



# Therapeutic Application of Mesenchymal Stem Cells Derived Extracellular Vesicles for Immunomodulation

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The immunosuppressive potential of mesenchymal stem cells has been extensively investigated in many studies *in vivo* and *in vitro*. In recent years, a variety preclinical and clinical studies have demonstrated that mesenchymal stem cells ameliorate immune-mediated disorders, including autoimmune diseases. However, to date mesenchymal stem cells have not become a widely used therapeutic agent due to safety challenges, high cost and difficulties in providing long term production. A key mechanism underpinning the immunomodulatory effect of MSCs is the production of paracrine factors including growth factors, cytokines, chemokines, and extracellular vesicles (EVs). MSCs derived EVs have become an attractive therapeutic agent for immunomodulation and treatment of immune-mediated disorders. In addition to many preclinical studies of MSCs derived EVs, their beneficial effects have been observed in patients with both acute graft-vs.-host disease and chronic kidney disease. In this review, we discuss the current findings in the field of MSCs derived EVs-based therapies in immune-mediated disorders and approaches to scale EV production for clinical use.

**Keywords:** extracellular vesicles, microvesicles, immunosuppression, autoimmune diseases, multiple sclerosis, transplant rejection, type 1 diabetes, graft-vs.-host disease

## INTRODUCTION

Mesenchymal stem cells (MSCs) bear great potential not only in regenerative medicine, but they also interfere with different pathways of the immune response and exhibit immunomodulatory activity (1, 2). Preclinical and clinical studies both indicate that MSCs have immunosuppressive activity, including suppression of T- and B-cell proliferation, modulation of regulatory T cell function maturation and activation, antigen presentation by dendritic cells, decrease secretion of proinflammatory cytokines and cytotoxicity (3). Up to now MSCs have been exploited in 67 clinical trials (<http://clinicaltrials.gov/>; accessed April 2019) of inflammation-associated diseases, autoimmune diseases and transplant rejection (**Supplementary Table 1**).

The accumulating evidence supports MSCs producing a strong paracrine action on neighboring cells through a broad range of growth factors, chemokines, cytokines and extracellular vesicles (EVs) (4). EVs are a heterogeneous population of spherical membrane vesicles, containing biologically active cargo of molecules (proteins, lipids, mRNA, microRNA, siRNA, miRNA, ssDNA, dsDNA) deliverable to target cells (5, 6). Due to the fundamental function of EV—mediation of intercellular communication, they are involved in numerous physiological and pathological

processes and pathways (7). MSCs derived EVs have been used to recapitulate some of the biological activity of parent cells, including stimulation of regeneration in models of renal, heart, liver and nervous tissue injury (8–12) a function which is arguable the most prominent function of MSCs.

Due to the genetic instability (13), undesired differentiation (14, 15), and pulmonary embolism risks (16), the application of MSCs in a clinical setting is restricted. Cell-free therapy based on EVs offers a promising alternative to stem cell-based therapy for inflammation-associated diseases, autoimmune diseases, and transplant rejection (17–19).

A caveat in pursuing EVs for clinical therapeutic applications has been the difficulty of producing sufficient yield. Therefore, in parallel with investigation of biological activity and therapeutic efficacy, approaches to scale EV production are also underway (17, 20, 21). Large scale EV production research aims to generate an industrially feasible approach that retains clinical-grade purity. As well as alternative approaches to isolate other EV-like and larger particles (21–23).

MSCs derived EVs are a promising therapeutic instrument which have advantages over cell therapy. In this review, we focus on the recent findings of therapeutic application of MSCs derived EVs for the treatment of immune-mediated disorders and the perspective application of different methods for large scale EVs production.

## MSCS-BASED IMMUNOSUPPRESSIVE THERAPY

*In vitro* and *in vivo* studies have been used to demonstrate the MSCs anti-inflammatory and immunomodulatory properties on both innate (macrophages, NK cells, dendritic cells) and adaptive immune cells (T-cells, B-cells) (24).

Using blocking antibodies and inhibitors it was shown that indoleamine 2,3-dioxygenase (IDO) (25), prostaglandin E2 (PGE2) (26), interleukin 10 (IL-10) (27), nitric oxide (NO) (28), and hepatocyte growth factor (HGF) (29) mediate the inhibitory action of MSCs on immune cells. The list of soluble factors which are associated with the immunomodulation capacity of MSCs is still to be fully elucidated.

The first clear evidence of MSCs immunosuppression of immune cells was produced *in vitro* by Aggarwal and Pittenger (30) by demonstrating human MSCs altered the cytokine secretion profile of co-cultured dendritic cells (DCs), T cells (TH1 and TH2), and natural killer (NK) cells to induce a more anti-inflammatory phenotype. In addition, the number of regulatory T cells (T-regs) was also increased (30). Successful immunosuppression of immune cells by MSCs *in vitro* lead to increasing the number of *in vivo* research and preclinical trials.

In more recent studies, MSCs isolated from human umbilical have been shown to inhibit inflammation in a rodent model of acute allergic rhinitis (31). Reduced expression of interleukin 4, tumor necrosis factor alpha (TNF- $\alpha$ ), and immunoglobulin E were detected in the serum of animals treated with MSCs (31).

Similar findings have also been reported in rodent models of diabetic nephropathy (DN) and rheumatoid arthritis (RA),

within which treatment with MSCs increased the concentration of anti-inflammatory cytokines (IL-10 in DN and RA and EGF in DN) This change was accompanied by a decrease in pro-inflammatory cytokines [IL-6, MCP-1, TNF- $\alpha$  and IL-1 $\beta$  in DN and of IL-6, TNF- $\alpha$ , TGF- $\beta$ , NF- $\kappa$ B, toll-like receptor-2, MMP-3, COMP-1, and RF (rheumatoid factor) in RA] (32, 33).

The effects of MSCs has also been tested in autoimmune encephalomyelitis (EAE) (the animal model of multiple sclerosis). Treatment with embryonic stem cell-derived MSCs in the cynomolgus monkey EAE model reduced the clinical symptoms of brain lesions and neuronal demyelination (34).

The first clinical trial using bone marrow derived MSCs was conducted in 2006 at the University of Cambridge (**Supplementary Table 1**). As a result of the trial the authors suggested improvements for the design of future studies to increase the efficacy of evaluation (35).

These recommendations have led to a growth in clinical trials in the area of MSCs based treatment of autoimmune disorders (**Supplementary Table 1**). China is currently leading on clinical trials within this field (26.87% of total number of trials), with a predominance (44.4%) toward the treatment of type 1 Diabetes (**Supplementary Table 1**). Trials conducted in other countries are reviewing MSCs effects in multiple sclerosis (35.82%), type 1 diabetes (22.39%), and rheumatoid arthritis (17.91%) (**Supplementary Table 1**).

Whilst the comparison of the data produced in these trials is complicated by variations in design (MSCs administration, dose and regimens). Importantly, to date there have been no reports of tumor development following MSCs infusion (36, 37).

Despite promising clinical studies, MSCs have not become a universal therapeutic agent for the treatment of immune-mediated disorders (38). In the main, this is due to concerns over safety (transformation, undesired differentiation and blood vessels occlusion), and the labor intensive, industrially inapplicable procedure of preparation. It is known that the beneficial effects of MSCs are largely mediated by paracrine factors. Cell-free therapy based on mediators of cell-cell communication, of which EVs are considered a promising approach, are now considered to be a safer alternative to entire stem cell therapy (17). MSCs derived EVs offer compelling advantages over cell therapies in terms of improved biodistribution, lower toxicity, higher stability in the circulation and scalable production (39). Autologous and allogeneic MSCs can be used for the production of EVs-based therapeutics. The biocompatibility of autologous MSCs offers an attractive therapeutic approach. However, from a manufacturing perspective EVs derived from allogeneic MSCs can be obtained in higher concentrations and isolated from MSCs obtained from younger donors which are more biologically active and potentially more readily available (40).

## MESENCHYMAL STEM CELLS DERIVED EXTRACELLULAR VESICLES

Originally it was thought the beneficial effects of MSCs based therapy in tissue regeneration was due to the engraftment and

differentiation of MSCs within damaged tissues. Subsequently, it was found that relatively few transplanted MSCs engraft in host tissues (41, 42). Together with evidence that media conditioned by MSCs is sufficient to stimulate regeneration (43, 44), the development of the paracrine hypothesis of the therapeutic effects of MSCs was formed (45), the hypothesis that is now widely accepted. Within the broad spectrum of factors secreted by MSCs, EVs have been highlighted as a potential therapeutic alternative to MSCs use (46).

EVs are bilipid membrane vesicles, encapsulating proteins (including transcription factors, growth factors, and enzymes) and genetic material (mRNA, siRNA, miRNA, ssDNA, dsDNA) (Figure 1A) (5, 5, 6, 59). It was shown that most RNAs (>80%) inside of MSCs derived EVs are 28S, 18S, 5.8S, 5S ribosomal RNA, alongside other small RNAs miRNA (44%), tRNA (47%) and Y RNA (8%). MSCs derived EVs were enriched with miRNA which regulate osteogenic differentiation: let-7a and c, mir-22, 199a, 196a, 199b, mir-27, 98, 100, 615, 125b, and 195 (Figure 1A) (60). Moreover, evidence suggests mitochondria (61), ribosomes (62), and proteasomes (63) might also be enclosed and transferred by EVs to target cells. Knowledge of the biological cargo carried by EVs is continually developing, the development of integrated proteome, transcriptome, and lipidome databases—such as Vesiclepedia, EVpedia, and Exocarta (<http://www.microvesicles.org/>; <http://evpedia.info>; <http://www.exocarta.org>) now provide current data on EVs from a variety of sources.

EVs are a heterogeneous population of vesicles, thought to contain two predominant subtypes—exosomes (40–150 nm) and microvesicles (400–2,000 nm) (17). Exosomes are vesicles of endosomal origin formed by invagination of the endosomal membrane inside of multi-vesicular bodies (MVBs) with subsequent release of exosomes as a result of fusion of the MVBs with the plasma membrane. Microvesicles are released by budding directly from the plasma membrane (17). EVs biogenesis and purification strategies have been described in several published reviews (64).

As both subpopulations of EVs overlap in both size and density, separation of the populations within a biological sample is difficult. Therefore, EVs is used as collective descriptive term to describe the population of small vesicles released by most types of cells including MSCs (17).

In addition to carrying nucleic acids, MSCs derived EVs also reflect the protein characteristics of MSCs and contain proteins, associated with the therapeutic effects of MSCs: surface receptors (PDGFRB, EGFR, and PLAU); signaling molecules (RRAS/NRAS, MAPK1, GNA13/GNG12, CDC42, and VAV2); cell adhesion molecules (CD29, CD44, CD73, FN1, EZR, IQGAP1, CD47, integrins, and LGALS1/LGALS3); and MSCs-associated antigens (CD9, CD63, CD81, CD109, CD151, CD248, and CD276) (Figure 1A) (63, 65). Recent transcriptomic and proteomic analysis of porcine MSCs derived EVs revealed that EVs were enriched in mRNAs encoding transcription factors and in proteins that support extracellular matrix remodeling, blood coagulation, inflammation, and angiogenesis (66).

Evidence also demonstrates that the biological activity of EVs is also similar to that of parental MSCs. EVs possess angiogenic, anti-apoptotic, and immunomodulatory properties similar to

parental MSCs (67). It was shown that mouse MSCs derived EVs are enriched with VEGF protein and miR-210-3p via which they stimulate angiogenesis in ischemic limbs (68). In a rat model of spinal cord injury, administration of MSCs derived EVs resulted in a decrease in cellular apoptosis and inflammation at the injured site. This was accompanied by a decrease in the expression levels of proinflammatory cytokines (TNF- $\alpha$  and IL-1 $\beta$ ) and an increase in anti-inflammatory cytokines IL-10 (69). In a model of traumatic acute lung injury (ALI) an increase of the survival rate and suppression of inflammatory response in experimental animals was also demonstrated (70). BM-MSCs derived exosomes reduced infarct size and improved cardiac function in rats after the acute myocardial infarction (71).

These results demonstrate the potential promise of MSCs derived EVs as an alternative to whole cell therapy. EVs efficiently mimic therapeutic effects of MSCs, whilst negating some of the safety concerns related to the use of MSCs, such as tumorigenicity and blood capillary embolism specific for MSCs based therapy (72). Currently, due to the lack of a nucleus, EVs do not fall into the category of advanced therapy medicinal products (ATMP) according to the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) (73). Unlike gene therapy and cells-based approaches, EVs are not currently considered to be high-risk biologic drugs, but further conventional demonstrations of safety and efficacy in preclinical and clinical trials are essential (74).

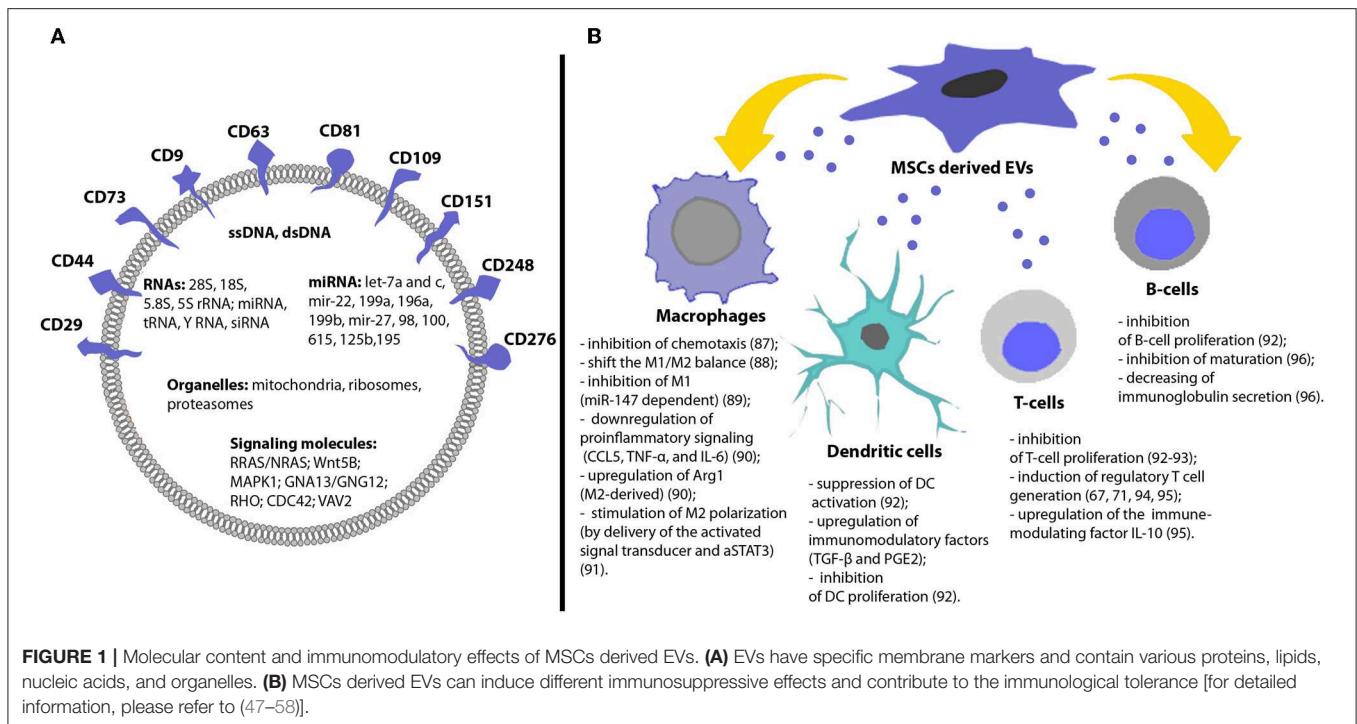
## IMMUNOMODULATORY ACTIVITY OF EXTRACELLULAR VESICLES

MSCs derived EVs have shown immunosuppressive effects on many types of immune cells: dendritic cells, T cells, B cells and macrophages (75) (Figure 1B). It was shown that MSCs-derived exosomes and microvesicles exert similar immunosuppressive functions (72). However, it should be noticed that exosomes were more efficient in suppressing inflammation *in vivo* in inflammatory arthritis (76).

Reis et al. showed that EVs impaired antigen uptake by DCs, inhibited DCs maturation, secretion of pro-inflammatory cytokines IL-6 and IL-12p70 and increased their production of anti-inflammatory cytokine TGF- $\beta$  (77). The immunomodulation was in part mediated by microRNAs (miR-21-5p, miR-142-3p, miR-223-3p, and miR-126-3p) which have known effects on DC maturation and were enriched in MSCs derived EVs (77) (Figure 1B).

Immunoregulation of T-cell mediated responses is an important tool to control autoimmune or inflammatory diseases. Treatment of T-cells with MSC derived EVs has been shown to result in a marked decrease in T-cell induced proliferation *in vitro* and downregulation of IFN- $\gamma$  and TNF- $\alpha$  (78). Khare et al. also demonstrated the inhibitory effect of MSCs derived EVs on the proliferation of activated PBMCs and isolated T and B cells (47) (Figure 1B).

Studies conducted by Zhang et al., found that MSC derived EVs induced an M2-like phenotype in monocytes, induced T-cells to differentiate into regulatory T-cells and attenuated



immune activity *in vivo* enhancing the survival of allogenic skin grafts in mice (79) (**Figure 1B**). Morrison et al. demonstrated that MSC derived EVs promote an anti-inflammatory and highly phagocytic macrophage phenotype through mitochondrial delivery (80). Taken together, these studies suggest that MSCs derived EVs retain the biological activity of the parental cells and are promising immunosuppressive instrument.

A number of different animal models have been used to study the immunomodulating activity of MSCs derived EVs (**Table 1**), demonstrating immunological activity and modification of the expression of both anti-inflammatory and pro-inflammatory cytokines. The immunomodulatory effect of EVs derived from human MSCs within animal model of pathogen/antigen induced tissue injury demonstrated a range of effects, including increases in survival in induced lung injury (81), decreased synovial lymphocyte counts and lowered TNF- $\alpha$  mRNA expression in synovitis joints (48), and increased regulatory T cells following concanavalin A induced liver injury (82). Additional preclinical studies in autoimmune uveoretinitis have shown MSCs derived EVs reduce the infiltration of inflammatory T cells in the eyes reducing the intensity of symptoms (83). Moreover, Shigemoto-Kuroda et al. demonstrated that MSC derived EVs suppressed Th1 and Th17 development, inhibited activation of APCs and T cells, increased expression of the immunosuppressive cytokine IL-10 and prevented development of uveoretinitis (84). **Table 1** summarizes some more recent findings in the field of preclinical studies of EVs-MSCs based therapy of immune-mediated disorders. As seen from **Table 1** EVs demonstrate the immunosuppression activity regardless of MSCs source. EVs derived from adipose-derived -MSCs, amnion-derived MSCs, and umbilical cord-MSCs are as effective as BM-MSCs (**Table 1**).

EVs circulate in body fluids disseminating throughout the body. To direct the immunosuppressive action of EVs, targeting strategies are actively being developed. Shamili et al. conjugated a myelin specific aptamer to the exosome surface and showed differences in the suppression of inflammatory response, demyelination process and severity of multiple sclerosis (95).

Clinical case studies to investigate the therapeutic potential of MSCs derived EVs have been conducted in patients with steroid-refractory acute graft-vs.-host disease (acute GvHD) (96). Treatment with EVs was reported to significantly improve GvHD symptoms in a 22-years female patient with severe cutaneous and intestinal GvHD, accompanied by a decrease in the level of IL-1b, TNF- $\alpha$ , IFN- $\gamma$  secreted by patient-derived PBMCs (96).

Phase II/III clinical study on 20 patients with chronic kidney disease (CKD) showed that treatment with MSCs derived EVs improved the glomerular filtration rate (eGFR), serum creatinine level, blood urea and urinary albumin creatinine ratio (UACR) (97).

Despite these promising clinical studies, there are still limitations that need to be overcome in order to develop EVs-based medicines: (1) establishment of a recommended isolation protocol for large-scale preparation, purification and storage of EVs; (2) standardized protocols of EVs quantification, molecular, and physical EV characterization; and (3) defined quality control (QC) criteria for clinical use (74).

## APPROACHES FOR INCREASING PLASMA MEMBRANE-DERIVED VESICLES YIELD

Limited yield and labor intensive procedures for EV isolation has made large-scale production by pharmaceutical companies



**TABLE 1** | Application of extracellular vesicles of mesenchymal stem cells for the therapy of immune-mediated diseases.

Source of EVs	Model of disease	Observed effect	References
<b>Inflammation associated diseases</b>			
Rat BM-MSCs	Cardiac ischemia	Inhibition of proliferation of T-cells <i>in vitro</i> , reduction of infarct size.	(85)
Pig AD-MSCs	Metabolic syndrome and renal artery stenosis	Attenuation of renal inflammation and fibrosis, improving of medullary oxygenation, renal blood flow, and glomerular filtration rate.	(86)
Human BM-MSCs	Local ischemic stroke (rat model)	Immune suppression 1 week after the injury, regeneration of blood vessels and nervous tissues.	(87)
Human BM-MSCs	Preterm brain injury (rat model)	Amelioration of inflammation, neuronal degeneration, reduction of microgliosis and prevention of astrogliosis.	(88)
Human BM-MSCs	Acute spinal cord injury	Attenuation of microglia activation, improving of locomotor recovery and mechanical sensitivity.	(89)
Mouse BM-MSC	Hepatic ischemia-reperfusion injury	Reduction of tissue necrosis, cells apoptosis, serum aminotransferase levels, expression of inflammatory cytokines (IL-6).	(90)
Rat amnion-derived MSCs	Liver fibrosis	Decrease of expression of inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, TGF- $\beta$ ), decrease of fiber accumulation, activation of Kupffer cells, and hepatic stellate cell.	(85)
<b>Autoimmune diseases</b>			
Human AD-MSCs	Multiple sclerosis (murine encephalomyelitis virus induced demyelination)	Immunomodulation, decrease of inflammatory infiltrates, reducing of brain atrophy, increase of cell proliferation in the subventricular zone.	(89)
Mouse AD-MSCs	Autoimmune encephalomyelitis	Reduction of the severity of EAE by inhibiting of T cells extravasation in the inflamed central nervous system after the preventive administration of EVs.	(91)
Human BM-MSCs	Type 1 diabetes (mouse model)	Delay of the onset of T1D in mice, inhibition of activation of antigen-presenting cells and suppression of development of T helper 1 (Th1) and Th17 cells.	(84)
Mouse BM-MSCs	Rheumatoid arthritis	Inhibition of T lymphocyte proliferation, decrease of inflammation.	(76)
Human AD-MSCs	Atopic dermatitis (mouse model)	Reduction of symptoms, the levels of serum IgE, the number of eosinophils, infiltration of mast cells, CD86+, and CD206+ cells in skin lesions. Reduction of expression of inflammatory cytokines (IL-4, IL-23, IL-31 and TNF- $\alpha$ ).	(92)
<b>Transplant rejection</b>			
Human Umbilical Cord-MSCs	aGVHD (mouse model)	Decrease of the symptoms, reduction of the mortality of the recipient mice, number of CD8+ T cells, reduction of serum levels of IL-2, TNF- $\alpha$ , IFN- $\gamma$ and increase of the level of IL-10.	(93)
Human BM-MSCs	aGVHD (mouse model)	Prolongation of the survival of mice with aGVHD and reduction of the pathologic damage in organs, suppression of CD4+ and CD8+ T cells, suppression of the functional differentiation of T cells from a naive to an effector phenotype.	(94)

BM-MSCs, bone marrow derived MSCs; AD-MSCs, adipose derived MSCs; TNF- $\alpha$ , tumor necrosis factor alpha; TGF- $\beta$ , Transforming growth factor beta; IFN- $\gamma$ , Interferon gamma; Th1, T helper cells; Th17, T helper 17 pro-inflammatory cells; EAE, encephalomyelitis; T1D, type 1 diabetes; aGVHD, acute graft-vs.-host disease.

problematic and restrains the wider use of EVs in preclinical and clinical applications. The amount of the MSCs derived EVs isolated in the first clinical case study from supernatant of  $4 \times 10^7$  MSCs was  $1.3\text{--}3.5 \times 10^{10}$  particles or 0.5–1.6 mg (96). This dosage of EVs was defined as 1 unit and the patient received 4 units in total (96). To increase the yield of vesicles from MSCs, Mendt et al. suggested the use of bioreactor cultures of BM-MSCs (98). The authors were able to get  $9.8\text{--}15.6 \times 10^{12}$  exosomes per bioreactor run (98), a concentration sufficient for the treatment of one patient using the original clinical case protocol (97).

Further investigations of the biochemical and biophysical properties of membrane proteins and membrane organization are leading to novel approaches and continued improvements in current approaches for large scale vesicle production. Del Piccolo et al. induced the release of vesicles from CHO cells using “vesiculation buffer” (23). Cells were rinsed with a hypotonic

buffer which induced cell swelling, then incubated the cells in a hypertonic solution. This osmotic buffer did not rupture the cells but stressed them sufficiently to increase the release of vesicles into solution (23). However, it remains to be determined how the increase in vesicle production alters the cargo and biological function of the resulting EVs.

Wu et al. produced vesicles from human retinal pigment epithelium (ARPE-19) cells by extruding the cell suspension through polycarbonate filter with 1  $\mu\text{m}$  or 2  $\mu\text{m}$ -pore size (20). The resulting population of vesicles consisted of two population—one with an average size  $0.2 \pm 0.1 \mu\text{m}$  and the smaller in number  $\text{--}0.8 \pm 0.5 \mu\text{m}$  (20). A similar approach was used to produce vesicles from bone marrow derived MSCs via extrusion through a polycarbonate membrane with 3  $\mu\text{m}$  pores. The authors were able to detect the functional active mitochondria inside the plasma membrane vesicles (21).

Plasma membrane-derived vesicles may be promising vector for the delivery of therapeutics. However, due to sporadic, unselective packing of cytoplasmic content into the plasma membrane-derived vesicles, these vesicles are not equal to the natural EVs, and the biological activity of such vesicles remains unknown and needs to be verified.

A more moderate approach to increase membrane vesicle production is treatment of cells with cytochalasin B (99). Cytochalasin B is a drug that causes disorganization of actin cytoskeleton (100). Treatment of cells with cytochalasin B resemble the disorganization of actin cytoskeleton by activated protease calpain during the natural process of microvesicle release (17, 101). Up to now EVs have been derived from HEK293 (99, 102, 103), 3T3 fibroblast (102), HUVECs (104), MDCKII-MDR1 (105), SH-SY5Y (22), and PC3 cells (106). Cytochalasin B-induced membrane vesicle (CIMVs) were used as vectors for nanoparticles and drugs delivery (102), decreasing the toxicity of chemotherapy *in vivo* by encapsulating doxorubicin (104). Cytochalasin B application to human cells promotes an increase in membrane vesicles yield by >100 fold (17). It was calculated that  $17 \pm 6\%$  of the cell membrane transformed to CIMVs (99). The CIMVs released have a diameter of 100–1,000 nm (96%) which is comparable with naturally occurring EVs (22). Our studies of CIMVs have shown that their uptake by target cells is via heterophilic interaction of CIMVs membrane receptors with the surface proteins of target cells, this has a greater impact on CIMVs entry into target cells (106). Moreover, the biological activity of CIMVs is sufficient to stimulate capillary tube formation *in vitro* and angiogenesis *in vivo* by delivering growth factors (22). The use of Cytochalasin B to induced membrane vesicles (CIMVs) has advantages over endogenous EV production, including easier procedure of isolation, increased yield, more homogeneous composition due to the unselective mechanism of cytoplasmic content enclosing. The advantages of CIMVs production together with the evidence of retention of biological activity (22) strongly indicates that CIMVs may represent the next step toward the clinical use of EVs as therapeutic tools. However, the full immunomodulating properties of MSCs derived CIMVs remains to be fully evaluated.

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## CONCLUSION

MSCs derived EVs retain the biological activity of parental MSCs and demonstrate a similar therapeutic potential. EVs stimulate the viability and proliferation of target cells and modulate the immune microenvironment. Therefore, the EVs of MSCs are potential therapeutic tools, which have advantages over cell therapy in terms of safety, ease of storage/transportation and clinical use. However, one of the major limitations of clinical adaption of EVs is the poor scalability of production. However, improved understanding of the physical properties of EVs and the mechanism of their biogenesis is leading to improved approaches to increase yield and uniformity of their production. Taken together, these data suggest that MSC derived EVs are a promising therapeutic tool for the treatment of immune-mediated disorders, including autoimmune diseases.

## AUTHOR CONTRIBUTIONS

MG wrote the manuscript and created the tables and the figure. VJ edited the manuscript. MG and AR conceived the idea and edited the manuscript and figure.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2019.02663/full#supplementary-material>

- levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res.* (2013) 10:301–12. doi: 10.1016/j.scr.2013.01.002
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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