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Effects of Mesenchymal Stromal Cells on Regulatory T cells: Current Understanding and Clinical Relevance

Running Head: MSC effects on T-reg

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Abstract:

The immunomodulatory potential of mesenchymal stromal cells (MSC) and regulatory T cells (T-reg) are well recognized by translational scientists in the field of regenerative medicine and cellular therapies. A wide range of pre-clinical studies as well as a limited number of human clinical trials of MSC therapies have not only shown promising safety and efficacy profiles but have also revealed changes in T-reg frequency and function. However, the mechanisms underlying this potentially important observation are not well understood and, consequently, the optimal strategies for harnessing MSC/T-reg cross-talk remain elusive. Cell-to-cell contact, production of soluble factors, re-programming of antigen presenting cells to tolerogenic phenotypes and induction of extracellular vesicles ("exosomes") have emerged as possible mechanisms by which MSC produce an immune-modulatory milieu for T-reg expansion. Additionally, these two cell types have the potential to complement each other's immunoregulatory functions and a combinatorial approach may exert synergistic effects for the treatment of immunological diseases. In this review, we critically assess recent translational research related to the outcomes and mechanistic basis of MSC effects on T-reg and provide a perspective on the potential for this knowledge base to be further exploited for the treatment of autoimmune disorders and transplants.

Introduction

The distinctive capacity of mesenchymal stromal cells (MSC) to differentiate into diverse cell lineages (adipocytes, chondrocytes and osteoblasts), repair damaged tissues, migrate to sites of injury and modulate a range of immune/inflammatory effector mechanisms has generated substantial interest among biomedical researchers in the field of regenerative medicine[1]. To date, as many as 969 clinical trials have been reported using MSC as a potential cell therapy for the treatment of diverse immunological and non-immunological disorders (https://clinicaltrials.gov/). Among the attributes of this versatile cell that make it a suitable candidate for cellular therapy are its ease of isolation from multiple accessible tissues, its amenability to large scale ex-vivo culture expansion, its low immunogenicity and its now well-documented safety profile. Though the International Society for Cellular Therapy (ISCT) has set minimum criteria for defining MSC which include expression of CD105, CD73 and CD90 and lack of expression of HLA-DR, CD11b, CD14, CD19 and CD34[1], a general consensus is lacking on how MSC can be consistently defined at the level of functionality and potency. This difficulty arises, in large part, from heterogeneity in primary MSC phenotype within different tissues and in ex-vivo culture conditions[2-4].

Among their functional properties, MSC have been consistently shown to suppress adaptive immune responses directly by inhibiting the proliferation of $CD4^+$ ("helper") and $CD8^+$ ("cytotoxic") T-cells and indirectly by modulating dendritic cell (DC)-mediated antigen presentation[5]. An additional putative mechanism whereby MSC may exert both short- and long-lasting influences on antigen-specific T-cell responses is through induction of regulatory T-cell (T-reg)[5]. In 2008, Di Ianni et al. demonstrated increased frequency of T-reg and prolonged maintenance of T-reg suppressive activities when human T-cell subpopulations were co-cultured with MSC[6]. As we describe later in this article, a relatively broad range of experimental studies has since been published to confirm this phenomenon and add mechanistic detail[7] and the topic continues garner interest[8-10]. Furthermore, ex vivo-expanded MSC and T-reg have both been shown to display potent immunomodulatory effects in a wide array of animal disease models and have been demonstrated in clinical trials to represent safe, feasible and potentially effective immunotherapies for human autoimmune diseases and transplants[2,11]. It would seem imperative, therefore, to better understand how the mechanisms underlying MSC-mediated induction of T-reg or combined MSC/T-reg cellular therapy may be successfully translated to the clinical arena. In this review, we re-evaluate the

existing concepts about the effects of MSC on T-reg expansion and the related mechanisms that may lead to T-reg induction by MSC under *in-vitro* and *in-vivo* conditions.

Current understanding of T-reg phenotypes

Based on their developmental origins, T-reg are classified in two major categories - one that develops de novo in the thymus as "natural" (n)T-reg and another that differentiates during activation in the periphery as "induced" (i)T-reg. While the primary identifying phenotype of T-reg in humans and rodents is $CD4^{+}CD25^{hi}FOXP3^{+}$, they have been found to be functionally and phenotypically heterogeneous[12]. For human, but not mouse, lower expression of the IL-7R (CD127) has also be shown to be a useful discriminator of T-reg from other $CD4^{+}$ T-cells[13]. Miyara et al. further divided T-reg based on the differential expression of CD45RA and FOXP3 into three main subpopulations: $CD45RA^{+}FOXP3^{lo}$ naive or resting T-reg; $CD45RA^{-}FOXP3^{+}$ activated or effector T-reg, and $CD45RA^{-}FOXP3^{lo}$ "non-suppressive," cytokine-secreting cells[14]. Subsequently, a number of other research groups have divided T-reg into different subpopulations based on the expression of specific surface markers. For example, surface expression of CD39, an ectoenzyme that hydrolyzes adenosine triphosphate (ATP) into adenosine monophosphate (AMP) has also been used to define T-reg subpopulations. Human $CD39^{hi}$ T-reg have been reported to suppress xenograft versus host disease in a mouse model while $CD39^{lo}$ T-reg were non-suppressive and highly unstable[15]. More recently, Mason and colleagues identified more than 22 different human T-reg subsets by mass cytometry[16]. The list of T-reg subsets as targets for therapeutic exploitation is growing yet there remain significant knowledge gaps regarding the phenotypic identities of T-reg subpopulations, their relevance to different disease processes and their potential for immunotherapeutic applications.

Pre-clinical and clinical evidence for therapeutically-relevant effects of MSC on T-reg

A number of animal model studies have documented increments in T-reg numbers after MSC administration. For example, in mice with collagen antibody-induced arthritis (CAIA), Nam et al. reported that MSC induced differentiation of $CD4^{+}$ T cells to T-reg *in-vitro* and that FOXP3 expression was upregulated in CAIA mice after MSC infusion[17]. Similar findings were reported by Roux et al. who observed induction of functional $CD4^{+}FOXP3^{+}$ T-reg when co-cultured *in-vitro* with human-induced pluripotent stem cell-derived MSC (hu-iPS-MSC). These findings were validated *in vivo* following hu-iPS-MSC administration to humanized mice[18]. In a rat model of high-risk corneal allo-transplantation, Lohan et al. reported that pre-transplant

intravenous administration of third-party allogeneic MSC resulted in increased rejection-free survival associated with higher proportions of T-reg in the graft draining lymph nodes[19]. Bai et al. reported increased T-reg numbers following administration of IL-17A-treated MSC to mice with renal IRI that was associated with greater protection from acute kidney injury and was dependent on COX2/PGE2[20]. Groups investigating the *in vitro* interactions of MSC with T-reg also reported T-reg induction by MSC derived from different sources[21,22].

As summarised in **Table 1**, a number of clinical studies have also shown an increase in T-reg numbers or percentages after systemic or localized administration of either autologous or allogeneic MSC in different disease and transplantation conditions. Notably, Shi et al. reported a significant elevation in the percentage of T-reg four weeks after umbilical cord-derived MSC infusion in twenty seven liver allograft recipients[23]. Likewise, Li et al. reported an increase in T-reg percentage with a significant decline in the percentage of Th17 cells in systemic lupus erythematosus (SLE) patients with refractory cytopenia after MSC infusion[24]. In a phase I/II clinical trial of third party MSC post kidney transplantation, Erpicum et al. reported increased frequency of T-reg 30 days after MSC infusion[25]. There are also reports, however, in which no alteration in T-reg was observed[26]. In a long-term follow up study of four kidney transplant patients treated with autologous MSC, Perico et al. reported an increase in T-reg percentage in two of the patients while it declined in the third patient and remained stable in the fourth[11]. Overall, more randomized, placebo-controlled clinical studies accompanied by detailed immunological monitoring are needed to determine the conditions under which MSC administration is most likely to consistently induce T-reg expansion as well as to determine whether changes in circulating T-reg are accompanied by changes to T-reg located in disease-relevant tissues.

Potential mechanisms for MSC-mediated effects on T-reg

As is evident from the literature summarized in the preceding section, a wide range of *in vitro* and *in vivo* studies have documented the potential for MSC to induce, expand or preferentially support the survival of T-reg in human and experimental animal species. Nonetheless, the kinetic and mechanistic details related to this phenomenon are incompletely understood and are likely to be complex and context-dependent. In **Figure 1** and in the subsections below, we summarize evidence for four basic (and non-mutually exclusive) mechanistic models for MSC effects on T-reg.

Cell-to-cell contact-dependent mechanisms have been reported to play an important role in the interactions between MSC and T-reg under *in vitro* and *in vivo* conditions. English et al. provided the first *in-vitro* evidence that direct contact between human MSC and purified $CD4^+$ T cells is important for the induction of T-reg as elimination of contact by a semipermeable membrane reduced the expression of FOXP3 mRNA to control levels[27]. In this study, PGE2 and TGF-β were also mechanistically implicated, suggesting a combined role for contact-dependent signals and soluble mediators. Subsequently, Lee et al. reported that expression of inducible co-stimulator ligand (ICOSL/CD275) by human MSC when co-cultured with $CD4^+$ T-cells is essential for T-reg induction under *in-vitro* condition as knockdown of ICOSL and use of transwell cultures significantly reduced T-reg induction and IL-10 production[28]. Mesenchymal stromal cells also express a wide range of other surface adhesion molecules including integrins, vascular cell adhesion protein (VCAM)-1, intercellular adhesion molecules (ICAM-1, ICAM-2), CD72, and CD58 (LFA-3), which have been shown to bind to T-cells with very high affinity and to play important roles in immune suppression. These molecules help to anchor T-cells to MSC and, in so doing, increase the potency of soluble factors to suppress T-cell proliferation and pro-inflammatory effector mechanisms. It is unknown, however, whether these adhesion events specifically promote T-reg induction and whether inhibiting MSC-T-cell adhesion interferes with this aspect of MSC-mediated immunomodulation. In contrast, signalling through Notch receptors is well documented to play a pivotal role in the development of T-reg[29] and MSC express a variety of Notch ligands including Jagged1, Jagged 2, and Delta-like (DLL) 1, 3 and 4. Notably, Del Papa et al. reported that induction of T-reg by human MSC was mediated by Notch1 and, subsequently, Cahill et al. demonstrated that the Notch ligand Jagged-1 was responsible for the expansion of T-reg by mouse MSC[30,31]. Finally, Rashedi et al. in a study of the influence of toll-like receptor (TLR) stimulation on MSC immunomodulatory effect, showed that indirect contact of MSC with human $CD4^+$ T cells in a transwell culture system was sufficient for T-reg induction but that direct contact resulted in expansion of T-reg numbers via a Notch-dependent mechanism[32].

Soluble factor-dependent mechanisms have been identified in a relatively large number of studies as playing a role in the effects of MSC on T-reg induction, proliferation, survival or suppressive potency.

(a) *Transforming growth factor beta 1 (TGF-β1):* This cytokine is secreted in an inactive latent form as pro-TGF-β1 which is cleaved into two fragments, of which the C-terminal homodimer represents mature TGF-β1 and the N-terminal homodimer is associated with

the latency-associated peptide (LAP) domain forming a small latency complex (SLC). Recently, it has also been recognized that glycoprotein A repetitions predominant (GARP) expressed by both MSC and T-reg plays a crucial role in the maturation and activation of the LAP/TGF-β1 complex by interacting with alpha-beta integrins (αVβ6 and αVβ8) expressed on many lymphocytes[33]. Thus, GARP expressed by MSC may assist in the promotion of T-reg by directing released TGF-β1 toward responsive T-cells. In the study of Cahill et al. in a mouse model of allergic airway inflammation, TGF-β1 neutralisation resulted in reduced mRNA and protein level of FOXP3 and CD25, further confirming that it plays a role in inducing T-reg differentiation[31]. Similarly, Hong et al. reported a significant increase in the number of $FOXP3^+$ T-reg when human $CD4^+$ T cells were co-cultured with dental pulp MSC which was reduced by blockade of TGF-β1[34].

(b) Prostaglandin E2 (PGE2): Co-culture studies of MSC with human peripheral blood mononuclear cells (PBMC) have indicated that PGE2 is an important mediator of T-reg promotion[35]. Yang et al. reported that human MSC reversed the suppressive deficiency of T-reg from multiple sclerosis patients by augmenting the production of multiple soluble factors including TGF-β1 and PGE2[36]. Similarly, Tumangelova-Yuzeir et al. reported that co-culturing of MSC derived from glioblastoma multiforme patients with PBMC from healthy donors resulted in secretion of PGE2 along with TGF-β1 that eventually increased the T-reg percentage and decreased Th-17 cell numbers[37]. In an *in-vivo* mouse model of colitis, An et al. reported that PGE2 secreted by feline adipose tissue-derived MSC reduced inflammation by increasing $FOXP3^+$ T-reg[38].

(c) Indoleamine 2,3-dioxygenase (IDO): The inducible enzyme IDO catalyses the rate-limiting step in tryptophan metabolism leading to accumulation of tryptophan catabolites including kynurenine, 3-hydroxyanthranilic acid and quinolinic acid. These catabolites enhance the effects of TGF-β1 leading to immune suppression and potentially to induction of T-reg[39]. Li et al. reported that human umbilical cord-derived MSC co-cultured with T-cells block cell cycle progression and induce apoptosis through an IDO-dependent mechanism[40]. In the same study, IDO-lentivirus-transfected MSC (IDO-MSCs) induced a higher percentage of $FOXP3^+$T-reg in human PBMC. Subsequently, low doses of IDO-MSC prolonged graft survival and induced tolerance by increasing antigen-specific T-reg in a rabbit kidney transplant model[41].

(d) Heme oxygenase-1 (HO-1): Another potential contributor to MSC-mediated induction of T-reg is the inducible enzyme HO-1, which catalyses the rate-limiting step of heme degradation to bilverdin. Mougiakakos et al. reported induction of different subsets of T-

reg in response to HO-1 produced by human MSC *in-vitro*[42]. In a co-culture study between human MSC and PBMC from asthmatic subjects, Li et al. reported that inhibition of HO-1 resulted in decreased T-reg proportions[43]. Moreover, BM-MSC adenovirally transduced to over-express HO-1 induced higher numbers of T-reg than control BM-MSC in a rat model of liver transplantation[44].

(e) Human leukocyte antigen G5 (HLA-G5): HLA-G5 is a soluble factor released by MSC that has potential to increase the number of $FOXP3^+$ cells. Selmani et al. reported *in vitro* induction of T-reg by human MSC secreting HLA-G5. Neutralisation of HLA-G5 partially restored T-cell proliferation in response to allogeneic stimuli[45]. Similarly, Chen et al. reported that *in vitro* expansion of T-reg in mixed lymphocyte reactions (MLR) by MSC was abrogated by blockade of HLA-G5 in systemic lupus erythematosus (SLE) patients[46].

(f) Leukemia Inhibitory Factor (LIF): Human MSC constitutively express LIF and it has been reported that LIF is elevated up to seven-fold when MSC are co-cultured with $CD3^+$ lymphocytes[47]. Additionally, restoration of human lymphocyte proliferation and a decline in $FOXP3^+$ T-reg was demonstrated in an MLR assay following addition of a LIF neutralizing antibody[47].

It is important to highlight that the soluble molecules that have been thus far recognized to play a role in MSC induction of T-reg are likely to share signalling pathways and engage in cross-talk with each other. For example, secretion of PGE2 induces the expression of IDO and kynurenine while TGF-β and IDO promote each other's gene amplification[48]. Similarly, release of TGF-β1 induces secretion of LIF which, in turn, stimulates increases PGE2 production[49]. Thus, while experimental studies have often focussed on providing evidence for a role for individual mediators, a more plausible mechanistic model is one whereby combinations of such factors - produced by MSC, T-cells and, as described below, secondary cell populations - act within a cascade of co-dependent events to support T-reg differentiation, survival and expansion while suppressing the differentiation and proliferation of other T cell subsets[27,50].

Antigen presenting cell (APC)-dependent mechanisms: In addition to directly interacting with T-cells, MSC also modulate the adaptive immune response through effects on APC (DC, macrophages and monocytes) that shift them to regulatory phenotypes associated with alternative surface receptor expression profiles and cytokine/chemokine secretion patterns. In 2009, Zhang et al. reported that mouse bone marrow-derived MSC re-programmed mature DC into APC with distinct jagged-2–dependent regulatory properties[51]. The findings were

corroborated by Zhao et al. who documented the generation of $FOXP3^+$ T-reg from $CD4^+CD25^-FOXP3^-$T-cells by bone marrow-derived MSC-conditioned regulatory DC. In this study, regulatory DC were also characterized by secretion of TGF-β1 and inhibition of T cell proliferation[52]. Further evidence came from Liu et al. who demonstrated both in vivo and in vitro induction of IL-10-dependent regulatory DC by a mouse embryonic fibroblast-derived MSC[53]. Cahill et al. reported induction of a semi-mature tolerogenic DC phenotype by MSC *in-vitro* that induced T-reg from $CD4^+CD25^-FoxP3^-$ T-cells. In a mouse model, these authors showed that Jagged-1 signalling played a key role in MSC expansion of T-reg[31]. It has also been shown by Meleif et al. that human MSC modulate monocyte/macrophages toward an alternatively-activated (M2) macrophages which promotes T-reg via secretion IL-10 and other soluble factors[54]. Consistent with this, Chiossone et al. also reported that MSC co-culture resulted in polarization of macrophages to an M2-like phenotype capable of suppressing effector T-cells and promoting T-reg[55]. Given recent evidence that intravenously administered MSC become trapped in the lungs and interact there with myeloid lineage immune cells[56], it is quite plausible that mechanisms of APC re-programming described in in vitro and in vivo experimental studies also operate in clinical settings to indirectly promote induction of T-reg and suppressive functions.

Extracellular vesicle (EV)-dependent mechanisms: A more recently-identified and potentially exciting mechanism whereby MSC may promote immune-regulatory effects is through the release of EV – particularly exosomes[57]. Although EV subtypes have complex and overlapping biochemical and physical properties, exosomes are typically defined as nanoparticles of 40-100 nm diameter that are generated by internal budding of the lipid membrane of late endosomes to form multi-vesicular bodies (MVB) which are subsequently released as exosomes upon fusion with the plasma membrane and a "biomolecular cargo" of proteins, glycans, lipids, messenger (m)RNAs and micro (mi)RNAs which have the potential to regulate immune cell gene transcription, intracellular signalling and effector functions[57]. In a 2014 study, Zhang et al. reported that MSC-EV delayed rejection following allogeneic skin grafts in mice with a concomitant polarization of activated $CD4^+$ T-cells to $CD4^+CD25^+FOXP3^+$ T-reg[58]. Subsequently, the same group observed that MSC-EV induction of T-reg in in vitro T-cell stimulation cultures was dependent on the presence of allogenic $CD11c^+$ APC. Furthermore, infusion of MSC-EV in a humanized mouse model of graft versus host disease resulted in reduced mortality associated with higher levels of human T-reg[59]. Interestingly, MSC-EV-conditioned human DC have also been shown to have

increased secretion of IL-10 and TGF-β1 leading to greater T-reg induction in pancreatic islet antigen-specific stimulation assays of T-cells from subjects with type 1 diabetes[60]. Finally, Hyvärinen et al. recently demonstrated that human MSC-EV downregulated the production of IL-22 and IL-23 by macrophages and polarized them to regulatory phenotype in a PGE2-dependent manner[61]. Though the potential for MSC-EV immunotherapy remains high, the significance of their role in T-reg induction in comparison to other mechanisms of MSC immunomodulation is not yet well understood.

Conclusion: Knowledge gaps and the potential for clinical translation of MSC effects on T-reg

Therapeutic applications of both autologous and allogeneic MSC have been actively pursued in many experimental human clinical trials during the past decade on the basis of their low immunogenicity, genetic stability, ease of production and diverse immunosuppressive/anti-inflammatory properties[1-5, 62,63]. A key concept underlying MSC efficacy in autoimmunity, transplantation and other inflammatory diseases has been their potential to induce or functionally enhance specialized populations of innate and adaptive immune cells with regulatory/suppressive functions[64-66]. In addition to $CD4^+$/$FOXP3^+$ T-reg, which we have focussed on in this review, evidence also exists that MSC may promote regulatory/tolerogenic populations of $CD8^+$ T cells, DCs and B cells as well as anti-inflammatory (M2) monocyte/macrophages and Th2-type $CD4^+$ effector T cells[62]. Nonetheless, a substantial number of completed and ongoing clinical trials and *in vivo* studies of MSC for immune-mediated diseases and transplants have been based, at least in part, on the premise that MSC increase the frequency of T-reg following systemic or localized administration. As summarized in **Table 1**, immune profiling studies from clinical trials involving relatively limited numbers of patients with transplant or immune-mediated disease have provided preliminary evidence for increased T-reg numbers or proportions following systemic or localized MSC administration[25,67]. The last few years have also witnessed swift progress in the number of clinical trials aimed at assessing the safety, feasibility and efficacy of *ex vivo*-expanded T-reg in transplantation and efforts have begun to translate this therapy to the clinic[68]. The evidence summarized here is encouraging to the extent of clearly establishing the potential for MSC to induce and/or increase proliferation of T-reg via a wide range of credible direct and indirect mechanisms. Given that the majority of therapeutically-administered MSC have short *in vivo* survival, such interactions between MSC and T-reg have high theoretical value through

sustained “downstream” suppressive effects on pro-inflammatory T-helper (Th)-1 and Th17 responses as well as modulatory effects on innate immune cells such as DC, monocytes and macrophages[69]. Though clinical application of MSC therapies to immune-mediated diseases appears promising, it remains unknown whether different types of MSC have more or less potent effects on T-reg or serve to promote distinct T-reg subpopulations. In the years ahead, this, and several other unresolved issues such as heterogeneity, stability, plasticity of MSC and T-reg populations, most effective dose of MSC for maximum induction of T-reg and long term safety of MSC-T-reg therapy need to be addressed with fresh perspectives from basic scientists and clinician investigators[63,70].

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Figure Legends

Figure 1: ***Schematic representation of different mechanisms used by MSC for T-reg Induction*** (a) Cell to cell contact: Interaction of different molecules such as ICOL and ICOS, Notch and Notch ligands expressed by MSC and T lymphocytes upregulates the production of IL-10 and T-reg proliferation. (b) Secretion of soluble factors: MSC secrete many soluble factors such as TGF-β1, PGE2, HO-1, HLA-G5 and LIF that induces T-reg expansion while suppressing other T cell proliferation (c) APC Dependent Induction: MSC effects on antigen presenting cells (dendritic cells, monocytes, macrophages) induce a regulatory phenotype on them however the molecules or factors responsible for this induction has not been identified yet. (d) Extracellular vesicle Induction: MSC-EV carrying RNA, proteins induces polarisation of $CD4^+$ T cells towards T-reg by increasing production of IL-10 while decreasing IL-17, IL-2, TNF-α, IFN-γ and IL-6. The figure was created with Biorender.com.

Conflict of Interests

The authors have no conflicts of interest to declare.

Data Availability Statement

This article does not include new data

Table 1: Summary of clinical trial reports in which effects of systemic or localized administration of autologous or allogeneic MSC on peripheral blood T-reg, with or without other immunological effects, were described in patients with medical or surgical conditions involving abnormal immune response or inflammation

Reference	Source	Disease	Key Findings	Route of Administration
Shi et al[23]	† Third party Allogeneic UC-MSC	Liver Transplantation	• Suppression of acute rejection in liver transplant recipients • **Increased T-reg and reduced Th17 cells** • Increased levels of TGFβ1 and PGE2	IV
Pers et al[71]	Autologous ASC	Severe Osteoarthritis	• **Increased percentage of T-reg at 3 months** • Increased percentage of $CD24^{high}CD38^{high}$ transitional B cells • Decreased percentage of classical $CD14^{+}$ monocytes	Intra-articular
Erpicum et al[25]	Third party Allogeneic BM-MSC	Kidney transplantation	• Improved early allograft function • **Increased frequency of T-reg at day 30** • No significant change in B cell population	IV
Ciccocioppo et al[67]	Autologous BM-MSC	Crohn's disease	• Reduction of perianal disease activity • **Increased percentages of mucosal and circulating T-reg**	Intra-fistular
Karussis et al[72]	Autologous BM-MSC	Multiple sclerosis and amyotrophic lateral sclerosis	• Possible migration of MSC in the occipital horns of the ventricles as visualised by magnetic resonance imaging • **Increased percentage of T-reg** • Decreased proliferative response of lymphocytes	Intra-thecal and IV
Wang et al[73]	Allogeneic UC-MSC	Systemic lupus erythematosus	• **Increased percentage of T-reg at 1 week and 1, 3, 6 and 12 months post-MSC** • Increased serum TGF-β increased at 1week, 3 and 12 months • TGF-β produced by MSCs mediated increased T-reg and PGE2-mediated decreased Th17 cells	IV

Liang et al[74]	Allogeneic BM-MSC	Systemic lupus erythematosus	• Lack of serious adverse effects after MSC infusion • **Increased T-reg at 3 and 6 months compared to baseline levels**	IV
Zhao et al[75]	Third party Allogeneic BM-MSC	Acute GvHD	• Reduced severity and incidence of chronic GvHD • **Higher T-reg frequencies in MSC treated compared to control**	IV
Ghoryani et al[76]	Autologous BM-MSC	Rheumatoid arthritis	• **Increased T-reg percentage 1 month post-MSC** • Decreased Th17 cells and IL-17 levels	IV
Gao et al[77]	Allogeneic UC-MSC	Chronic GvHD	• Decreased incidence of GvHD • **Increased T-reg frequency** • Increased frequency and number of $CD27^+$ memory B lymphocytes • Decreased NK cell frequency	IV
Kong et al[78]	Allogeneic UC-MSC	Type 2 diabetes mellitus	• Increased plasma C peptide • **Increased T-reg numbers**	IV
Weng et al[79]	Allogenic BM-MSC	Dry eyes associated chronic GvHD	• **No change in $CD4^+CD25^+$T-reg** • Increased $CD8^+CD28^-$ regulatory T cells percentage	IV
Li et al[24]	Allogeneic UC-MSC and BM-MSC	Systemic lupus erythematosus	• **Increased T-reg and decreased Th17**	IV
Xu et al[80]	Autologous BM-MSC	Liver cirrhosis	• Improvement in liver function • **Increased T-reg numbers and reduced Th17 proliferation** • Elevated serum TGF-β	Infusion via hepatic artery
Fang et al[81]	Allogeneic UC-MSC	Decompensated Hepatitis B cirrhosis	• **Increased percentage of T-reg** • Reductions in serum IL-6 and TNF-α levels • Elevated serum levels of IL-10 and TGF-β	Hepatic artery, portal vein and IV
Xiao et al[82]	Allogeneic BM-MSC	Refractory aplastic anemia	• **Increased percentage of T-reg**	IV

Detry et al[83]	Third party unrelated MSC	Liver Transplantation	• **No change in T-reg number and phenotype**	IV
Peng et al[84]	Donor derived Allogeneic BM-MSC	Kidney Transplantation	• **No change in percentages of $CD4^+$T-reg, NK cells and $CD8^+$ T cells** • Increased B cell proportion	Intra-arterial and IV
Perico et al[85]	Autologous BM-MSC	Kidney transplantation	• **Increased percentage of T-reg** • Inhibition of memory $CD45RO^+RA^+CD8^+$ T cell expansion	IV
Soeder et al[86]	Third party Allogenic BM-MAPC	Liver Transplantation (single patient)	• **Increased percentage of T-reg days 1, 3 and 9 post-transplant**	Intra-portal and IV
Perico et al[11]	Autologous BM-MSC	Kidney Transplantation	• **Increased T-reg/memory CD8+ T cell ratio**	IV

† Abbreviations: UC-MSC = umbilical cord-derived mesenchymal stromal cells; TGFβ1 = transforming growth factor beta 1; PGE2 = prostaglandin 2; T-reg = regulatory T-cells; Th17 cells = T helper 17 cells; IV = intravenous; ASC = adipose-derived stromal cells; BM-MSC = bone marrow-derived mesenchymal stromal cells; GvHD = graft versus host disease; IL-17 = interleukin 17; NK cell = natural killer cell; BM-MAPC = bone marrow-derived multipotent adult progenitor .

a

IL-10

T-reg

ICOSL-ICOS

T lymphocyte

Jagged1-Notch

IL-6, IL-12

Th17

b

IL-10, TGF-β1,
PGE2, NO, IDO

Suppression of T
cell proliferation

HLA-G (Surface)

HLA-G (Soluble)

IL-10, TGF-β1,
PGE2, HO-1, LIF

T-reg Expansion

Mesenchymal stromal cells

Cell to Cell Contact

Extracellular-vesicle Induction

Soluble Factors

APC Dependent Induction

Coculture

Regulatory T cells

c

MSC derived EV

EV

T lymphocyte

IFN-γ, IL-2,
TNF-α, IL-6, IL-17

Inhibition of T cell
proliferation and
Th17
differentiation

IL-10

T-reg expansion

d

Regulatory
Macrophages

Regulatory
Dendritic Cell

TGF-β1, IL-10

T-reg expansion