

# Preclinical Efficacy and Mechanisms of Mesenchymal Stem Cells in Animal Models of Autoimmune Diseases

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Mesenchymal stem cells (MSCs) are present in diverse tissues and organs, including bone marrow, umbilical cord, adipose tissue, and placenta. MSCs can expand easily *in vitro* and have regenerative stem cell properties and potent immunoregulatory activity. They inhibit the functions of dendritic cells, B cells, and T cells, but enhance those of regulatory T cells by producing immunoregulatory molecules such as transforming growth factor- $\beta$ , hepatic growth factors, prostaglandin E<sub>2</sub>, interleukin-10, indolamine 2,3-dioxygenase, nitric oxide, heme oxygenase-1, and human leukocyte antigen-G. These properties make MSCs promising therapeutic candidates for the treatment of autoimmune diseases. Here, we review the preclinical studies of MSCs in animal models for systemic lupus erythematosus, rheumatoid arthritis, Crohn's disease, and experimental autoimmune encephalomyelitis, and summarize the underlying immunoregulatory mechanisms.

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## MESENCHYMAL STEM CELLS

Stem cells have self-renewal potential and differentiate into one or more specialized cell types (1). There are two types of stem cells, embryonic and adult. Embryonic stem cells,

which are isolated from blastocysts, are able to differentiate into any organ-specific cells, whereas adult stem cells show restricted proliferation and lineage differentiation (2). Adult stem cells undergoing mesodermal lineage-specific differentiation to osteocytes, adipocytes, or chondrocytes are named mesenchymal stem cells (MSCs) (3). Although embryonic stem cells can differentiate into any cell types in humans, teratoma formation and ethical concerns limit their wide clinical application (4). In contrast, MSCs can be used for tissue repair and regeneration without these restrictions.

MSCs can be isolated from various human tissues, such as bone marrow, adipose tissue, umbilical cord blood, and placenta. Typical MSC markers include CD73, CD90, and CD105; however, MSCs do not express the hematopoietic markers CD34 and CD45. Upon exposure to specific differentiation media, MSCs differentiate into osteocytes, chondrocytes, adipocytes or other cell types (5,6). Cultured MSCs adhere to tissue culture plates (7,8). MSCs secrete various soluble factors that promote angiogenesis and mitosis and reduce apoptosis, and can be used for tissue repair or regeneration (9-11). MSCs can produce a number of immunoregulatory molecules and have advantages for clinical applications, such as easy preparation and low immunogenicity (12-15). Overall, these properties make MSCs good ther-

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Abbreviations: BM, bone marrow; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; HO, heme oxygenase; IDO, indolamine 2,3-dioxygenase; IFN, interferonIL, interleukin; MSC, mesenchymal stem cell; PG, prostaglandin; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TGF, transforming growth factor; Th, helper T; TNF, tumor necrosis factor; Treg, regulatory T

apeutic candidates for the treatment of transplantation rejection, graft-versus-host diseases, and autoimmune diseases (13,16). In this review, we discuss the potential use of MSCs for the treatment of autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Crohn's disease, and experimental autoimmune encephalomyelitis (EAE).

### EFFICACY OF MESENCHYMAL STEM CELLS IN ANIMAL MODELS OF AUTOIMMUNE DISEASES

MSCs show promising therapeutic activity in animal models of SLE (Table I), RA (Table II), Crohn's disease, and EAE (Table III). Overall experimental strategies can be summarized as follows. Human MSCs are usually isolated from BM, but sometimes from umbilical cord or adipose tissues. MSCs are injected intravenously or intraperitoneally at a dose of approximately  $1 \times 10^6$  cells per mouse.

#### Systemic lupus erythematosus

SLE is a severe autoimmune disease characterized by multi-organ dysfunctions including renal, cardiovascular, neural, musculoskeletal, and cutaneous involvement (17). SLE is charac-

terized by activation and proliferation of autoreactive T and B cells (18). MRL/*lpr* mice, which have a mutation in the *lpr* gene, spontaneously develop an autoimmune disease that is very similar to human SLE (19). Zhou and colleagues investigated the effect of human BM-derived MSCs on the pathogenesis of SLE in MRL/*lpr* mice (19). Human MSCs reduced proliferation of T cells from MRL/*lpr* mice *in vitro*. MSCs injected intravenously reduced serum levels of anti-dsDNA antibodies and proteinuria in MRL/*lpr* mice. Immunohistochemical analysis showed low expression levels of TGF- $\beta$ , vascular endothelial growth factor, and complement C3 in renal tissues. Gu and colleagues isolated MSCs from human umbilical cord and injected them intravenously into MRL/*lpr* mice, which decreased the levels of proteinuria, serum creatinine and anti-dsDNA antibodies, and the extent of renal injury (17). In contrast, MSCs increased the number of Treg cells in the spleen. Ji and colleagues investigated how MSCs inhibited T cell proliferation *in vivo* (18). Intravenous injection of BM-derived MSCs reduced the serum anti-dsDNA antibody level. MSCs inhibited G<sub>1</sub>/S transition in T cells in the spleen and lymph nodes through a decrease in CDK2 expression. Furthermore, MSCs inhibited the Akt/GSK3 $\beta$  signaling pathway of T cells from MRL/*lpr* mice. These studies

**Table I.** Preclinical efficacy of mesenchymal stem cell (MSC) in animal models for systemic lupus erythematosus

MSC source	Recipient animals	Autoimmune disease	Cell No*	Route	Experimental periods	Outcomes	Ref.
Human-BM	MRL/ <i>lpr</i>	SLE	1	iv	224	Reduce antibody production in serum and nephritis.	(1)
Human-UC	MRL/ <i>lpr</i>	SLE	1	iv	203	Reduce T cell number and increase Treg cell number in spleen. Reduce antibody production and inflammatory cytokine production in kidneys. Reduce nephritis.	(2)
Human-AD	(NZB $\times$ NZW) <sub>F1</sub>	SLE	2	iv	378	Reduce anti-dsDNA antibody production in blood. Increase anti-inflammatory cytokine production in serum. Increase Treg cell proportion & anti-inflammatory cytokine production in spleen. Increase survival rate of mice	(3)
Mouse-BM	MRL/ <i>lpr</i>	SLE	2	iv	30	Reduce antibody production in serum. Inhibit cell cycle of T cells. Inhibit AKT/GSK3 $\beta$ signaling of T cells.	(4)
Mouse-BM	(NZB $\times$ NZW) <sub>F1</sub>	SLE	1	ip	273	Increase anti-dsDNA antibody production in blood. Increase plasma cell number in bone marrow. Increase proteinuria & nephritis	(5)
Mouse-BM	(NZB $\times$ NZW) <sub>F1</sub>	SLE	1.25	iv	210	Not affect antibody production in serum, proteinuria level, and mortality rate of mice.	(6)

\* ( $\times 10^6$  cells/mouse). iv: intravenous injection, ip: intraperitoneal injection, Treg: regulatory T cells, SLE: systemic lupus erythematosus, BM: bone marrow, UC: umbilical cord blood, AD: adipose tissue, MSC: mesenchymal stem cell

**Table II.** Preclinical efficacy of mesenchymal stem cells in animal models for rheumatoid arthritis

MSC source	Recipient animals	Autoimmune disease	Cell No*	Route	Experimental periods	Outcomes	Ref.
Human-AD	DBA/1	RA	1	iv	50	Reduce inflammatory cytokine production and increase anti-inflammatory cytokine production in serum. Increase Treg cells proportion in spleen and lymph nodes.	(7)
Human-UC	DBA/1	RA	1	ip	62	Reduce inflammatory cytokine production and increase anti-inflammatory cytokine production in serum. Increase Treg cell proportion in blood and spleen.	(8)
Human-AD	DBA/1	RA	2	iv	42	Reduce inflammatory cytokine expression in joint and serum. Reduce antibody production in spleen. Increase Treg cell proportion in spleen.	(9)
Mouse-BM	DBA/1	RA	5	iv	42	Reduce inflammatory cytokine production in serum. Increase Treg cell proportion in spleen.	(10)
Mouse-BM	K/BxN	RA	1	iv	29	Not affect T cell infiltration in ankle joints	(11)

\*( $\times 10^6$  cells/mouse). iv: intravenous injection, ip: intraperitoneal injection, Treg: regulatory T cells, RA: rheumatoid arthritis, BM: bone marrow, UC: umbilical cord blood, AD: adipose tissue, MSC: mesenchymal stem cell

**Table III.** Preclinical efficacy of mesenchymal stem cells in animal models for Crohn's disease and experimental autoimmune encephalomyelitis

MSC source	Recipient animals	Autoimmune disease	Cell No*	Route	Experimental periods	Outcomes	Ref.
Human-BM	Balb/c	Crohn's disease	1-3	ip	10	Reduce the production of inflammatory cytokines and antibody in serum and tissue. Increase anti-inflammatory cytokine production in serum and tissue. Increase Treg cells proportion in lymph nodes.	(12)
Mouse-BM	C57BL/6	Crohn's diseases	1	iv	10	Reduce T cell proportion and increase Treg cell proportion in blood.	(13)
Mouse-BM	C57BL/6	EAE	1	iv	90	Reduce the proportion of T cells and macrophages in brain, spleen, lymph nodes and spinal cord.	(14)
Mouse-BM	SJL/J	EAE	1	iv	80	Reduce T cell proliferation, inflammatory cytokine & antibody production in spleen and lymph nodes.	(15)

\*( $\times 10^6$  cells/mouse). iv: intravenous injection, ip: intraperitoneal injection, Treg: regulatory T cells, EAE: experimental autoimmune encephalomyelitis, BM: bone marrow, UC: umbilical cord blood, AD: adipose tissue, MSC: mesenchymal stem cell

suggest that MSCs can ameliorate SLE pathogenesis by inhibiting the functions of T and B cells and activating Treg cells in the MRL/*lpr* mouse model. Controversial data were shown in NZB x NZW F1 (NZB/W) mouse models of SLE (20). MSCs from Balb/c mice were injected into NZB/W mice before and after disease onset, which showed worsen disease progression by showing increase of anti-dsDNA antibody production, plasma cell number, proteinuria level, and nephritis (20). MSCs from C57BL/6 mice also did not affect the anti-

body production, proteinuria level, and the mortality rates (21). However, human MSCs ameliorated SLE without adverse effect in NZB/W mice (22). Human MSCs increased the survival rate and the proportion of Treg cells, but decreased the levels of anti-dsDNA antibody and proteinuria (22).

### Rheumatoid arthritis

RA is characterized by the loss of self-tolerance, chronic inflammation in the joints, subsequent cartilage destruction, and

bone erosion. The crucial process underlying RA initiation is the abnormal activation of DCs, T cells, B cells, macrophages, and neutrophils (23). The anti-inflammatory effects of MSCs in RA have been mainly studied in the collagen-induced arthritis model in DBA/1 mice. González et al. injected human adipose tissue-derived MSCs intravenously into collagen-injected DBA/1 mice. Systemic infusion of these cells significantly reduced the incidence and severity of experimental arthritis by decreasing production of various inflammatory cytokines and chemokines, and reducing the ratios of Th1/Th17 cells. MSCs induced production of anti-inflammatory IL-10 in lymph nodes and joints, and *de novo* generation of antigen-specific Treg cells. Liu and colleagues injected human umbilical cord-derived MSCs intraperitoneally into DBA/1 mice (24). Systemic infusion of these cells reduced the severity of arthritis. MSCs reduced the levels of proinflammatory cytokines and chemokines (TNF- $\alpha$ , IL-6 and monocyte chemoattractant protein-1), increased the levels of IL-10, shifted from Th2 to Th1 type responses, and induced Treg cells. Zhou and colleagues injected human adipose tissue-derived MSCs intravenously into DBA/1 mice (25). These MSCs reduced the incidence and severity of arthritis by inhibiting production of various inflammatory mediators and reducing antigen-specific Th1 cell expansion. MSCs also induced production of anti-inflammatory cytokines and generation of antigen-specific Treg cells. Human BM-derived MSCs showed similar efficacy in a collagen-induced arthritis model in DBA/1 mice as umbilical human cord- or adipose tissue-derived MSCs (26). These studies suggest that MSCs can ameliorate RA pathogenesis in DBA/1 mice by inhibiting the production of inflammatory cytokines (which are mainly produced by macrophages and T cells) and activating Treg cells. However, contradictory data was reported in adjuvant-induced and spontaneous (K/BxN) arthritis model and showed that MSCs were effective when administered only before disease onset, which suggested that MSCs lost their immunoregulatory properties when infused into inflammatory microenvironments (27).

### Crohn's disease

Crohn's disease is a chronic form of inflammatory bowel disease and characterized by dysfunction of intestinal T cells, abnormal cytokine production, and inflammation in small intestine and the colonic mucosa (28). Pathogenesis of Crohn's disease is related to abnormal activation of Th1 cells, macrophages and neutrophils, uncontrolled production of in-

flammatory cytokines and chemokines, and an imbalance between effector T cells and suppressive Treg cells (29). In a preclinical study of the effect of MSCs on Crohn's disease, colitis was induced in mice by trinitrobenzene sulfonic acid, and human adipose tissue-derived MSCs were injected intraperitoneally after the onset of the disease (28). Systemic infusion of MSCs ameliorated the clinical and histopathologic severity of colitis, abrogated body weight loss, diarrhea and inflammation, and increased survival. MSCs down-regulated Th1-driven inflammatory responses and decreased the production of inflammatory cytokines and chemokines. MSCs also impaired Th1 cell expansion and activated Treg cells (28). Systemic infusion of human BM-derived MSCs ameliorated the pathogenesis of dextran sulfate sodium salt-induced experimental colitis and induced T cell apoptosis via the FASL-FAS pathway *in vivo* (30). These studies indicate that MSCs can ameliorate chemically induced colitis by inhibiting the inflammatory functions of macrophages and T cells in mice.

### Experimental autoimmune encephalomyelitis

EAE is an autoimmune disease of the central nervous system, which is mediated by T cells and macrophages (31). EAE was induced in mice by injecting myelin oligodendrocyte glycoprotein-35-55 peptide, and human BM-derived MSCs were injected intravenously before and after the onset of the disease (31). MSCs ameliorated EAE progression showing low infiltration of inflammatory cells when injected before its onset, but their efficacy was relatively weak when they were injected after the onset (31). Human BM-derived MSCs were injected into another animal model of EAE, mice immunized with the peptide 139-151 of the proteolipid protein (32). MSC-injected mice ameliorated disease progression and low relapses, with decreased infiltration of inflammatory cells and decreased demyelination and axonal loss. MSCs reduced antigen-specific T-cell response and antibody titers. These studies suggest that MSCs can ameliorate neuro-inflammation by inhibiting the infiltration and functions of inflammatory cells in animal models.

### IMMUNOREGULATORY MECHANISMS

MSCs have diverse immunoregulatory activities, which vary depending on immune cell subpopulations. MSCs affect differentiation, maturation, and function of dendritic cells (DCs) (33). MSCs inhibit the initial differentiation of CD14<sup>+</sup> monocytes into immature DCs (34) and also inhibit DC maturation

by decreasing the expression of HLA-DR, CD1a, CD80, CD83, and CD86, and the production of IL-1 $\beta$ , interleukin (IL)-12, and tumor necrosis factor (TNF)- $\alpha$  by DCs (35-38). MSCs impair the activation of lipopolysaccharide-treated DCs, reduce antigen presentation to CD4<sup>+</sup> and CD8<sup>+</sup> T cells and inhibit secretion of inflammatory cytokines (33,39). MSCs also reduce the expression of CCR7 and prevent DC homing to lymph nodes, where the main functions of DCs are to activate T cells (39). MSCs might affect DC functions via soluble mediators, such as IL-6, macrophage colony-stimulating factor (M-CSF), and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) (40,41).

MSCs can regulate T cell functions via two ways. First, MSCs directly inhibit the functions of antigen-specific T cells (42,43). Second, MSCs inhibit T cell functions indirectly by stimulating the expansion of regulatory T (Treg) cells (36). The inhibitory effect of MSCs on T cells has been demonstrated in an animal model of graft-versus-host disease, which is mainly caused by abnormal activation of T cells (44). MSCs reduce allograft rejection in a mouse model (13). In an RA model, MSCs inhibit the activation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and increase the number of and IL-10 production by Treg cells (45). MSCs affect T cell functions by producing transforming growth factor (TGF)- $\beta$ , PGE<sub>2</sub>, indolamine 2,3-dioxygenase (IDO), heme oxygenase-1 (HO-1), and chemokines (46). Inflammatory cytokines, including interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$  released from cells in damaged tissues, strongly induce IDO expression by MSCs (47). HO-1 produced by human MSCs is able to promote the expansion of Treg cells (48). MSCs release CCL2, trigger T cell migration, directly contact with T cells, and induce T cell apoptosis through Fas-FasL interaction (30). Inflammatory cytokines, such as TNF- $\alpha$  and IL-1 $\beta$ , up-regulate the expression of ICAM-1 and VCAM-1 in MSCs, which strengthens their interaction with T cells (49).

MSCs can also modulate B cell functions (mainly antibody production). B cell proliferation is not affected by naïve MSCs, but is inhibited by IFN- $\gamma$ -treated MSCs in transwell culture systems (47). MSCs inhibit B cell proliferation by arresting the G<sub>0</sub>/G<sub>1</sub> phase of the cell cycle, but not by inducing apoptosis (50). MSCs inhibit B cell differentiation to plasma cells producing IgM, IgG, and IgA, and inhibit the expression of CXCR4, CXCR5, and CCR7 by B cells, resulting in reduced homing to lymph nodes (50). However, MSCs do not affect the expression of co-stimulatory molecules and cytokine production by B cells. MSCs directly inhibit B cell functions by producing soluble factors, as indicated by transwell experi-

ments. MSC also indirectly affect B cell functions by inhibiting T cells, which play a key role in antigen-specific antibody production by B cells, and plasmacytoid DCs, which are crucial for B cell maturation (50,51).

## CONCLUSIONS

MSCs are one of the promising therapeutic candidates for the treatment of autoimmune diseases and show beneficial effects in animal models of SLE, RA, Crohn's disease, and EAE. MSCs show beneficial therapeutic activity in animal models of these autoimmune diseases through inhibiting abnormally activated immune functions of dendritic cells, T cells, and B cells. However, several contradictory papers were also noted in preclinical efficacy evaluation study of MSCs in autoimmune animal models (20-22,27). Such contradiction may be due to several factors. First, properties and characteristics of MSCs might be diverse according to sources (organs or species) and culture conditions. Second, each laboratory used different experimental methods; route, number, and timing (before and after disease onset) of injection. Third, animal models were diverse according to the studies. Each animal model that is used in preclinical studies has typical pathophysiology and has both strength and weakness. Therefore, no single model may represent human disease and predict accurately the efficacy of MSCs. Overall, this observation strongly emphasizes the importance of the usage of qualified or standardized MSCs and the usage of appropriate animal models. In addition, several basic questions remain to be answered. First, it is critical to understand the normal physiological functions of MSCs *in vivo*, since there is limited information on the physiological roles of MSCs *in vivo*. Second, with respect to the therapeutic mechanisms and efficacy, survival time, distribution and tissue homing, and action site (local or systemic) of MSCs should be further studied *in vivo*. Third, it is also important to understand the trafficking of MSCs into lymphoid tissues, where they interact with immune cells. Understanding the mechanisms of MSC action in treatment of autoimmune diseases will help to expand clinical application of MSCs to autoimmune diseases.

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## CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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