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Review

Immunobiology of mesenchymal stem cells

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Mesenchymal stem cells (MSCs) can be isolated from almost all tissues and effectively expanded *in vitro*. Although their true *in situ* properties and biological functions remain to be elucidated, these *in vitro* expanded cells have been shown to possess potential to differentiate into specific cell lineages. It is speculated that MSCs *in situ* have important roles in tissue cellular homeostasis by replacing dead or dysfunctional cells. Recent studies have demonstrated that *in vitro* expanded MSCs of various origins have great capacity to modulate immune responses and change the progression of different inflammatory diseases. As tissue injuries are often accompanied by inflammation, inflammatory factors may provide cues to mobilize MSCs to tissue sites with damage. Before carrying out tissue repair functions, MSCs first prepare the microenvironment by modulating inflammatory processes and releasing various growth factors in response to the inflammation status. In this review, we focus on the crosstalk between MSCs and immune responses and their potential clinical applications, especially in inflammatory diseases.

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Facts

- The immunoregulatory properties and the tissue reparative functions of mesenchymal stem cells (MSCs) are induced by inflammatory cytokines.
- MSCs express immunosuppressive molecules and various growth factors that facilitate tissue repair and maintain immune homeostasis.
- The plasticity of immunoregulatory roles of MSCs is relied on the inflammatory status.

Open Questions

- How do MSCs benefit patients suffering from inflammatory diseases?
- Do MSCs always possess the immunosuppressive properties?
- Does the efficiency of MSC engraftment at the injured tissue sites determine the therapeutic effects of MSCs?

Properties of Mesenchymal Stem Cells

MSCs exist in almost all tissues, and have the capacity of self-renewal and the potential to differentiate into multiple cell types. Under certain physiological and experimental conditions, MSCs differentiate into specialized cells,[1] although to a lesser extent than embryonic stem cells (ES) and induced pluripotent stem cells (iPS). Nevertheless, their differentiation capacity has encouraged scientists and clinicians to seek suitable protocols to apply these cells to treat various diseases. Recently, several studies have demonstrated that MSCs can be activated and recruited to sites of tissue damage where they regenerate new tissues and repair the defects.

Stem cells bring new hope for the treatment of many diseases. The clinical use of ES or iPS cells, however, is hampered by their tendency to form teratomas, by allogeneic rejection problems and by ethical issues. In comparison with ES cells and iPS cells, MSCs are devoid of the ethical, teratomas-formation and histocompatibility issues. MSCs can be isolated from nervous tissue, adipose tissue, bone marrow, amniotic fluid, umbilical cord, placenta, menstrual blood and even dental pulps.[2–5] Morphologically, MSCs have the appearance of fibroblasts. Although there is no specific marker that identifies MSCs, these cells do express certain patterns of surface markers. Because of their potent self-renewal capacity, MSCs can be passaged many times without significant alteration of their major properties.[6] MSCs have the

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Abbreviations: MSCs, mesenchymal stem cells; ES, embryonic stem cells; iPS, induced pluripotent stem cells; EGF, epidermal growth factor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; IGF, insulin growth factor-1; Ang-1, angiopoietin-1; KGF, keratinocyte growth factor; SDF-1, stromal cell-derived factor-1; GvHD, graft *versus* host disease; SLE, systemic lupus erythematosus; iNOS, inducible nitric oxide synthase; NO, nitric oxide; PGE2, prostaglandin E2; LIF, leukemia inhibitory factor; DTH, delayed-type hypersensitivity; IDO, indoleamine 2,3-dioxygenase; TSG6, tumor necrosis factor-inducible gene-6; HO-1, hemeoxygenase-1; G-CSF, granulocyte colony-stimulating factor; IBD, inflammatory bowel diseases; EAE, experimental autoimmune encephalomyelitis

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potential to differentiate into several different cell types, such as adipocytes, chondrocytes, osteoblasts, myocytes and neurons (Figure 1).[7–11] These criteria are used to define cultured MSCs, however, the exact anatomical locations of these cells *in situ* remain unclear. Experiments to track MSCs *in vivo* have revealed that these cells reside mostly close to blood vessels,[12,13] a trait that is similar to pericytes. Pericytes in culture are similar to cultured MSCs in term of their morphological features, cell surface markers as wells as differentiation potential into osteoblasts, chondrocytes and adipocytes.[12,13] However, not all pericytes have the unique properties of MSCs and not all MSCs are equivalent to pericytes. The key distinction is that pericytes locate strictly in the basement membrane of capillary and post capillary, whereas MSCs can be isolated from interstitial tissues and tissues surrounding arteries and veins.[14–16] In addition, the proposed functions of pericytes are heterogenous and varied from regulating vessel stabilization to vascular integrity and tone, which are different from the functions of MSCs.[14] MSCs are involved in many physiological and pathological processes, including cellular homeostasis maintenance, aging, tissue damage and inflammatory diseases.[1,17,18] Although their differentiation potential is less broad than that of ES cells and iPS, MSCs, nevertheless, hold great promise for clinical applications. The most prominent therapeutic effect of MSCs is exerted through their immunoregulatory functions. The aim of this review is to elucidate the bidirectional regulatory interactions between MSCs and immune responses. We specifically emphasize recent reports of *in vitro* investigations and *in vivo* preclinical studies that reveal the mechanisms of this MSC-immune response interaction. We also discuss their implications for the clinical uses.

Communication between MSCs and Damaged Tissues

Because of their broad tissue distribution, multipotent differentiation capacity and well-established effects in preclinical and clinical studies, MSCs are believed to have critical roles in repairing damaged tissues.[18] Tissue injury is always associated with the activation of immune/inflammatory cells, not only macrophages and neutrophils but also adaptive immune cells, including $CD4^+$ T cells, $CD8^+$ T cells and B cells, which are recruited by factors from apoptotic cells, necrotic cells, damaged microvasculature and stroma.[19,20] Meanwhile, inflammatory mediators, such as TNF-α, IL-1β, free radicals, chemokines and leukotrienes, are often produced by phagocytes in response to damaged cells and spilled cell contents.[21] Thus, these inflammatory molecules and immune cells, together with endothelial cells and fibroblasts, orchestrate changes in the microenvironment that result in the mobilization and differentiation of MSCs into stromal and/or replacement of damaged tissue cells. These MSCs can be tissue-resident or be recruited from the bone marrow. However, the mechanisms by which MSCs are mobilized and recruited to damaged sites are not known. In addition, how they survive and differentiate into distinct cell types is still not clear. Once MSCs have entered the microenvironment of injured tissues, many factors, including cytokines such as TNF-α, IL-1, IFN-γ, toxins of infectious agents and hypoxia can stimulate the release of many growth factors by MSCs, including epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin growth factor-1 (IGF-1), angiopoietin-1 (Ang-1), keratinocyte growth factor (KGF) and stromal cell-derived factor-1 (SDF-1).[22–25] These growth factors, in turn, promote the development of fibroblasts, endothelial cells and tissue progenitor cells, which carried out tissue regeneration and repair (Figure 2, Table 1).

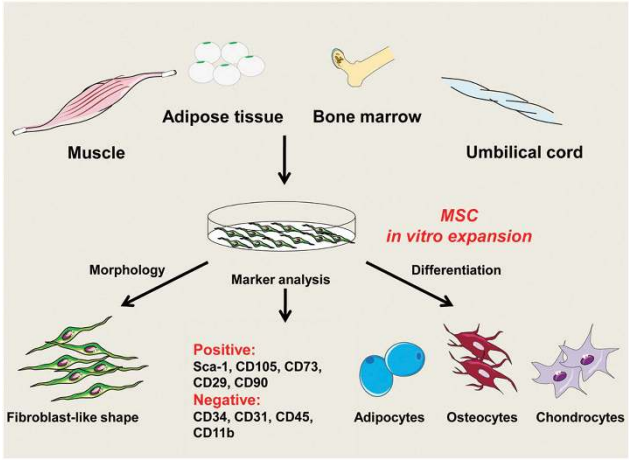

Figure 1 The properties of MSCs. MSCs can be isolated from various tissues including adipose, bone marrow, umbilical cord, muscle and tooth root. After *in vitro* expansion, MSCs can be defined by several characteristics. Morphologically, MSCs are fibroblast like. They also express a panel of markers: positive for Sca-1, CD105, CD73, CD29 and CD90, and negative for CD31, CD34, CD45 and CD11b. In addition, MSCs have the potential to differentiate into adipocytes, chondrocytes, osteoblasts and other cell types

The barrier function of the endothelial monolayer in the capillary bed is often broken down in damaged tissues, allowing the release of protein-rich plasma and some leukocytes from the blood. MSCs produce various factors, like Ang-1, VEGF, HGF, EGF, PDGF, FGF, KGF and TGF-β, which directly affect endothelial cells. These paracrine trophic factors are potentially important in maintaining endothelial integrity and promoting angiogenesis through their ability to regulate endothelial cell proliferation and extracellular matrix production, reduce endothelial permeability or prevent interactions between leukocytes and endothelial cells.[26,27] Apart from angiogenesis mediated by endothelial cells, in response to such trophic factors, fibroblasts also have essential functions in maintaining tissue integrity and promoting wound healing through their secretion of extracellular matrix and matrix metalloproteinase. Some *in vivo* studies have suggested that growth factors secreted by MSCs can be applied to improve wound healing and recovery from myocardial infarction.[28–30]

The long-term functional recovery of damaged tissue and organs is likely to depend on the differentiation of tissue-intrinsic progenitors or stem cells. Although engrafted stem cells can differentiate into tissue cells, they also produce growth factors, including stem cell factor (SCF), macrophage colony-stimulating factor (M-CSF), SDF-1, leukemia inhibitory factor (LIF), Ang-1 and many chemokines, that intrinsically trigger tissue repair.[22,31–33] HGF, a well-demonstrated growth

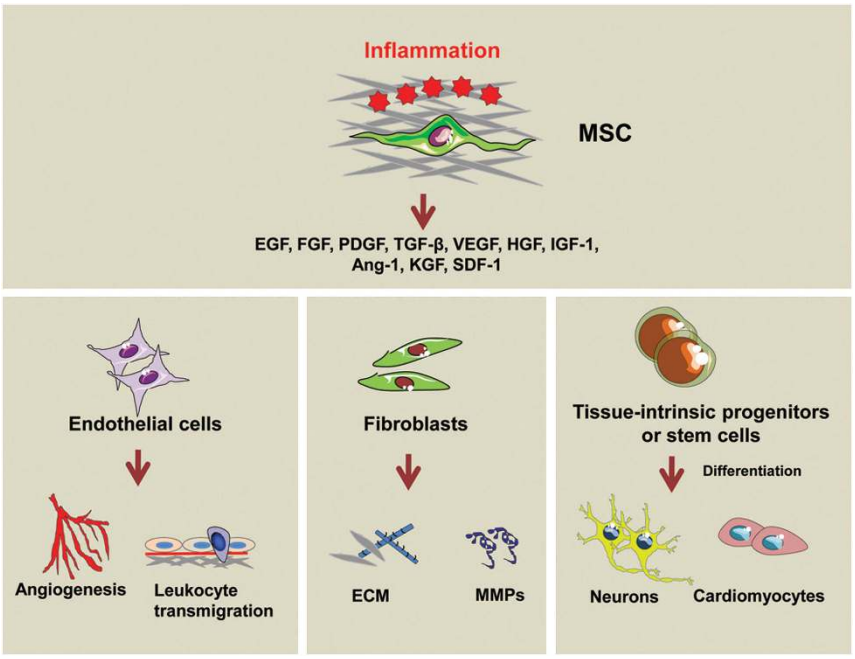

Figure 2 Tissue reparative properties of MSCs. Under the stimulation of different inflammatory cytokines at the damaged tissue sites, the newly immigrated MSCs release a plethora of growth factors, including EGF, FGF, PDGF, TGF-β, VEGF, HGF, Ang-1, KGF, SDF-1, IGF-1 and others. These growth factors orchestrate endothelial cells, fibroblasts as well as stem cells to promote tissue regeneration and repair through enhancing angiogenesis, inhibiting leukocyte transmigration and eliciting intrinsic progenitor cell/stem cell differentiation

Table 1 Summary of growth factors critical for MSC-mediated tissue repair

Growth factors	Roles in MSC-mediated tissue repair
EGF	Wound healing,[114] tissue regeneration,[115,116] neurogenesis[117]
PDGF	Tissue repair[118]
FGF	Tissue repair,[114] intrinsic stem cell survival and regeneration[119]
TGF-β	Wound healing[120,121]
VEGF	Angiogenesis, wound healing[121–123]
HGF	Vasculogenesis,[124] intrinsic neural cell regeneration[34]
IGF-1	Wound healing,[114] neurogenesis[125]
KGF	Wound healing[126]
Ang-1	Angiogenesis, tissue repair[123]
EPO	Angiogenesis[127]
GDNF	Neuroprotective effect[128]
SDF-1	Neuroprotective effect,[129] wound healing[130,131]
IL-8	Wound healing[114]

Abbreviations: Ang-1, angiopoietin-1; EGF, epidermal growth factor; EPO, erythropoietin; FGF, fibroblast growth factor; GDNF, glial cell line-derived neurotrophic factor; HGF, hepatocyte growth factor; IGF, insulin growth factor-1; IL-8, interleukin-8; KGF, keratinocyte growth factor; MSC, mesenchymal stem cell; PDGF, platelet-derived growth factor; SDF-1, stem cell-derived factor-1; TGF-β, transforming growth factor β, VEGF, vascular endothelial growth factor

factor in MSC-based tissue repair, was recently shown to be effective in modulating endogenous neural cell remyelination for the enhancement of functional recovery in both experimental autoimmune encephalomyelitis (EAE) and spinal cord demyelination.[34] Taken together, these observations demonstrate complex interactions that exist between MSCs and the damaged tissue during the tissue repair process. The multitude of paracrine factors produced by MSCs, which provoke tissue-resident progenitor cells or other relevant cells to initiate tissue repair, may explain the dramatic beneficial effects of MSCs on tissue repair, even in the absence of local MSC engraftment.[34,35]

Some tissue injuries, including those induced by chemical toxicity and trauma, are considered not immune cell-related; however, strong inflammation still occurs in these damaged tissues.[36,37] Thus, better elucidation of the detailed mechanisms underlying the inflammation-modulated production of growth factors by MSCs will provide a better perspective for the clinical application of MSCs or their paracrine factors in tissue regeneration.

MSCs and Inflammation Niches

Besides the reparative functions of MSCs in inflammatory niches, the increasing evidences demonstrate that MSCs have potent immunomodulatory properties. For example, MSCs retain dendritic cells (DCs) in an immature state by inhibiting the expression of MHC class II, CD1-α, CD40, CD80 and CD86, and by suppressing proinflammatory cytokine production.[38] TNF-α-stimulated MSCs can recruit more monocytes/macrophages to tumor site to formulate the immunosuppressive microenvironments, thereby enhancing tumor growth.[39] Moreover, MSCs induce IL-10-secreting macrophages in both *in vitro* and *in vivo* study.[40] Besides DCs and macrophages, NK cells can also be suppressed by MSCs through soluble factors, such as TGF-β and prostaglandin E2 (PGE2).[41] Similar results are observed with human cells that MSCs inhibit IL-2- or IL-15-driven NK cell proliferation.[42] In adaptive immune responses, MSCs have been already known to inhibit T-cell proliferation triggered by many

types of stimuli, to downregulate IFN-γ production and to induce Tr1 cells and Foxp3$^+$ regulatory T cells.[43–45] In addition, they can decrease B-cell proliferation by cell–cell contact and secreted soluble factor, although the effects change dramatically according to culture conditions.[46]

Recently, it has been suggested that immunosuppressive functions of MSCs are triggered by the surrounding microenvironment, where abundant inflammatory factors are released from immune cells.[47,48] Initially, studies of the mechanism of immune modulation by MSCs were conflicting. Some studies of graft *versus* host disease (GvHD) and systemic lupus erythematosus (SLE) showed the benefits of MSCs in inhibiting vigorous immune responses *in vivo*.[48–50] In other studies, although suppression of lymphocyte proliferation by MSCs could be observed *in vitro*, prolongation of graft survival and rescue from GvHD *in vivo* were not achieved.[51,52] These investigations show that the immunosuppressive property of MSCs could be affected by specific disease-related tissue microenvironments. It has been reported that although allogeneic MSCs are rejected by the host immune system in MHC class I- and II-mismatched recipient mice, they exhibited similar therapeutic effect on EAE as that of autologous MSCs.[53,54] That is, even though MSCs have the ability to downregulate immune responses, these cells may not be immune privileged. In MSC-based therapy, the dosage of cells is also important. Using a rat brain injury model, researchers recently have found that there is an efficacy plateau, above which additional delivered MSCs could not further improve the outcome.[55] Moreover, MSCs with high passage number showed diminished stem cell activation and myocardial protection.[56] Hence, immunosuppression by MSCs can be influenced by conditions, such as the source from which MSCs are isolated, the number of passages in culture before they are used, the dosages of MSCs administered and the specific pathological conditions of the recipients. Nevertheless, the immunomodulatory roles of MSCs have attracted great interest from basic and clinical researchers.

Immunosuppressive Properties of MSCs

Recently, our studies have found that the ability of MSCs to inhibit immune cell activity is licensed by inflammatory environment. IFN-γ in combination with one of the proinflammatory cytokines, TNF-α, IL-1α or IL-1β, can stimulate MSCs to elicit very high levels of immunosuppressive factors, as well as a burst of chemokine and adhesion molecule expression, including CXCR3 ligands, CCR5 ligands, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).[48,57] Their concerted action leads to an accumulation of immune cells in close proximity to MSCs, thus fabricating a microenvironment in which the effects of the locally acting factors produced by MSCs are amplified and leading to potent immunosuppression (Figure 3).

Surprisingly, the molecules that mediate MSC-induced immunosuppression are not the same in different species. We have found that murine MSCs use inducible nitric oxide synthase (iNOS) produced nitric oxide (NO), which is highly immunosuppressive at high concentrations through largely undefined mechanisms.[58–60] In murine models of delayed-type hypersensitivity (DTH) and GvHD, when iNOS

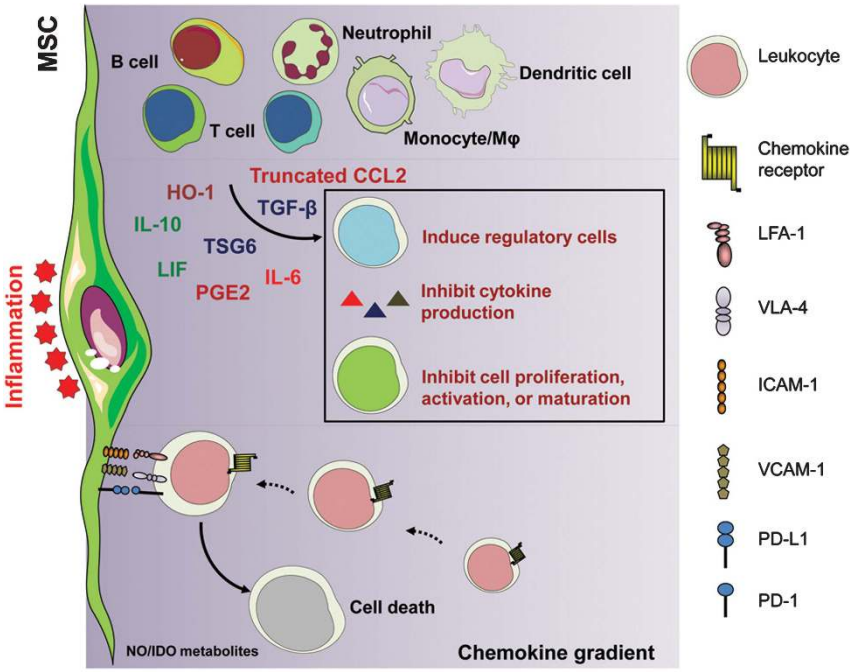

Figure 3 Immunosuppressive properties of MSCs. Damaged tissues are always accompanied by infiltration of immune cells and MSCs. Inflammation triggers the production of high levels of chemokines and adhesion molecules in MSCs, including CXCR3 ligands, CCR5 ligands, ICAM-1 and VCAM-1. These molecules induce the accumulation of immune cells in close association with MSCs, whereby high concentrations of NO (in murine MSCs) or depletion of tryptophan (in human MSCs) leads to the inhibition of immune cells. Other immunosuppressive factors such as IL-10, TSG6, IL-6, LIF, PGE2, HO-1 and truncated CCL2 could also affect immune cell activation, proliferation and functions

activity was abolished in MSCs by either chemical inhibition or genetic ablation, the therapeutic effects disappeared.[46,61,62] Meanwhile, iNOS-deficient MSCs also showed less therapeutic effects on autoimmune arthritis in mouse.[63] These findings suggest that NO is a major player in mediating the immunosuppressive function of murine MSCs. Interestingly, metalloproteinase-mediated paracrine proteolysis of CCL2 was also found to be important in murine MSC-based therapy on EAE.[64]

Of note, although murine MSCs use NO to exert their immunosuppressive function, human MSCs harness indoleamine 2,3-dioxygenase (IDO) instead.[47,65,66] IDO is an enzyme that catalyzes the degradation of tryptophan, an essential amino acid, along the metabolic pathway to kynurenine. Immunosuppression is believed to result from the depletion of tryptophan and the accumulation of tryptophan metabolites locally,[67] but their related mechanisms have not been fully investigated. Besides IDO, tumor necrosis factor-inducible gene-6 (TSG6), a supposedly anti-inflammatory protein, can be induced in human MSCs by TNF-α. Deletion of TSG6 in human MSCs reverses their ability to repair myocardial infarct, corneal damage and fails to prolong the corneal allograft survival.[68–70] In addition, TSG6 production by human MSCs can ameliorate zymosan-induced mouse peritonitis by modulating the toll-like receptor 2 (TLR2)/nuclear factor κB (NF-κB) signaling pathway in resident macrophages.[71] However, the exact role of TSG6 in human MSC-mediated immunoregulation merits further investigations. Other factors, such as HLA-G, LIF and some others, are also documented to mediate the immunosuppression by human MSCs *in vitro*[32,72,73] (Table 2). LIF was found to be secreted by mouse ES cell-derived neuron progenitors and exerts the therapeutic benefit to EAE,[35] yet the role of this molecule in murine MSC-mediated immunoregulatory effects in remains elusive.

Studies implicated that, except above mentioned immunosuppressive factors in murine or human MSCs, some molecules are shared in murine MSC- and human MSC-mediated immunosuppression, such as PGE2, IL-10, hemeoxygenase-1 (HO-1), programmed cell death 1 ligand 1 (PD-L1) and IL-6[40,61,74–77] (Figure 3, Table 2). Particularly, PGE2 has been shown to be produced by inflammatory factor-stimulated murine and human MSCs. PGE2 released by mouse or human MSCs alone can reprogram macrophages to produce more IL-10, inhibit DC maturation as well as shift the balance between Th1 and Th2.[40,74,75] Meanwhile, it is worth noting that, in presence of PGE2, the effects of IDO in MSC-mediated immunoregulation of T-cell proliferation and NK cell activation can also be enhanced.[78,79] Additional studies of the mechanism of PGE2 expression in MSCs and its role in immunoregulation will be helpful for better clinical applications of MSCs. Soon after the discovery of the immunosuppressive function of MSCs, it was found that there was a clear relationship between IL-10 and the immunosuppressive behavior of MSCs. IL-10 production was found *in vivo* by MSC administration or *in vitro* when MSCs cocultured with splenocytes. However, the role of IL-10 is still controversial. Some studies indicate that MSCs alone or with LPS/IL-3 stimulation do not secret IL-10, whereas other studies suggested that MSCs could produce high levels of IL-10 when they are cocultured with activated lymphocytes.[80–82] Thus, it is obscure that the increased IL-10 production is actually from MSCs or from immune cells cocultured with MSCs. However, it has been shown that IL-10 blockade did not affect MSC-mediated immunosuppression on lymphocytes.[83] Clearly, further studies should illustrate detailed mechanisms of these inhibitory factors in MSC-based immunosuppressive functions, orchestrate the network of when, where and how MSCs implement its beneficial roles in clinical applications.

The immunosuppressive property is just one facet of MSC-mediated immunomodulation, however, the emerging evidence points out that MSCs can promote immune responses in the presence of low levels of inflammation,[84] indicating the plasticity

Table 2 Summary of factors critical for MSC-mediated immunosuppression

Immunomodulatory factors	Species	Roles in MSC-mediated immunosuppression
iNOS	Murine MSCs	Inhibits T-cell proliferation[48,61,62]
CCL2	Murine MSCs	Inhibits $CD4^+$ Th17 cells[64]
IDO	Human MSCs	Inhibits T-cell proliferation;[47] promotes type II macrophage differentiation;[132] impair NK cell activity[79]
Semaphorin-3A	Human MSCs	Inhibits T-cell proliferation[133]
B7-H4	Human MSCs	Inhibits T-cell activation and proliferation[134]
HLA-G	Human MSCs	Inhibits PBMC response[72,135]
LIF	Human MSCs	Inhibits T-cell proliferation[32]
TSG6	Human MSCs	Regulates macrophages,[71] inhibits inflammation[136]
Galectin(s)	Human MSCs	Inhibits T-cell proliferation[73,133]
HO-1	Murine MSCs, human MSCs	Inhibits T-cell response;[61] induces IL-10^+ Tr1 and TGF-beta$^+$ Tregs[43]
IL-6	Murine MSCs, human MSCs	Inhibit the differentiation of dendritic cells;[137] inhibit T-cell proliferation[138]
TGF-β	Murine MSCs, human MSCs	Induces Tregs;[139–141] inhibits NK cell activation and function[41]
IL-10	Murine MSCs, human MSCs	Inhibits T-cell responses, decreases Th17 cell differentiation[80,82,142]
PGE2	Murine MSCs, human MSCs	Induces $Foxp3^+$ Tregs;[143] inhibits NK cell function;[41,79] induces type II macrophages;[40,144] inhibit DC maturation[75]
PD-L1/2	Murine MSCs, human MSCs	Inhibits Th17 cells;[145] inhibits T-cell proliferation[146,147]
FasL	Murine MSCs, human MSCs	Induces T-cell apoptosis[76,148]

Abbreviations: CCL2, chemokine ligand 2; DC, dendritic cells; FasL, Fas ligand; HLA-G, human leukocyte antigen G; HO-1, heme oxygenase-1; IDO, indoleamine 2,3-dioxygenase; iNOS, inducible nitric oxide synthase; LIF, leukemia inhibitory factor; MSCs, mesenchymal stem cells; PGE2, prostaglandin E2; PD-L1/2, programmed cell death 1 ligand1/2; PBMC, peripheral blood mononuclear cells; TSG6, TNF-α stimulated gene/protein 6

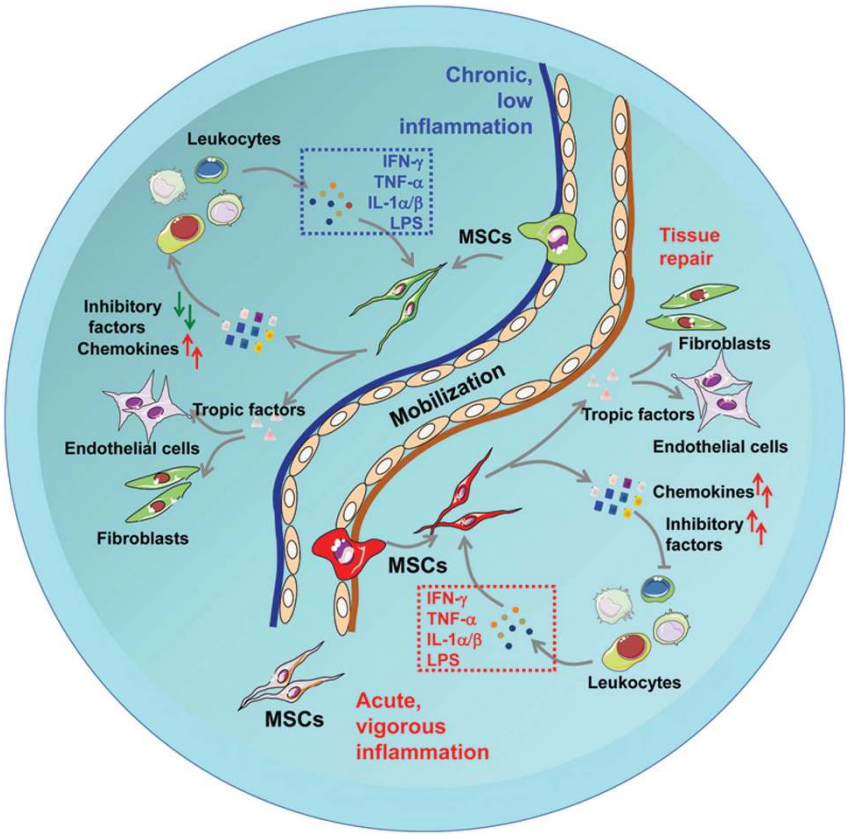

Figure 4 A proposed model of the interaction between MSCs and immune responses during tissue repair. Once tissue injury occurs, MSCs are mobilized. Vigorous inflammation licenses MSCs to possess the abilities to downregulate immune responses, a process mediated by high levels of chemokines and immune inhibitory factors. In addition, growth factors are also released by MSCs, which promote endothelial cells and mesenchymal stem/stromal cells to repair injury. Insufficient inflammatory cytokines during chronic inflammatory sites, however, could stimulate MSCs to produce chemokines and tropic factors in absence of sufficient immune inhibitory factors. As such, chronic inflammation may lead MSCs to protract the disease recovery, or even worsen the disease course

of the immunoregulatory functions of MSCs. When inflammation is low or the expression of the above mentioned immunosuppressive factors is inhibited, MSCs could dramatically promote immune responses (Figure 4). This conceptual change has significant implications for proper clinical application of MSCs.

Immune Enhancing Properties of MSCs

One of the early clinical studies of MSCs is to treat GvHD patients.[85] However, the therapeutic effects are not always achieved. In some clinical trials, MSCs were ineffective in GvHD patients. In some cases, MSCs accelerated graft rejection, even coadministrated with cyclosporine A (CsA).[51,86] The question is why the immunosuppressive function of MSCs is not always achieved. Under certain conditions, indeed, the immunosuppressive function of MSCs does not occur, instead, an enhanced immune response is observed. For example, under inflammatory conditions rendered by high dose of concanavalin A (ConA) or proinflammatory cytokines, MSCs exerted strong immunosuppressive effect. However, with low dose of ConA or the addition of IL-10, the suppressive effect of MSCs was abrogated.[87] This can also happen when the levels of inflammatory cytokines are insufficient to stimulate MSCs to secret enough NO, although still produced chemokines.[84] Previous experiment also indicated that vigorous inflammation is important in eliciting the immunosuppressive function of MSCs *in vivo*. Less protection to GvHD was observed if MSCs were administrated at the day of bone marrow transfer.[52] Good therapeutic effect can be achieved when MSCs were infused after disease development.[48] Therefore, the inflammation status determines the immunomodulatory tendency of MSCs. Notably, with low dose of IFN-γ, the antigen presentation ability could be induced in MSCs. In fact, IFN-γ-stimulated MSCs can be regarded as conditional antigen presenting cells.[88] Antigen-pulsed IFN-γ-treated MSCs can induce antigen-specific cytotoxic $CD8^+$ T cells *in vivo* and thereby making MSCs candidates for the treatment of cancer or infectious diseases.[89] Thus, except immunosuppressive roles, MSCs could also upregulate immune responses. Further studies on the molecular mechanisms regulating the immunoregulatory property of MSCs could have dramatic impact on the clinical application of these unique cells.

Engraftment of MSCs at Sites of Injury or Inflammation

It has been reported that trophic factors produced by MSCs can be used to implement therapeutic effects to inflammatory diseases, whereas in damaged tissue engrafted MSCs can exert concerted action, orchestrating with immune cells,

stromal cells, endothelial cells and tissue progenitor cells to promote tissue repair.[34,48] Hence, the successful engraftment of MSCs to inflammatory niches and sites of injury are important considerations when analyzing the beneficial effects of MSCs. Unlike the well-characterized phenomenon of leukocyte homing, the mechanism of MSC homing, by *de novo* or exogenously delivered MSCs, is still unclear. However, MSCs expressing sialyl Lewis(x), a molecule capable of promoting leukocyte migration in the inflamed tissue, exhibit higher efficiency in homing to inflamed tissues.[90] It is important to note that the peripheral blood of vascular injured mice yields more MSCs than that of control mice, a finding that is likely due to enhanced levels of granulocyte colony-stimulating factor (G-CSF) following injury.[91,92] Further studies are needed to lend support to the concept that injury stimulates the appearance of MSCs in the circulating blood. In addition to host MSC mobilization, more and more studies have focused on evaluating the engraftment capability and beneficial effects of exogenously delivered MSCs in animal disease models, including inflammatory bowel diseases (IBD), EAE, collagen-induced arthritis, type I diabetes and GvHD, and in clinical trials with patients suffering from GvHD, acute myocardial infarction, multiple sclerosis and Crohn's diseases.[48,93–97]

The therapeutic efficacy can be influenced by culture conditions, which are now known to significantly influence MSC function, because exogenously administrated MSCs always have to be expanded and passaged *in vitro*. It has been reported previously that extensive passages of MSCs adversely affected MSC activation and protection in ischemia/reperfusion, a phenomenon attributed to reduced growth factor production by MSCs with high passage numbers.[56] Besides, MSCs can acquire or lose certain surface receptors during culture, which might affect their chemotaxis ability.[98] Indeed, compared with freshly isolated MSCs, cells maintained in culture display impaired homing ability.[98,99] Related to this, CXCR4, a receptor for SDF-1that presents at high levels in the bone marrow and ischemic tissues, always disappears from the surface of MSCs after culture. However, hypoxic culture condition can promote MSC engraftment through enhancing CXCR4 and CX3CR1 expression.[100] In addition, when cultured MSCs are treated with a panel of cytokines, CXCR4 level can also be recovered. This effect helps to promote the bone marrow engraftment of MSCs in irradiated NOD/SCID mice and allows easier hematological recovery of transplanted MSCs.[101] Moreover, TNF-α, TGF-β and IL-1β can stimulate MSCs to secret high levels of matrix metalloproteinase, which can endow these cells the ability to migrate through extracellular matrix in response to chemokines.[102] Furthermore, in *in vivo* study, pretreating MSCs with TNF-α/or a cocktail of cytokines resulted in enhanced MSC engraftment efficiency and improvement in acute pyelonephritis.[103] Thus, many factors can influence MSC mobilization and engraftment. Therefore, it is critical to accurately assess and tightly control the properties of cultured MSCs for clinical application; otherwise, the physiological microenvironments they encounter may cause them to behave in unexpected ways.

Still other factors, such as injection site, timing and cell number administered, may also affect the engraftment and therapeutic effects of MSCs that are depending on the specific disease status. The various routes of injection that have been tried, including intravenous, intraperitoneal, intra-arterial and *in situ*, each affects the efficiency of MSC homing or localization to target organs.[98] Among them, intravenous delivery is convenient and successful in treating certain type of diseases, but better engraftment efficiency can sometimes be obtained by intra-arterial and *in situ* injections, such as myocardial infarction, kidney transplantation and brain injury.[104–107] Administration of MSCs *in situ*, although highly sometimes effective in both engraftment and therapy, is less clinically applicable as it is so invasive and introduces cells in a microenvironment that could be unsuitable for survival.[108] In another study, in spinocerebellar ataxia, intravenous transplantation was more effective in promoting the survival of cerebellar Purkinje cells and MSC engraftment than that of intracranial injection.[109] The intraperitoneal injection route has been rarely used, but some recent studies used it to treat muscular dystrophy and IBD in the mouse model, resulting in effective engraftment and therapeutic effects.[110–112] Therefore, when MSCs are used to treat distinct diseases, their administration routes should be well-selected. Another influence on MSC effectiveness is the stage of disease: delivery of MSCs at an early stage following an event causing ischemia or EAE has shown enhanced engraftment rates or therapeutic effects, whereas administrated at the relapse stage of EAE, their beneficial effects are reduced.[64,93,113] Finally, the dose of MSC administration should also be considered, because more MSC administration does not show a better therapeutic effect in the brain injury animal model.[55] Taken together, these studies clearly demonstrate that when MSC-based therapies are used in preclinical experiments and clinical trials, the source of MSCs, the dose, route and timing of MSC administration should all be carefully considered.

Conclusions

We have highlighted the current understanding of the interaction between MSCs and immune responses. The differences, between murine MSCs and human MSCs, in the mechanisms mediating immunosuppression were discussed. As IDO is so central to the immunoregulatory function of human MSCs, the precise roles of tryptophan depletion and tryptophan metabolites in orchestrating such immunosuppression merit further investigation. Although MSCs disappear quickly after administration, their immunosuppressive effects linger for considerably longer. Considering that MSC supernatant alone can be effective in treating some diseases, it is possible that MSCs themselves may not be indispensable in mediating the therapeutic effect. In other words, is MSC differentiation-based repair sufficient for disease treatment, or do MSC-produced factors that modify the tissue microenvironment and lead to recovery intrinsically. Efforts of scientists in the coming years are anticipated to elucidate the precise roles of MSCs, both their reparative and immunoregulatory functions, and the mechanisms that impart and govern their clinical efficacy in the treatment of disease.

Conflict of Interest

The authors declare no conflict of interest.

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