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Extrinsic and intrinsic mechanisms by which mesenchymal stem cells suppress the immune system

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Abstract

Mesenchymal stem cells (MSCs) are a group of fibroblast-like multipotent mesenchymal stromal cells that have the ability to differentiate into osteoblasts, adipocytes, and chondrocytes. Recent studies have demonstrated that MSCs possess a unique ability to exert suppressive and regulatory effects on both adaptive and innate immunity in an autologous and allogeneic manner. A vital step in stem cell transplantation is overcoming the potential graft-versus-host disease, which is a limiting factor to transplantation success. Given that MSCs attain powerful differentiation capabilities and also present immunosuppressive properties, which enable them to survive host immune rejection, MSCs are of great interest. Due to their ability to differentiate into different cell types and to suppress and modulate the immune system, MSCs are being developed for treating a plethora of diseases, including immune disorders. Moreover, in recent years, MSCs have been genetically engineered to treat and sometimes even cure some diseases, and the use of MSCs for cell therapy presents new perspectives for overcoming tissue rejection. In this review, we discuss the potential extrinsic and intrinsic mechanisms that underlie MSCs' unique ability to modulate inflammation, and both innate and adaptive immunity.

Keywords

graft versus host disease; immunity; inflammation; mesenchymal stem cells; stem cell transplantation

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I. INTRODUCTION

Stem cells (**SCs**) were first used as a form of cell therapy in 1968 when bone marrow stem cells (**BMSCs**) were used to treat lymphoma. The transplanted bone marrow contained SCs, which were able to produce all cell components of the blood and supporting matrix of the bone marrow. Today, BMSCs are routinely transplanted to treat a variety of blood and bone marrow diseases, blood cancers, immune disorders, and lysosomal storage diseases. Recently, peripheral blood SCs and umbilical cord blood SCs have also been used to treat some of the same blood-based diseases. These treatments involve the use of versatile hematopoietic SCs that can readily differentiate into all blood cell types and efficiently repopulate the recipient's bone marrow, making SCs an excellent tool for cell therapy.

Over the past decade, much attention has been given to mesenchymal stem cells (**MSCs**), a group of fibroblast-like multipotent mesenchymal stromal cells.^{1,2} MSCs were originally identified as multipotent stromal precursor cells in bone marrow by Friedenstein and coworkers in the 1970s.³⁻⁵ These cells present the *in vitro* ability to differentiate into osteoblasts, adipocytes, and chondroblasts. Currently recognized markers for MSCs are negativity for the cell surface markers CD45, CD34, CD14, CD11b, CD79 α , CD19 and HLA-DR, and positivity for CD44, CD90, CD105, CD79, and CD29. MSCs possess a unique ability to exert suppressive and regulatory effects on both adaptive and innate immune cells in an autologous and allogeneic manner.⁶

The MSC cell surface receptors involved in immune regulation are listed in Table 1. MSCs can be isolated from a variety of tissues, such as bone marrow (**BM**), umbilical cord (**U**),⁷ adipose tissue (**AT**),⁸ dental pulp (**DP**),⁹ hair follicle (**HF**), and corneal stroma,^{10,11} making them readily available. MSCs isolated from BM, AT, DP, and HF have the advantage that they can be used for autologous transplantation. Moreover, DPSCs and HFSCs may be obtained as a by-product after removal of molar teeth and by noninvasive surgery, respectively, making them very attractive sources of SCs.

II. IMMUNOSUPPRESSIVE PROPERTIES OF MESENCHYMAL STEM CELLS

An important step in regenerative medicine is to engineer cells or tissues which are then transplanted to restore normal tissue function. However, graft-versus-host disease limits transplantation success. MSCs in addition to powerful differentiation capabilities also present immunosuppressive properties, which aid them in surviving host immune rejection. Therefore, the use of MSCs for cell therapy presents new perspectives for overcoming tissue rejection.

The first report to describe the immunosuppressive properties of MSCs was by Le Blanc, who found that transplantation of allogeneic MSC infusions, at the time of mismatched skin grafts in baboons, suppressed immune cells, preventing the graft-versus-host attack.¹² Since then, evidence has mounted with regard to how MSCs modulate immune cells, and over the past 10 years, MSCs have been shown to alter the cytokine secretion profile of subpopulations of inflammatory cells into a more anti-inflammatory phenotype,¹³ to

stimulate macrophages into attaining higher phagocytic activity,^{14,15} to impair proliferation and differentiation of B cells, and to inhibit natural killer function.¹⁶

MSCs have been shown to modulate the innate immune system. They actively regulate macrophages,^{17,18} neutrophils,^{19,20} dendritic cells,²¹⁻²³ and natural killer cells,^{24,25} thereby modulating the first line of defense. MSCs also regulate the adaptive immune system, i.e., B and T lymphocytes.²⁶ In the case of T cells, MSCs primarily promote the activation of T regulatory cells.^{27,28} In the case of B cells, MSCs inhibit B-cell proliferation, plasma-cell differentiation, and antibody production, all in a T-cell-dependent manner.²⁹ To date, MSCs have been shown to regulate the innate and adaptive immune responses primarily in a cytokine-dependent manner through the secretion of transforming growth factor-beta (**TGFβ**), interferon gamma (**IFNγ**), prostaglandin E2 (**PGE2**), interleukin (**IL**)-10 and tumor necrosis factor-alpha (**TNFα**). (See Table 2.) However, we have recently shown that UMSCs have unique immunosuppressive properties that can prevent xenograft rejection, making these cells extremely attractive for cell therapy.²⁷ Our study demonstrated that UMSCs actively regulate immune cells, inhibiting M1 polarization and promoting M2 polarization in an extracellular matrix-dependent manner.

Macrophages present different functional states, which dictate their involvement in either promoting the immune response (recruiting and activating inflammatory cells) or resolving the immune response (secreting anti-inflammatory cytokines and having increased apoptotic potential).³⁰⁻³² The M1 and M2 macrophage phenotypes were first coined to differentiate between these two macrophage functional states. During the early stages of inflammation, M1 macrophages are essential for development of the acute response; however, failure to then downregulate this initial response and activate M2 macrophages, primarily involved in tissue repair, leads to chronic pathogenesis. Therefore, the ability of MSCs to stimulate M2 polarization and inhibit M1 polarization is of great pharmaceutical significance. However, MSCs have also been shown to secrete a plethora of cytokines that directly regulate immune cells both in the surrounding tissue and in the circulation. Therefore, it has been well established that MSCs directly modulate immune cells, both through the rich environment they produce (extrinsic mechanisms) and by means of the extensive cytokine panels they have been shown to secrete (intrinsic mechanisms). In the following sections, we will discuss the different intrinsic and extrinsic mechanisms described to date for MSCs, which directly modulate immune cells.

A. Intrinsic Mechanisms

Depending on the environment that MSCs are exposed to, they may adopt either an immunosuppressive or pro-inflammatory phenotype.³³ Pro-inflammatory cytokines activate MSCs via the Toll-like receptor 3 (**TLR3**), which leads to an immunosuppressive phenotype: suppression of proliferation, activation of dendritic cells, macrophages, B cells, T cells, NK cells, NKT cells, and neutrophils. Thus, in a pro-inflammatory environment, MSCs respond by secreting anti-inflammatory cytokines, such as TGF-β, IL-10, leukemia inhibitory factor (**LIF**), galectin-1, galectin-3, and semaphorin-3A (Figure 1).³⁴⁻³⁹ All of these factors have been shown to suppress T cell proliferation and are upregulated following MSC stimulation with TNF-α and IFN-γ.⁴⁰ On the other hand, TLR4 activation may lead to

a pro-inflammatory MSC phenotype, where MSCs secrete pro-inflammatory factors, such as IL-6 and IL-8, and promote neutrophil and T cell activation enhancing the immune response.^{33,41}

IFN- γ levels have been shown to coordinate the pro- and anti-inflammatory properties of MSCs through a feedback loop (Figure 1).⁴² In addition, a delicate balance between MSCs, T-regulatory cells, and IFN- γ levels regulates the immune stimulatory and inhibitory properties of MSCs through this feedback loop.⁴³ In the presence of low levels of IFN- γ , MSCs express MHC-II, which enables them to present antigens upon insult, leading to the activation of T-regulatory cells. The activated T-regulatory cells express IFN- γ , which then suppresses MHC-II presentation by MSCs, thereby hampering the inflammatory process and concluding the feedback loop.

The immunosuppressive effects of BMSCs have been shown to be dependent on Notch-RBP-J (recombination signal binding protein-J κ) signaling, which regulates the production of IL-6 and PGE2, using a murine lethal acute graft-versus-host disease model.⁴⁴ Secretion of high levels of IL-6 by MSCs has been shown to downregulate the expression of MHC II, CD40, and CD86 on mature dendritic cells and reduce T-cell proliferation,⁴⁵ while PGE2 inhibits both T-cell proliferation and activation.^{46,47} MSCs secreting PGE2 have been shown to be a potential effector for T-cell suppression.⁴⁸ Interestingly, PGE2-sensitive T cells have been characterized by low production of IL-2 and IL-4, while PGE2-resistant T cells secrete high levels of IL-2, IL-4 or both.⁴⁹ MSCs upregulate PGE2 upon IFN- γ and TNF α treatment, suggesting that T-cell regulation depends on the inflammatory microenvironment. MSCs have also been shown to induce macrophage reprogramming by PGE2 secretion, and the presence of either LPS or TNF α in the microenvironment is essential.⁵⁰

MSCs have also been shown to inhibit the maturation of dendritic cells through the stimulation of IL-10 secretion, and by activating the JAK1 and STAT3 signalling pathway.⁵¹ BMSCs can differentiate mature dendritic cells into a distinct regulatory dendritic cell population that has lower CD1a, CD80, CD86 and CD40 expression, higher CD11b expression and does not stimulate T-cell proliferation.⁵² The nitric oxide pathway has also been shown to play a role in regulating T-cell activation. IFN γ -stimulated MSCs express high levels of nitric oxide synthase, which in turn suppresses T-cell responsiveness, and MSCs have been shown to prevent graft-versus-host disease in both an IFN γ - and nitric oxide-dependent manner.⁵³ MSCs have also been shown to regulate follicular helper T cells through the secretion of indoleamine 2,3-dioxygenase.⁵⁴

UMSCs have been shown to be the MSCs with the greatest immune suppressive properties, and this has been correlated to the high levels of HLA-G (non-classical HLA with strong immune-inhibitory properties) that these cells express.⁵⁵ Interestingly, UMSCs upregulate HLA-G expression when treated with IFN γ .⁵⁵ Human UMSCs (**hUMSCs**) express an immunosuppressive isoform of HLA-I, but not HLA-DR (major histocompatibility complex [MHC] class II cell surface receptor), indicating that these cells have low immunogenicity.⁵⁶ Furthermore, the expression of immune response-related surface antigens, such as CD40, CD40 ligand, CD80, and CD86 is absent on hUMSCs, allowing hUMSCs to escape the host

immune attack.^{56,57} Similar to hUMSCs, hBMSCs and hATSCs are negative for immunologically relevant surface markers.⁵⁸

MSCs have been shown to modulate inflammatory processes not only through the secretion of cytokines, but also by secreting systemic factors that can neutralize the activity of inflammatory cytokines.⁵⁹ This study reveals that MSCs secrete systemic agents that neutralize pro-inflammatory mediators, which in turn attenuate generalized inflammation. MSCs secrete a soluble form of TNF receptor 1, which neutralizes TNF α , and again the presence of LPS in the microenvironment enhances the expression of TNF receptor 1 by MSCs.⁵⁹

The intrinsic properties of MSCs for modulating inflammation are not restricted to cytokine secretion. We have also shown that umbilical cord mesenchymal stem cells (UMSCs) secrete exosomes.⁶⁰ Recently, other groups have shown that these exosomes secreted by MSCs can modulate the inflammatory response (Figure 1). MSC-derived exosomes improve functional recovery after traumatic brain injury by reducing inflammation and by promoting both endogenous angiogenesis and neurogenesis.⁶¹ MSC-derived exosomes have also been shown to be active effectors of immune regulation, stimulating T-cell proliferation, inducing B cell-mediated tumor suppression, inducing apoptosis in activated cytotoxic T cells, promoting differentiation of monocytes into dendritic cells, and stimulating T regulatory and myeloid-suppressive cells.⁶²⁻⁶⁵ Infusion of immunologically active MSC-derived exosomes enhances the survival of allogeneic skin grafts in mice, inducing high levels of anti-inflammatory IL-10 and TGF- β 1.⁶⁶ These exosomes induce an attenuated pro-inflammatory cytokine response and enhance anti-inflammatory IL-10 expression, reminiscent of an M2 macrophage profile, which are capable of promoting tissue repair while limiting injury. Additionally, Peche et al found that treatment of heart allograft recipients with intravenously administered donor-type dendritic cell-derived exosomes delays acute allograft rejection and induces significant prolongation of allograft survival in a rat model.⁶⁷ They also observed a significant decrease in both IFN- γ mRNA expression and graft-infiltrating leukocytes in the exosome-treated rats, suggesting immunomodulation of allograft rejection. MSC-derived exosomes have also been shown to inhibit the proliferation of Concanavalin A-activated lymphocytes.⁶⁸ Currently, MSC-derived exosomes are also being developed as drug delivery agents.

In a proteomic study, analysis of the content of BM-MSC-derived exosomes from control and multiple myeloma patients revealed that BM-MSC-derived exosomes from multiple myeloma patients contained higher expression of oncogenic proteins, cytokines, and protein kinases, such as IL-5, MCP-1, junction plakoglobin and fibronectin, than control patients.⁶⁹ MSC-like cells isolated from islet of Langerhans release highly immunostimulatory exosomes that activate autoreactive B and T cells that have been endogenously primed in a non-obese diabetic mouse model.⁷⁰ Both the serum levels of exosomes and exosome-induced IFN- γ production have been positively correlated with disease progression in prediabetic mice, suggesting that the exosomes could be autoantigen carriers acting as an autoimmune trigger.⁷⁰ The immunomodulatory properties of MSC-derived exosomes have also been previously tested in a model of stimulated T cells, revealing that exosomes exert

an inhibitory effect on the activation, proliferation, and differentiation of T cells reducing IFN- γ release.⁷¹

Recently, gene ontology analysis has been used to predict micro-RNA (miRNA) targets in MSCs and MSC-derived exosomes that could be related to development and cell survival.⁷² MSC-derived exosome miRNAs were mostly associated with immune system regulation. These exosome-derived miRNAs suppressed specific targets when transferred to recipient cells, demonstrating the direct functionality of these mediators in cell-to-cell communication.⁷³ Moreover, multipotent MSC-derived exosome-mediated transfer of miR-133b to neural cells has been shown to contribute towards neurite outgrowth.⁷⁴

B. Extrinsic Mechanisms

Over the past decade, extensive evidence has demonstrated that there is significant cross-talk between MSCs and inflammatory cells, and that pro- and anti-inflammatory microenvironments significantly alter the expression profile of MSCs. Many cytokines have been implicated in this cross-talk between MSCs and inflammatory cells; however, the precise mechanism by which MSCs suppress inflammatory cells, and also the mechanism by which MSCs sense pro- or anti-inflammatory environments, remain to be fully elucidated. Interestingly, over the past couple of years a new layer has been added to this MSC and inflammatory cell cross-talk: the extracellular matrix (**ECM**). MSCs have been shown to secrete a rich environment, which also plays a fundamental role in differentiation and immune suppression. We have recently demonstrated that UMSCs secrete a specific ECM that is composed of hyaluronan (**HA**), heavy chains (**HCs**), tumor necrosis factor-inducible gene 6 protein (**TSG-6**), and pentraxin 3 (**PTX3/TSG14**).²⁷ (See Figure 2.) This specific glycocalyx inhibits the maturation of M1 macrophages and promotes the maturation of both M2 macrophages and T-regulatory cells.

Recently, Kota et al demonstrated that BM-MSCs secrete a HA-rich glycocalyx (extensive pericellular HA coat) when treated with FGF-2, and proposed that this pericellular HA forms a protective niche.⁷⁵ This group also demonstrated that MSCs secrete HA cable-like structures which sequester/trap inflammatory cells.⁷⁵ This group activated MSCs with Poly I:C in the presence of serum containing Inter- α -Inhibitor (**I α I**) and MSC-secreted TSG-6, which cross-linked these HA chains and also catalyzed the transfer of heavy chains onto the HA, enabling the formation of the cable-like structures, as previously shown by de la Motte et al.⁷⁶ The formation of this HC/HA/TSG-6 matrix has also recently been shown to be essential for the settlement and differentiation of MSCs in muscles.⁷⁷ In this case, TSG6, HA and I α I were crucial factors for MSCs to create a microenvironment that was critical for transplantation success.

Oh et al have shown that solely injecting recombinant human TSG6 into the corneas of mice following chemical burns ameliorates the inflammatory response and improves corneal opacity.⁷⁸ This same group was able to reduce the rejection of mouse allogeneic corneal transplants by intravenous administration of MSCs, which were shown to be trapped primarily in the lungs and to increase TSG-6 levels.⁷⁹ Dyer et al have recently shown that free TSG-6 impairs both the binding of CXCL8 to cell surface glycosaminoglycans (**GAGs**) and its transport across the endothelium, thereby impeding formation of the CXCL8 gradient

required for neutrophil chemotaxis and extravasation.⁸⁰ Also, BM-MSCs treated with pro-inflammatory macrophages lead to an increase in GAG expression.⁸¹

The fact that MSCs secrete a rich ECM microenvironment, which is essential for the settlement, differentiation, and immunosuppressive properties of MSCs, comes as no surprise. The ECM is crucial for directing cellular processes and maintaining tissue integrity.⁸²⁻⁸⁷ Moreover, the severe developmental phenotypes shown by knock-out mice for heparan sulfate, an important ECM component, is a testament to the importance of the ECM for creating microenvironments.^{88,89} Therefore, more studies should be directed toward unveiling the intricate ECM secreted by MSCs, which enables them to perform their vital tasks-- immune suppression and tissue repair. Moreover, given the importance the ECM plays on the tissue microenvironment, many groups are developing HA-based tissue scaffolds to use as a means of MSC delivery, which increase transplantation success.^{90,91} Therefore, the development of HA-based cell-delivery vehicles for regenerative therapies is an exciting, growing field with prospects of increasing transplantation efficiency. TSG-6 has recently been suggested as a biomarker for predicting the efficacy of MSCs for treating sterile inflammatory models of corneal injury.⁹²

III. Therapeutic Potential of Mesenchymal Stem Cells

A. Treatment of Ocular Surface Diseases

1. Anterior Segment—The therapeutic potential of MSCs for treating the ocular surface has been widely explored, primarily because the cornea is easily accessible and is an immune-privileged tissue. MSCs have the ability to differentiate into epithelial cells⁹³⁻⁹⁵ and keratocytes.⁹⁶ Our previous work has shown that UMSCs can be used to successfully treat the congenital lysosomal storage disorder mucopolysaccharidosis VII (**MPS VII**).⁶⁰ This study shows that UMSCs transplanted into the corneal stroma of MPS VII mice secrete exosomes containing the deficient enzyme, which are taken up by host keratocytes enabling GAG catabolism, thereby reducing GAG accumulation in both the ECM and keratocytes.⁶⁰ Moreover, one month after transplantation of UMSCs into mouse corneal stroma, these cells assume keratocyte-like morphology and express a keratocyte cell marker (Figure 3).

Great strides have been made in applying MSCs to treatment of corneal disorders, such as experimental limbal stem cell deficiency (**LSCD**).⁹⁷ BMSCs and limbal epithelial stem cells are similarly efficacious for treating limbal cell deficiency after alkali burn.⁹⁸ The ability of BMSCs to treat LSCD is attributed not only to transdifferentiation, but also to suppression of local inflammation and secretion of growth factors, thereby supporting the growth of residual corneal epithelial cells.⁹⁸ The use of MSCs for treating corneal diseases has recently been reviewed by our group, and the discussion below focuses primarily on the use of MSCs for treating inflammatory response in the ocular surface (Figure 4).⁹⁹

Systemically administered MSCs have been shown to home into the inflamed cornea.^{100,101} We have recently demonstrated the efficacy of human UMSCs to limit inflammation after alkali burn using the mouse model.²⁷ The use of BMSCs was further demonstrated for limiting damage after alkali burn.^{92,102} Both human MSCs and human MSC-conditioned media were shown to improve cell survival after ethanol injury to corneal epithelial

progenitor cells.¹⁰³ Human MSC infusions have also been shown to prevent mouse allogenic corneal transplant rejection by suppressing inflammation.⁷⁸

The success of these studies involving grafting human MSCs into animal models resides in the fact that MSCs actively modulate immune cells, enabling them to survive xenograft rejection. Both mouse and rabbit BMSCs transplanted onto the ocular surface have been shown to suppress local inflammation.^{104,105} Moreover, our recent work has demonstrated that UMSCs inhibit the polarization of M1 macrophages and lead to the maturation of M2 macrophages.

Great expectations reside in generating treatments that increase the levels of “therapeutic cells,” such as M2 macrophages, as a means of resolving inflammation as opposed to simply using immunosuppressors to hamper the whole immune system. Current findings indicate that MSCs show great improvement over current treatments, since they actively modulate immune cells into restorative phenotypes rather than simply suppressing the immune system. The use of MSCs has recently been widely explored for preventing host-versus-graft disease. For these studies, MSCs are administered due to their immune-suppressive properties for preventing the rejection of transplanted tissues. BMSCs have been shown to promote the survival of a fully allo-MHC-mismatched corneal allograft by suppressing both local and systemic inflammation.¹⁰⁶

2. Posterior Segment—The use of MSCs to treat the posterior chamber of the eye has also been explored. Vitreoretinal and chorioretinal diseases are chronic, progressive conditions related to refractory retinal degeneration due to pathophysiological conditions. Some stem cell therapy strategies involving human embryonic stem cells (**hESC**) have been successfully used to treat age-related macular degeneration (**AMD**) and Stargardt's macular dystrophy^{107,108}; however, MSC targeting as a choice of delivery is still under development and has not yet reached clinical trial level.

Recently, delivery of MSCs labelled with magnetic iron oxide nanoparticles has been investigated for targeting dystrophic retinas.¹⁰⁹ These particles were well tolerated, and the MSCs remained viable, retaining their differentiation abilities after intravitreal and/or intravenous administration. The use of MSCs for treating non-neovascular dry AMD has also been explored. Dry AMD is primarily caused by the accumulation of reactive oxygen free radicals and lipid peroxide in retinal pigment epithelium (**RPE**) cells, leading to the activation of chronic inflammation and apoptosis, damaging outer nuclear layer photoreceptors.¹¹⁰ MSCs have the ability to cross-differentiate into RPE-like cells in vivo with similar morphological and phagocytic capabilities.^{111,112} This has also been accomplished by altering MSC gene expression prior to transplantation.^{113,114}

Currently, cells are transplanted as subretinal masses, thereby limiting the number of cells injected and consequently the levels of photoreceptors that can be rescued in areas surrounding injection sites. This emphasizes the need for in-depth studies regarding the delivery method^{115,116} and the need/efficacy of gene manipulation of MSCs prior to treatment.

Another posterior segment malady is retinitis pigmentosa, which affects mainly photoreceptors, making it a more difficult target for regeneration. Recent studies have tried to access invasive¹¹⁷⁻¹²¹ and non-invasive¹²² delivery therapies, with MSCs being capable of surviving primarily in the outer layer of the retina. These cells were able to differentiate into RPE cells and photoreceptors, but whether there was any functional recovery of the retina remains to be investigated.

B. Treatment of Other Diseases

MSCs are still being explored for their great potential in cell replacement therapy, but their ability to alter the immune response is being vastly exploited for therapeutic purposes. Currently, clinical trials are underway that explore the use of MSCs for the treatment of steroid-refractory graft-versus-host disease.¹²³ Moreover, MSC pre-clinical studies have also been carried out showing that MSCs are effective for treating ischemic heart disease, ischemic kidney injury, type 2 diabetes mellitus, and, more recently, several models of sepsis.^{54,124-126} All these studies in one way or another have taken advantage of the valuable immunosuppressive properties of MSCs.

MSCs have also been suggested as a new alternative for repairing skin wounds. Topical administration of UMSCs together with skin microparticles after degree III deep burn wounds leads to the development of newborn skin and its appendages. New methods of delivering MSCs and supporting matrices are also currently being explored. Laminin-PAMs have been used to support MSCs in an in vivo model of transient stroke in order to increase MSC survival. This strategy achieved prolonged vascular endothelial growth factor (VEGF) release, increasing angiogenesis around the implantation site and supporting the migration of immature neurons towards the ischemic site.¹²⁷

It is of particular interest that MSCs present tumor-homing properties and can therefore be used in tumor-targeting treatments. Qiao et al showed that MSCs transfected with adenoviruses carrying the OPG gene migrate to tumor sites and express OPG protein in mice bearing osteosarcoma, reducing tumor growth and inhibiting bone destruction.¹²⁸ MSCs have also been shown to be useful for the delivery of therapeutic genes in a colon cancer liver metastasis mouse model.¹²⁹ There are many other examples of MSCs being a potential vehicle for therapeutic delivery to tumors, including to brain tumors,¹³⁰ lung tumors,¹³¹ hepatocellular carcinoma,¹³² and prostate cancer.¹³³ These studies describe the use of MSCs for delivering not only therapeutic genes, but also therapeutic drugs and theranostic agents.

The characteristic of MSCs for suppressing the activity of several T-lymphocyte populations both in vivo and in vitro has been explored for therapeutic applications in treating T-cell mediated diseases,¹³⁴⁻¹³⁸ such as, graft-versus-host disease,^{123,139} and Crohn's disease,¹⁴⁰ and to prevent rejection after organ transplantation.¹⁴¹

IV. Future Directions of Mesenchymal Stem Cell Research

Advances in MSC biology have raised expectations that diseases, injuries, and the need for an organ transplant can be ameliorated by transplanting MSCs. Recent advances in MSC research have broken the stem cell therapeutic paradigm with several groups, revealing that

the therapeutic benefits of MSCs do not derive exclusively from extensive cell engraftment. Furthermore, studies have shown that solely soluble factors secreted by MSCs are sufficient for therapeutic benefits; for example, TSG-6 secreted by MSCs trapped in the lungs decreases early and excessive neutrophil infiltration into the heart of a mouse myocardial infarction model.⁷⁹ Moreover, recently, MSC-derived exosomes have been successfully used to suppress the immune response.^{62,142-146} The ability of MSC-derived exosomes to suppress immune cells has attracted much attention for the development of MSC-derived exosomes for alternative treatments. MSC-derived exosomes are being tailored for development as vehicles for drug delivery; for example, siRNA has been delivered to the mouse brain by systemic injection of exosome-packaged siRNA.¹⁴⁷ Furthermore, solely extracellular vesicles released by MSCs have been shown to promote angiogenesis following myocardial infarction and protect the cardiac tissue from ischemic injury.¹⁴⁸

In recent years, within the field of MSCs, much attention has been dedicated to isolating active components from MSCs as therapeutic agents as opposed to using the MSCs themselves, of which TSG-6 is an excellent prototype. Many in vivo studies have shown that injecting TSG-6 alone reproduces many of the beneficial effects of MSCs. Further studies have demonstrated that administration of solely enriched MSC-secreted soluble compounds can suppress inflammation and lead to regeneration.^{149,150} Studies have also started to isolate active components from MSCs for promoting cell differentiation; for example, active components isolated from UMSCs can induce osteogenic differentiation of BM-MSCs. These studies therefore suggest that active components can be extracted from MSCs for treating chronic inflammatory disorders and promoting differentiation.¹³⁰

Currently, there has been a growing trend toward developing MSCs for therapy due to their ability to actively suppress inflammation and not for their capacity to differentiate into bone, cartilage, or adipose tissue. MSC research avenues are now dedicated to exploring the immunomodulatory capability of these cells, and many preclinical and clinical studies are using MSCs as therapeutic agents for the treatment of autoimmune or degenerative diseases. Moreover, due to the self-renewal capacity of MSCs and their ability to differentiate into tissues of mesodermal origin, MSCs show great potential for being developed into a cell-based therapy for immune disorders or degenerative diseases. Adipose-derived MSCs have been effectively used to treat a murine model of Crohn's disease by downregulating pro-inflammatory cytokines, such as TNF- α , IL-12 and VEGF, and upregulating anti-inflammatory cytokine IL-10.¹⁵¹ Adipose-derived MSCs have also been used for ameliorating radiation-induced pulmonary fibrosis in Sprague-Dawley rats, whereby hepatocyte growth factor (**HGF**) and PGE2 levels increased and TNF- α and TGF- β 1 levels decreased after MSC infusion.¹⁵² Another example is the use of BM-MSCs to treat chronic obstructive pulmonary disease, where the BM-MSCs alleviate airway inflammation and emphysema by promoting the down-regulation of cyclooxygenase-2 (**COX-2**) and COX-2-mediated PGE2 production in alveolar macrophages through p38 and ERK.¹⁵³

MSCs provide therapeutic potential beyond immunosuppression and cell therapy. BM-MSCs have been used to treat a non-immune cuprizone model of multiple sclerosis, where intravenously transfused BM-MSCs were able to migrate to demyelinating sites, engraft and promote remyelination and reduce demyelination by oligodendrocytes. This study suggests

that MSCs induce oligo/neuroprotection, demonstrating that MSCs can also support functional improvement of resident cells.¹⁵⁴ BM-MSCs have also been shown to promote functional improvement following myocardial infarction,¹⁵⁵ and the outcome can be further improved by using genetically modified or treated MSCs, such as HGF gene-modified BMMSCs,¹⁴⁶ heat shock protein 27 (Hsp27) gene-modified MSCs,¹⁵⁶ receptor activity-modifying protein 1 (**RAMP1**) gene-modified MSCs,¹⁵⁷ or MSCs previously treated with IL-1 β and TNF- α .¹⁵⁸ Moreover, solely the supernatant of MSCs transfected with adenovirus carrying human heme oxygenase-1 gene has been shown to improve cardiac function suggesting an effect of paracrine-acting mediators secreted by MSCs.¹⁵⁹

MSCs are attracting a great deal of attention from pharmaceutical companies, and commercial BM-MSC cell lines are now available, e.g., stempeucel®, which are adult allogeneic mesenchymal stem cells that are being used in a number of phase II clinical trials with clinical trials planned for 2015/2016. Stempeucel® have recently been used in a phase I/II study for treating humans after acute myocardial infarction with intravenous transplantation.¹⁶⁰ These preliminary studies with commercial BM-MSCs have demonstrated that they are safe and well tolerated when administered intravenously.

V. Conclusion

A recent paradigm shift in MSC research has generated interest in using MSCs primarily for their immunosuppressive properties and secreted components, and not solely for use as cell replacement therapy. Moreover, interest is growing in isolating the active components from MSCs for therapeutic purposes, as opposed to using MSC cell engraftment. These new therapeutic avenues have generated great enthusiasm for MSC research.

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Figure 1.

Schematic of the intrinsic mechanisms by which MSCs modulate the inflammatory response. MSCs respond differently to pro-inflammatory and anti-inflammatory environments. In an anti-inflammatory environment (low levels of IFN- γ) TLR4 activation in MSCs leads to MHC-II expression and the presentation of antigens to T cells, which in turn secrete IFN- γ creating a pro-inflammatory environment. In a pro-inflammatory environment TLR3 activation leads to the secretion of anti-inflammatory cytokines such as IL-10, TGF- β , Sema3A and galectin-1 and -3 thereby creating an anti-inflammatory environment and inhibiting T-cell, neutrophil and macrophage (M ϕ) recruitment. Thus, the delicate balance of IFN- γ in the MSC environment dictates MSC phenotype

Figure 2.

Schematic of the extrinsic mechanisms by which MSCs modulate the inflammatory response. MSCs express a rich glycocalyx composed of HA/HCs/TSG-6/PTX3 which actively suppresses inflammatory cells. A soluble form of HA/HCs/TSG-6/PTX3 inhibits maturation of M1 macrophages and promotes the maturation of M2 macrophages. The HA cables produced sequester macrophages maintaining them in a non-polarized state (M0).

Figure 3. UMSCs *in vitro* and *in vivo*. (A) UMSCs in culture prior to transplantation into mouse corneal stroma. (B) 1 month after transplantation UMSCs assume keratocyte-like morphology and express a keratocyte cell marker in the corneal stroma.

MSCs, Ethanol Injury (TSG-6 and STG-1 > proliferation & inhibit apoptosis of Epi) [103]
BMSCs, Corneal chemical injury (TSG-6 suppresses inflammation) [92]
BMSCs Alkali burn, (Transdifferentiation, residual cell support, immunosuppression) [98]
BMSCs Alkali burn, (Suppression of oxidative injury) [105]

Figure 4.

Schematic of MSC therapeutic developments for treating inflammatory disorders of the ocular surface. Various clinical applications have been developed for treating inflammatory disorders associated with the corneal epithelium (listed below the image) and stroma (listed above the image). However MSC treatments for curing endothelial defects remain to be developed. Each line represents a study with the type of MSC used listed in bold, followed by the condition being treated and finally the mechanism by which the MSCs resolve the condition listed in parenthesis.

Table 1

Cell surface receptors involved in MSC inflammatory cell cross-talk

Surface receptor	Role
• <i>MSCs</i>	
HLA class II	Present antigens to T-cells ¹⁶¹
CXCR4	Stimulate cell migration ^{162,163}
CD44	Cell-cell communication ¹⁶⁴⁻¹⁶⁶
Integrin	Cell-cell communication ^{167,168} ; $\alpha 5\beta 1$ ¹⁶⁹
Notch	Epithelial-to-mesenchymal transition (EMT) ¹⁷⁰
ICAM-1/2	Homing through migration and adhesion ¹⁷¹
VCAM-1 (CD106)	Mobilization of granulocyte-macrophage colony-forming units ^{172,173}
ALCAM (CD166)	Regulation of tumor cell invasion ^{174,175}
CD90	Mechanical stimuli receptor ¹⁷⁶ ; immunosuppressive activity ¹⁷⁷
CD105	Allogenic modulation of immune cells ¹⁷⁶
CD45	Antigen receptor signal transduction and lymphocyte development ¹⁷⁸
CD34	Decrease cell adhesion ¹⁷⁹
CD14/ CD11b	LPS receptor for promoting TLR4 endocytosis and interferon expression ¹⁸⁰⁻¹⁸²
• <i>T-cells</i>	
CCR10	Migration of T cells, ¹⁸³ chemokine receptor ¹⁸⁴

Table 2

Immunoregulatory cytokines secreted by MSCs

Secreted cytokines	Role
HLA	Present antigens to T-cells ¹⁸⁵ ; inhibitory interactions and NK cell activation ^{186,187}
TGF- β	Induce epithelial to mesenchymal transition (EMT), ¹⁸⁸ pluripotency maintenance ¹⁸⁹
IL-10/Galectin-3	Immunosuppression ¹⁹⁰⁻¹⁹⁴
Leukemia inhibitory factor (LIF)	Transplantation tolerance ^{37,195}
Semaphorin-3A	Endogenous antiangiogenic agent ^{196,197}
TNF- α /INF- γ	Immunomodulation ^{13,198}