSCs produced elevated PGE2 when co-cultured with T lymphocytes and phytohemagglutinin, or in MLR, and inhibitors of PGE2 production mitigated MSC-mediated immune modulation (Aggarwal and Pittenger, 2005; Sato et al., 2007). MSCs were able to inhibit cytokine-induced proliferation, cytotoxic activity and cytokine production of freshly isolated NK cells by MSC-derived PGE2 (Spaggiari et al., 2008). PGE2 was shown to have a non-redundant role in the induction of CD4+CD25+FoxP3+ T cells, which were able to suppress alloantigen-driven proliferative responses in MLR (English et al., 2009).

CCL2

MSC-conditioned medium inhibits experimental autoimmune encephalomyelitis -derived CD4+ T cell activation by suppressing STAT3 phosphorylation via MSC-derived CCL2. The loss of function of CCL2 (-/-) MSCs in EAE mice further confirmed the key role of MSC-derived CCL2 in inhibition of T cell proliferation (Rafei et al., 2009).

HLA-G

HLA-G is a nonclassical major histocompatibility complex class I antigen. HLA-G can be detected on MSCs by reverse transcriptase-polymerase chain reaction, immunofluorescence, flow cytometry, and enzyme-linked immunosorbent assay in the supernatant. Anti-HLA-G blocking antibody significantly raised lymphocyte proliferation in MSCs/MLR, which provides evidence supporting involvement of HLA-G in the immunosuppressive properties of MSCs (Nasef et al., 2007).

Heme oxygenase-1 (HO-1)

HO-1, a potent immunosuppressive enzyme, was recently identified as a key contributor for MSC-mediated suppression of alloactivated T cells. MSCs were positive for HO-1, and HO-1 inhibition on MSCs was sufficient to completely block their immuno-suppressive capacity (Chabannes et al., 2007). In a HO-

1-dependent fashion, human MSCs were shown to induce regulatory T cells, which possess manifold immunomodulatory properties (Mougiakakos et al., 2011).

Furthermore, galectins (Gieseke et al., 2010), TSG-6 (Lee et al., 2009), insulin-like growth factor-binding proteins (Gieseke et al., 2007) and leukemia inhibitory factor (Nasef et al., 2008) have also been reported to contribute to the inhibitory mechanism of MSCs.

Extracellular vesicles (EVs)

Besides soluble factors, EVs secreted by MSCs have also been reported to be involved in MSC-mediated immunomodulatory actions. EVs are paracrine or endocrine signalling vehicles that might transport defined signalling molecules from one cell to another. EVs released from murine MSCs are capable of inhibiting activation and proliferation of auto-reactive lymphocytes, inducing the apoptosis of activated T cells, and promoting the production of Treg (Mokarizadeh et al., 2012). EVs derived from human bone marrrow MSCs were able to modulate the Th1/ Th2 balance by decreasing the Th1 response, up-regulating the percentage of Treg, and down-regulating the proportion of Th17 cells (Favaro et al., 2014). As for B cells, EVs obtained from human bone marrrow MSCs possess a inhibitory capability on the proliferation and differentiation of B cells and production of IgM, IgG and IgA dose dependently (Budoni et al., 2013). When *in vitro* immunomodulatory effects of MSCs were compared with those of EVs from the same MSCs, it was shown that MSCs had the higher ability to suppress the T cells proliferation induced by PHA than the corresponding EVs, whereas MSCs displayed similar inhibitory activity on B cell proliferation as EVs (Conforti et al., 2014). Furthermore, EVs had a lower suppressive effect on antibody secretion of B lymphocytes (Conforti et al., 2014).

Conclusion

In the past few years, there has been a dramatic improvement in the understanding of immunomodulatory properties and the potential clinical use of MSCs, although some studies are controversial. The current data have shown promise for patients with autoimmune diseases and inflammatory diseases, and the preliminary results are encouraging. However, there is still a long way to go before the extensive application of MSCs in the clinic based on their immunological functions.

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