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Therapeutic Factors Secreted by Mesenchymal Stromal Cells and Tissue Repair

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ABSTRACT

Systemic administration of MSCs resulted in remarkable functional improvements in injured tissues without either long-term engraftment or differentiation in many clinical and experimental situations. Emerging evidence suggest that most of the beneficial effects of MSCs could be explained by secretion of soluble factors that have multiple effects including modulation of inflammatory and immune reactions, protection from cell death, and stimulation of endogenous progenitor cells. In this review, we focus on the therapeutic factors that account for the beneficial effects of MSCs in animal models of human diseases. J. Cell. Biochem. 112: 3073–3078, 2011.

KEY WORDS: MSCs; SECRETORY FACTORS; ADULT PROGENITOR CELLS

Currently, considerable efforts are being made to develop cell therapies using multipotent mesenchymal stromal cells often referred to as mesenchymal stem cells (MSCs) [Dominici et al., 2006; Prockop et al., 2010]. They are readily isolated from small aspirates of a patient's bone marrow, expand rapidly in culture, and differentiate into several cellular phenotypes [Dominici et al., 2006]. Therefore, they were originally sought to repair injured tissues by engrafting and differentiating. Engraftment with differentiation was observed in some prenatal systems or in animal models with local infusions of high concentrations of the cells [Kopen et al., 1999; Mackenzie and Flake, 2001; Prockop, 2009; Shake et al., 2002]. However, in most clinical and experimental situations, systemic administration of MSCs resulted in functional improvements without evidence of long-term engraftment or differentiation [Iso et al., 2007; Lee et al., 2009b; Prockop, 2009; Prockop et al., 2010]. In addition, improvements of injured tissues take place too rapidly to be explained by differentiation of MSCs. Therefore, emerging evidence suggest that most of the beneficial effects could be explained by secretion of therapeutic factors that have multiple effects including modulation of inflammatory and immune reactions, protection from cell death, and stimulation of endogenous progenitor cells [Prockop et al., 2010]. Moreover, it has been shown that MSCs secrete a large number of cytokines under normal culture conditions [Caplan, 2009]. More importantly, they can be activated to express high levels of additional therapeutic factors by cross-talk with injured cells or microenvironments. In this review, we focus on the therapeutic factors that can explain the beneficial effects of MSCs observed in animal models of human diseases.

ANTI-INFLAMMATORY AND IMMUNOSUPPRESSIVE EFFECTS

Continued research on MSCs during the past decade revealed their remarkable ability to modulate immune and inflammatory reactions. Since MSCs were originally shown to suppress activation of T cells in vitro and prolong the survival of skin grafts in vivo [Bartholomew et al., 2002; Le Blanc et al., 2003], they have been tested in various models of diseases that involve inflammatory and immune components. The majority of the studies suggest that administered MSCs quickly respond to stress or injury and suppress excessive immune responses without significant engraftment.

The notion that MSCs have immunoregulatory abilities is remarkable, but not surprising. Vast evidence suggest that MSCs can actively participate in maintaining the homeostasis of local microenvironment. First, the ability of bone marrow-derived MSCs to support hematopoietic stem cells (HSCs) in culture has been known for a long time [Dexter et al., 1977; Sacchetti et al., 2007]. By secreting certain factors, MSCs can preserve the undifferentiated state of HSCs and support their proliferation. In fact, it is now accepted that MSCs play a role as organizers of HSC niche in vivo [Sacchetti et al., 2007; Mendez-Ferrer et al., 2010]. Second, MSCs are known to have similarities with immune cells. For example, they

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have either constant or inducible expression of Toll-like receptors and cytokine/chemokine receptors [Docheva et al., 2008] that rapidly trigger the production of secretory factors in MSCs after exposure to pathogen-associated molecules (LPS, zymosan, peptidoglycan, etc.) or pro-inflammatory cytokines (TNF-α, IFN-γ, etc.).

Therefore, MSCs have a machinery that allows them to actively respond to the stress or injury in a manner similar to immune cells e.g., by sensing pathogens or injury signals and by secreting a variety of cytokines or chemokines. Among these factors, many have been identified as therapeutic proteins secreted by MSCs that modulate the inflammation and immune reactions in models of acute inflammation, autoimmune diseases, and organ transplantation.

ANTI-INFLAMMATORY PROTEIN TNF-α STIMULATED GENE 6 PROTEIN (TSG-6)

Recently, we reported that systemic administration of human MSCs (hMSCs) resulted in functional improvements in a mouse model of acute myocardial infarction (MI) without significant engraftment [Lee et al., 2009b]. We employed real-time PCR assay to track the fate of intravenously (IV) injected MSCs. Surprisingly, only a few cells were found in the injured heart, while the majority was trapped in the lungs. However, hMSCs trapped in the lungs underwent major changes in their patterns of gene expression. Among the up-regulated genes, TSG-6 had attracted our attention, because it was previously shown to produce multipotent anti-inflammatory effects: (a) it inhibits the inflammatory network of proteases primarily by increasing the inhibitory activity of inter-α-inhibitor, (b) it binds to fragments of hyaluronan and thereby abrogates their pro-inflammatory effects, and (c) it suppresses neutrophil infiltration into sites of inflammation. We further demonstrated that hMSCs did not secrete TSG-6 under normal culture conditions, but they were rapidly activated to secrete TSG-6 by inflammatory signals (TNF-α, IL-1β, or LPS) or environmental stress such as aggregation or hypoxia [Bartosh et al., 2010]. In a mouse model of MI [Lee et al., 2009b], hMSCs attenuated excessive inflammatory responses in which infiltrating neutrophils generate MMPs that degrade the myocardium by secreting TSG-6, and therefore reduced the infarction size in the heart. In addition, hMSCs with an siRNA knock-down of the TSG-6 gene had no effect on inflammatory responses and infarct size. Systemic administration of recombinant human (rh) TSG-6 duplicated the effects of hMSCs.

The anti-inflammatory effects of hMSCs via secreting TSG-6 were also observed in mouse and rat models of chemical and mechanical injury of the cornea [Oh et al., 2010; Roddy et al., manuscript submitted]. Either intraperitoneally (IP) or IV administered hMSCs significantly suppressed neutrophil infiltration, production of pro-inflammatory cytokines, and development of corneal opacity. The results are consistent with the previous observations in a mouse model of MI. There was no engraftment of hMSCs in corneas following IP or IV injection. The hMSCs transduced with the TSG-6 siRNA had no significant effect on corneal opacity and inflammation. Intraocular, topical, or systemic administration of rhTSG-6 reproduced the remarkable effects in suppressing inflammation and reconstructing the corneal surface.

In a mouse model of zymosan-induced peritonitis [Choi et al., 2011], we further demonstrated a novel mechanism whereby hMSCs via TSG-6 attenuated the cascade of inflammation. In this model, resident macrophages are primarily responsible for initiating inflammatory cascade by secreting pro-inflammatory cytokines. Administration of either hMSCs or TSG-6 quickly suppressed TLR2-mediated NF-κB translocation in resident macrophages, and thereby inhibited secretion of TNF-α and other chemokines responsible for neutrophil recruitment. These effects were dependent on the interaction between TSG-6 and CD44 expressed on the macrophage surface.

Considering that excessive inflammatory responses contribute to pathological changes in many diseases, the anti-inflammatory effect of TSG-6 secreted by hMSCs at the initial phase of acute inflammation may explain the therapeutic effects of MSCs without long-term engraftment.

INTERLEUKIN 1 RECEPTOR ANTAGONIST (IL-1RA)

IL-1RA is a naturally occurring inhibitor of IL-1, and is known to be expressed by mouse and hMSCs [Ortiz et al., 2007]. IL-1RA expressed by MSCs blocked an IL-1α-dependent proliferation of T cell-line and inhibited release of TNF-α from activated macrophages in vitro. Also, IL-1RA-expressing mouse MSCs protected the lungs from inflammation and fibrosis in a model of bleomycin-induced lung injury in mice. In fact, IL-1RA expressed by MSCs was more effective in suppressing inflammation than systemic or viral delivery of recombinant IL-1RA. Considering IL-1 and TNF-α are at the nexus of most inflammatory responses, MSCs as a cellular vector for IL-1RA could be potential therapeutic agents in the treatment of human diseases such as lung injury and diabetes mellitus [Ortiz et al., 2007; Volarevic et al., 2010].

PROSTAGLANDIN E2 (PGE2)

Among the factors MSCs produce to suppress immune reactions, PGE2 is one of the molecules most studied. The hMSCs secrete high level of PGE2 alone or in response to IL-6 [Bouffi et al., 2010], IFN-γ, TNF-α, or LPS [Nemeth et al., 2009]. Reports have demonstrated that MSCs via PGE2 exert their immunosuppressive effects in vitro in multiple ways by (a) suppressing the proliferation and activation of mitogen-induced or antigen-specific proliferation of T cells [Najar et al., 2010], (b) switching the host response from a Th1/Th17 toward a Th2 immune profile [Bouffi et al., 2010], (c) inducing regulatory T cells (Tregs) [English et al., 2009], (d) inhibiting the maturation and differentiation of dendritic cells (DCs) [Spaggiari et al., 2009], (e) promoting the production of IL-10 in macrophages [Nemeth et al., 2009], and (f) inhibiting the proliferation and cytotoxic function of natural killer (NK) cells [Spaggiari et al., 2008].

The in vivo evidence of PGE2-mediated effects of MSCs were reported in models of sepsis, experimental autoimmune encephalitis (EAE), and experimental arthritis. The notable observation was made by Nemeth et al. [2009]. They found that MSCs reduced the mortality in mice with sepsis, but the beneficial effects of MSCs were eliminated by depleting macrophages or blocking IL-10 signaling. Subsequently, they demonstrated that MSCs released PGE2 in response to inflammatory signals such as TNF-α and LPS, and thereby acted on the host macrophages through the prostaglandin

EP2 and EP4 receptors to increase their production of the anti-inflammatory cytokine IL-10. Recently, more groups reported the PGE2-mediated immunosuppressive effects of MSC in models of EAE [Matysiak et al., 2011] and collagen-induced arthritis (CIA) [Bouffi et al., 2010]. In both studies, they confirmed the PGE2-mediated effects utilizing direct or indirect inhibition of PGE2 production.

INDOLEAMINE 2,3-DIOXYGENASE (IDO)

Recently, it has been shown that MSCs express IDO upon stimulation with IFN-γ [Meisel et al., 2004]. IDO has been identified as a T-cell inhibitory factor by catabolizing the essential amino acid tryptophan required for T cell proliferation [Meisel et al., 2004; Ryan et al., 2007]. Several reports demonstrated an IDO-mediated inhibition of MSCs on T cell response in mixed lymphocyte reactions (MLRs) [Meisel et al., 2004]. Furthermore, IDO-mediated immunosuppressive effects of hMSCs was confirmed in disease models associated with T cell activation such as organ transplantation including heart transplantation in rats [Popp et al., 2008] and kidney transplantation in mice [Ge et al., 2010]. In both models, blocking IDO in MSCs abrogated the graft acceptance, and therefore IDO was verified as a therapeutic factor in prolonging the graft survival in models of organ transplantation. However, Gieseke et al. [2007] reported that hMSCs exerted important immunomodulatory functions independently of IFN-γR1 signaling and IDO expression.

NITRIC OXIDE (NO)

Emerging evidence showed that the mechanisms of MSC-mediated immunosuppression vary among different species [Ren et al., 2008]. Immunosuppression by human- or monkey-derived MSCs is mediated by IDO, whereas mouse MSCs utilize NO under the same culture conditions. Ren et al. [2008] showed that mouse MSCs abundantly expressed iNOS upon simulation of IFN-γ and other pro-inflammatory cytokines such as TNF-α, IL-1α, or IL-1β, while hMSCs mainly secreted IDO. In a mouse model of graft-versus-host disease (GVHD) [Ren et al., 2008], only wild-type MSCs reduced GVHD, whereas MSCs lacking either the IFN-γ receptor or iNOS were not effective. However, a specific NOS inhibitor, l-NMMA, did not completely restore the T cell proliferation by primary MSCs, suggesting that there should be additional factors involved in this suppression [Sato et al., 2007].

TRANSFORMING GROWTH FACTOR-β1 (TGF-β1)

It has been shown that TGF-β1 contributes to the hMSC-mediated immune modulation. Especially, Groh et al. [Groh et al., 2005] showed that MSCs are activated by monocytes suppressed T cell responses by secreting TGF-β1. Nasef et al. [Nasef et al., 2007a] further demonstrated that TGF-β1 was particularly involved in the inhibition of T lymphocyte proliferation during cell contact with hMSCs along with IL-10. Later, English et al. [English et al., 2009] showed that TGF-β1 and PGE2 derived from MSCs induced Tregs that suppressed T cell response. Nemeth et al. [Nemeth et al., 2010] reported that IV injected MSCs suppressed Th2-driven allergic responses in a mouse model of ragweed-induced asthma by secreting TGF-β1. Inflammatory cell-derived IL-4 and/or IL-13 induced secretion of TGF-β by MSCs, which could not only induce the differentiation and help the survival of Tregs, but also block the pro-inflammatory Th2 response at the same time. In addition, Sotiropoulou et al. [Sotiropoulou et al., 2006] reported that MSCs had suppressive effects on NK cells by secreting TGF-β1 and PGE2.

HUMAN LEUKOCYTE ANTIGEN-G (HLA-G)

Nasef et al. [Nasef et al., 2007b] showed that HLA-G contributed to MSC-mediated inhibition of immune response in vitro. HLA-G may play a role in immune tolerance in pregnancy, because it was initially found on trophoblasts where it contributes to tolerance at the materno-fetal interface. Interestingly, Ivanova-Todorova et al. [Ivanova-Todorova et al., 2009] reported that progesterone stimulated MSCs to express increased levels of both cell surface and cytoplasmic HLA-G. Furthermore, Selmani et al. [Selmani et al., 2008] further demonstrated the HLA-G-mediated immunomudulatory effects of MSCs using blocking of HLA-G. In addition to their action on the adaptive immune system, MSCs, through HLA-G5, affected innate immunity by inhibiting both NK cell-mediated cytolysis and IFN-γ secretion.

ANTI-APOPTOTIC AND REGENERATIVE EFFECTS

In addition to anti-inflammatory and immunosuppressive effects, inhibition of cell death and stimulation of endogenous progenitors also contribute to tissue repair. Chen et al. [Chen et al., 2003] demonstrated that systemic administration of MSCs promoted functional recovery by reducing neuronal apoptosis and stimulating endogenous progenitor proliferation in brains of rats after stroke. Recently, more groups [Munoz et al., 2005; Semont et al., 2010] reported similar observations in hippocampus of healthy immunodeficient mice and in a model of radiation-induced gastrointestinal tract injury. The aforementioned reports did not define the mechanism of MSCs. However, they suggested the possibility that the secretion of soluble factors by MSCs may account for beneficial effects since there was no significant engraftment of MSCs. Indeed, many trophic factors, including several well-known growth factors, have been suggested as responsible for MSC-mediated tissue repair by stimulating endogenous tissue progenitors or protecting injured cells from death. Also, several groups reported that MSC-derived stromal cell-derived factor-1 (SDF-1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), or insulin-like growth factor 1 (IGF-1) exerted protective effects in many disease models including MI [Yu et al., 2009; Tsubokawa et al., 2010; Angoulvant et al., 2011], acute kidney injury [Imberti et al., 2007; Togel et al., 2007], and brain injury [Deng et al., 2010; Wakabayashi et al., 2010; Wang et al., 2010; Bakondi et al., 2011]. In addition, using an overexpression technique [Liang et al., 2010; Tang et al., 2011], some groups indirectly demonstrated secretion of trophic factors as a mechanism of MSC-mediated tissue repair. Notably, using MSCs transduced with lentiviral SDF-1 short hairpin RNA (shSDF-1), Bakondi et al. [2011] directly showed that SDF-1 was a main therapeutic factor of MSCs in a model of stroke. They found that conditioned medium generated from shSDF-1-treated cells showed significantly less protective effect, compared to conditioned medium from control MSCs. In the murine model of cisplatin-

induced kidney injury, Imberti et al. [2007] demonstrated that administering IGF-1 gene-silenced MSCs limited their protective effect on renal function and tubular structure compared to control MSCs. Block et al. [2009] also observed that hMSCs cocultured with UV irradiated fibroblasts markedly reduced the apoptosis of injured cells by secreting stanniocalcin-1 (STC-1). From microarrays and western blot analysis of MSCs, they found that hMSCs were activated to increase synthesis and secretion of STC-1. Blocking STC-1 with either antibodies or siRNA reversed the anti-apoptotic effects of MSCs in UV-irradiated fibroblasts and lung epithelial cells incubated at low pH in hypoxia.

However, since most trophic factors have been known as having protective roles that promote cell proliferation and protect cells from apoptosis, these factors may not only protect the host cells, but also support MSC survival after transplantation, and thereby prolong the therapeutic effects of MSCs in vivo.

CONCLUSION

Unlike embryonic or HSCs, MSCs seem to have a limited longevity and differentiation potential in vivo. However, MSCs have shown remarkable effects in a broad spectrum of diseases without significant engraftment. As we discussed in this review, a variety of secretory factors may explain these observations (Fig. 1). Considering MSCs could be activated diversely by many signals from injured tissues, it is reasonable to presume that a combined action of many factors, rather than a sole action of one factor, could contribute to beneficial effects of MSCs observed in various diseases. For example, in the acute inflammatory phase, pro-inflammatory environment may stimulate MSCs to secrete TSG-6 and PGE2 or STC-1 and SDF-1, which in turn suppress excessive immune responses and protect the injured tissues. On the other hand, MSCs secrete SDF-1, VEGF, or other cytokines to stimulate recruitment, proliferation, and differentiation of endogenous progenitor cells, hence promoting tissue regeneration. Immunosuppressive effects could be also mediated by other factors. Both direct T cell inhibition by IDO or PGE2 and immune tolerance induced by TGF-β and PGE2 may contribute to MSC-mediated immunosuppressive effects. Furthermore, additional factors that have not been discussed in this review could contribute to tissue repair by the same or different mechanisms. Indeed, factors such as leukemia inhibitory factor [Nasef et al., 2008], heme oxygenase-1 [Mougiakakos et al., 2011], keratinocyte growth factor [Lee et al., 2009a], and chemokine (C–C motif) ligand 2 [Rafei et al., 2009] released from MSCs have been shown beneficial in several models of diseases.

Taken together, therapeutic factors play a critical role in mediating the action of MSCs in tissue repair. Defining these therapeutic factors secreted by MSCs helped us to better understand MSC function. However, it is still not clear how MSCs are being activated in vivo to secrete these factors: This process can be dependent on the type of the disease or the local environment in the host. Addressing these issues will further help to utilize MSCs for treating human diseases.

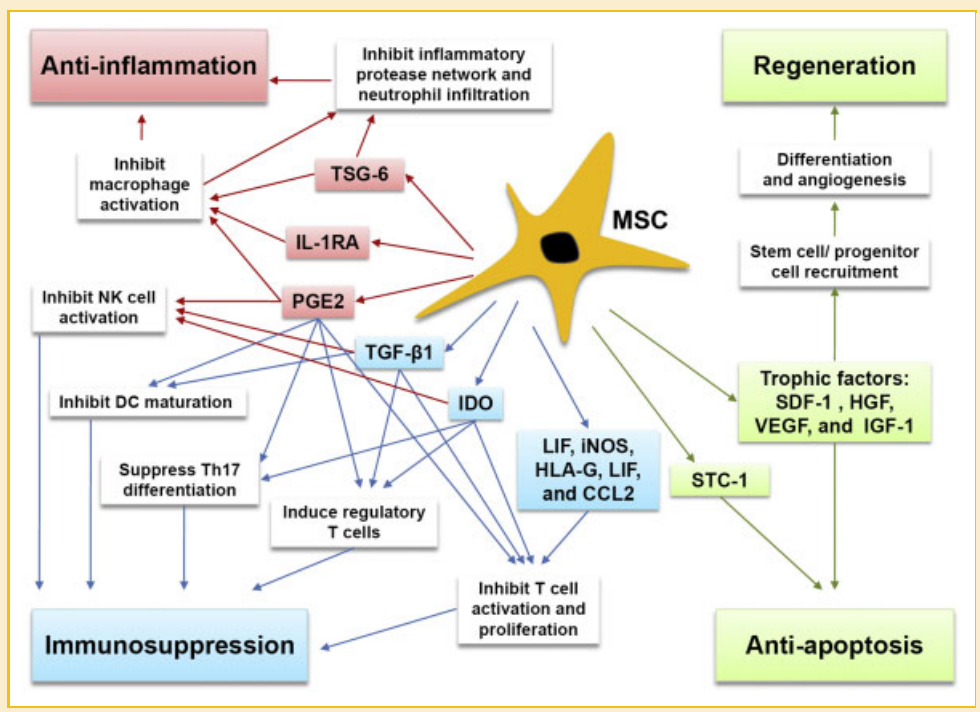

Fig. 1. MSCs exert their therapeutic effects by secreting a variety of factors.

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