Int. J. Dev. Biol. 64: 465-469 (2020)
https://doi.org/10.1387/ijdb.200242jc

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Reciprocal interactions between mesenchymal stem cells and macrophages

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ABSTRACT Mesenchymal stem cells (MSCs) are used as therapeutic agents for the treatment of a wide spectrum of diseases, as well as for the regeneration and healing of burns and wounds. MSCs have an immunomodulatory effect and influence the phenotype and functions of immune cells, including macrophages, which in turn prime and license the MSCs. We discuss the new findings on the feedback loop between MSCs and macrophages and its consequences on the outcome of MSC therapies.

KEY WORDS: *mesenchymal stem cell, MSC, macrophage, MSC therapy, immunomodulation*

Introduction

The mesenchymal stem cells (MSCs), discovered in 1968/1970 by Alexander Friedenstein, are present in the umbilical cord, placenta, peripheral blood, bone marrow, and adipose tissue. They have the ability to differentiate into many different cell types, and as such are very promising candidates for the treatment of many diseases (Sharma *et al.*2014). In 2019 there were over 920 clinical trials in the USA using MSCs for the minimally invasive treatment of ALS, Alzheimer's, bronchopulmonary dysplasia, burns, chronic renal failure, cancer, Crohn's disease, cystic fibrosis, multiple sclerosis, osteoarthritis, bone/cartilage, liver, lung, brain, and heart diseases, diabetes, systemic lupus, graft versus host disease, ischemic cardiomyopathy, and spinal cord injuries among others. The MSCs can be easily expanded in the laboratory to the clinically desirable numbers. Depending on the clinical need, the MSCs can be administered intravenously (to subsequently home into the diseased organ/tissue), as the transplantable scaffolds or via direct injection into the injury site. Recently, MSCs have also been tested for the treatment of the acute respiratory distress syndrome in COVID-19 patients. Although in the early days of the therapeutic use of MSCs it seemed that the MSCs function through the replacement of the damaged cells, accumulating knowledge and data from the *in vivo* and *in vitro* studies indicate that MSCs repair damaged tissue through the secretion of immunomodulatory factors, which affect the activity of the immune cells (T cells, dendritic cells, and macrophages), inhibit inflammation, and stimulate the recovery of damaged cells. Studies also indicate that the immunoregulatory functions of the MSCs are not intrinsic and that the "naïve" MSCs have to be primed/licensed by the inflammatory environment. Here we describe what is currently known about the crosstalk between the MSCs and the macrophages during tissue repair and regeneration in various diseases, *in vitro* and in vivo.

Macrophages play both pro-inflammatory and anti-inflammatory roles, thus participate in tissue inflammation and damage, but also homeostasis, repair, and healing. The diversification and plasticity of macrophage phenotypes are key for their diverse functionality (Jinnouchi *et al.*, 2020). The M1 macrophages are pro-inflammatory and the M2 macrophages are anti-inflammatory and promote tissue repair and healing. The Mox macrophages develop from M1 and M2 macrophages in response to oxidative tissue damage and play a role in the development of chronic inflammatory diseases and atherosclerosis (Kadl *et al.,* 2010). The M4 macrophages differentiate in the presence of CXCL4 (platelet factor 4, PF4). The M4 macrophages, similar to Mox macrophages, are present in the atherosclerotic lesions but are also abundant in the leprosy patients (de Sousa *et al.*, 2018). The hemoglobin-associated macrophages

Abbreviations used in this paper: EV, extracellular vesicle; MSC, mesenchymal stem cell; TNT, tunneling nanotubes.

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Submitted: 5 September, 2020; *Accepted:* 21 October, 2020; *Published online:* 14 December, 2020.

ISSN: Online 1696-3547, Print 0214-6282

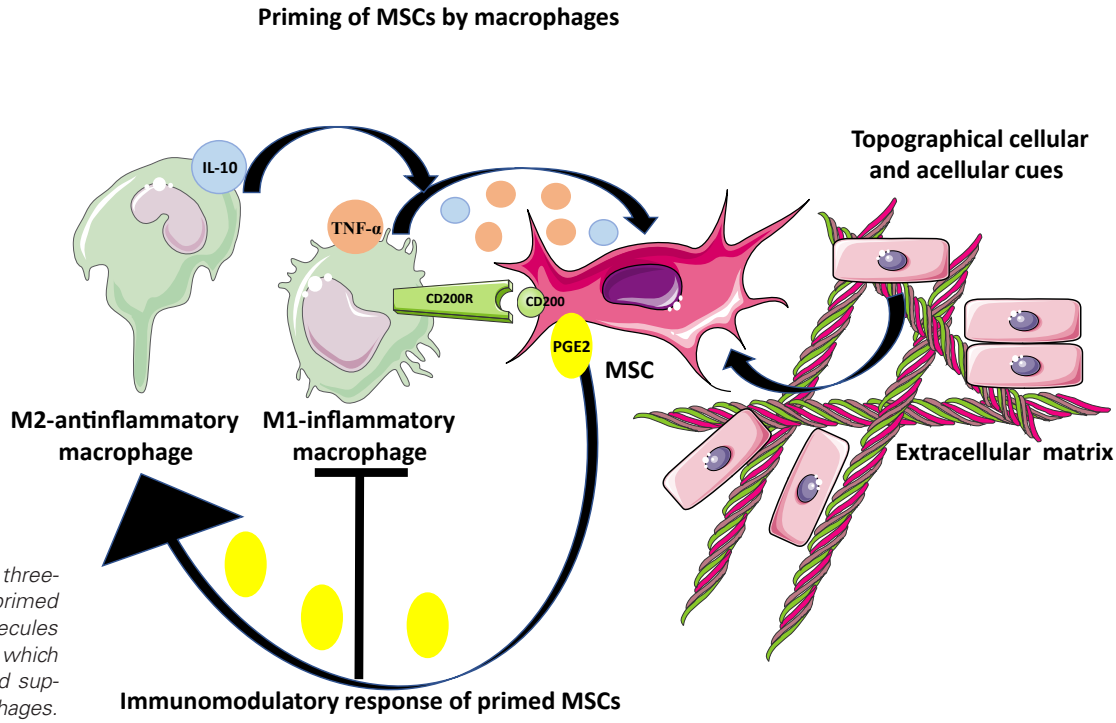

Fig. 1. Reciprocal interactions between mesenchymal stem cells (MSCs) and macrophages. *The diagram shows the example of interactions between MSCs and macrophages. The naïve MSCs are primed by inflammatory (TNF-α) and anti-inflammatory (IL-10) cytokines produced by M1 and M2 macrophages, respectively. The MSC priming also occurs through direct cell-to-cell contact via M1 macrophage receptor CD200R and its ligand CD200 produced by MSCs. The cellular and acellular topographical cues, which also prime the MSCs are delivered from the three-dimensional surrounding. The primed MSCs produce regulatory molecules such as prostaglandin 2 (PGE2), which promote anti-inflammatory and suppress the inflammatory macrophages.*

M(Hb) that differentiate in response to hemoglobin produce a low level of the inflammatory and high level of anti-inflammatory cytokines, promote angiogenesis, and increase vessel permeability and leakage (Habib and Finn, 2014; Guo *et al.,* 2018). The suppressor (regulatory) macrophages, Mregs, produce a high level of the anti-inflammatory cytokine, inhibit the immune response, do not induce fibrosis, and are known to induce tolerance to the transplanted organs (Mosser and Edwards, 2008; Hutchinson *et al.*, 2011). There is also a subpopulation of tumor-associated macrophages (TAMs), which are believed to fuse with cancer cells bestowing motility and metastasis (Kloc *et al.*, 2016). Considering the diversity of macrophage phenotypes and functions it is not surprising that the modulatory effect of MSCs on macrophages, and vice versa, may negatively or positively influence the outcome of MSC therapies.

MSC priming and modulation by macrophages

The MSCs isolated from the healthy donor are naïve and lack the immunomodulatory properties. Before the naïve MSCs can perform their therapeutic (healing or regenerative) functions they have to be primed by the cytokines and factors secreted by the immune cells of the inflammatory milieu. The macrophages, which are the key regulator of healing, and, depending on the subtype, either advance or resolve inflammation, are the main source of the MSC priming factors. Studies of human bone marrow-derived MSCs encapsulated in the hydrogen gels and primed with media conditioned by activated human macrophages, showed that the pro-inflammatory cytokine TNF-α, produced by the pro-inflammatory macrophages is the main priming factor and that the anti-inflammatory IL-10 produced by the anti-inflammatory macrophages, enhances the priming effect of TNF-α. The effectiveness of MSCs priming was assessed by the ability of MSCs to suppress inflammatory macrophages. The primed, but not the naive MSCs, inhibited the pro-inflammatory and sustained the anti-inflammatory phenotype of macrophages (Saldana *et al.,* 2019). Studies also showed that the primed MSCs produce prostaglandin 2 (PGE2) that reprograms monocytes and pro-inflammatory macrophages into the anti-inflammatory phenotype (Saldana *et al.,* 2019; Carty *et al.*, 2017). Recently, Valles *et al.,* (2020) showed that TNF-α secreted by pro-inflammatory macrophages induced MSCs elongation and enhanced their migration and attachment, while the anti-inflammatory IL-10 promoted the osteogenic activity of MSCs. Studies of the MSCs therapy in mouse abortion models showed that the direct cell-to-cell contact between MSCs and pro-inflammatory macrophages strengthened, in comparison to the conditioned media (in the transwell chamber), the immunosuppressive properties of MSCs (Li *et al.*, 2019). These studies also showed that the direct interaction between MSCs and inflammatory M1 macrophages occurs through the binding between the CD200 (a type-1 membrane glycoprotein), expressed on the surface of MSCs, and its receptor CD200R expressed on M1-macrophages (Li *et al.*, 2019). This indicates that the communication between MSCs and macrophages are not only paracrine, through the secreted factors, but also through the cell-to-cell contacts (Fig. 1). There are also studies indicating that the behavior and functions of MSCs cells are regulated by the topographical cues of the surrounding. The MSCs can sense the three-dimensional (3D) topography and adjust, accordingly, their special micro-arrangement and secretion of anti-inflammatory cytokines (Fig. 1; Valles *et al.*, 2015). In the living organism, the cells of tissues and organs are surrounded by the extracellular matrix, which is composed of tissue/organ-specific conglomerate of collagen fibers, enzymes, and glycoproteins, which form a composition- and topology- unique, three-dimensional environment (Frantz *et al.,* 2010, Wang *et al.*, 2011). Valles *et al.,* (2015) studied the effect of substrate tri-dimensionality on the interactions of MSCs with the

macrophages. They seeded MSCs on highly porous, synthetic 3D scaffolds made of cross-linked polystyrene, or 2D surface, and co-cultured them with the macrophages in a trans-well insert system. They showed that the 3D microarchitecture of the scaffold drives MSCs to secret a higher level of the anti-inflammatory molecules prostaglandin (PGE_2) and tumor necrosis factor- (TNF) stimulated gene-6 (TSG-6), decreases secretion of the inflammatory and chemotactic factors such as IL-6 and MCP-1 in the co-cultured macrophages, and decreases their migratory activity (Valles *et al.,* 2015). Not only the microarchitecture of the EMC but also its molecular composition has a major immunomodulatory effect on the MSCs and macrophages and their interactions. The ECM's collagen fibers can bind the macrophage receptors and affect the secretion of metalloproteinases. The EMC's glycosaminoglycans such as hyaluronic acid, depending on their molecular weight, can stimulate anti- or pro-inflammatory macrophage phenotype. Additionally, the ECM contains hidden domains, which are similar to cytokines and can be unmasked by metalloproteinase-driven proteolysis. After the unmasking, such cytokine-like molecules can modify the immune response (García-García and Martin, 2019). These findings indicate that the composition and tri-dimensionality of the surrounding enhances the immunoregulatory properties of MSCs and create the anti-inflammatory milieu and underline the importance of the EMC/scaffold-induced mechanisms for tissue regeneration and wound healing (Li *et al.,* 2019; Qiu *et al.*, 2018; García-García and Martin, 2019).

Modulation of macrophages by MSCs

The modulatory effect of MSCs on the macrophages has been described in various disease models (Eggenhofer and Hoogduijn, 2012). The modulatory effect of MSCs on macrophages is partially mediated by prostaglandin E2(PGE-2) and involves reprogramming of the macrophage metabolic and respiratory pathways, which are different in M1 and M2 macrophages (Maggini *et al.,* 2010; Eggenhofer and Hoogduijn, 2012; Vasandan *et al.,* 2016; Chen *et al.,* 2017). Thus, MSCs therapies are often used to treat various diseases and injuries. Studies in a rat myocardial infarction model showed that coculturing the macrophages with the bone marrow-derived stem cells depressed inflammatory macrophage phenotype and upregulated the expression of anti-inflammatory marker Arginase-1 (Arg1). The transplantation of the macrophage-MSC coculture to the hearts of rats with induced myocardial infarction decreased cardiac fibrosis, improved angiogenesis, and increased the number of anti-inflammatory macrophages. Authors suggest that the macrophages primed by MSCs can be applied as the adjuvant for cardiac therapies (Lim *et al*., 2018). Another study, also in the rat myocardial infarction model, showed that the coculture of the bone marrow-derived macrophages with the MSCs suppressed the expression of the inflammatory factors (IL-1β, IL-6, monocyte chemoattractant protein-1, and inducible nitric oxide synthase (iNOS)), and increased the expression of anti-inflammatory factors (IL-4, IL-10, CD206, and Arg1. This study also showed that the macrophages adjacent to the injected into the heart MSCs had much higher expression of anti-inflammatory marker Arg1(Cho *et al*.2014). The modulatory effects of MSCs on the macrophages has been also shown in diabetic cardiomyopathy (DCM). Jin *et al.,* (2019) showed that in the rat DCM model, the MSCs induce macrophage polarization into the anti-inflammatory M2 phenotype via the prostaglandin-endoperoxide synthase (COX-2) and PGE2 pathway and protect against myocardial injury. The immunoregulatory properties of MSCs make them also excellent therapeutic options for rheumatoid arthritis, osteoarthritis, and other autoimmune diseases, and bone and cartilage disorders and cancers (see review in Djouad *et al*., 2009; Berthelot *et al*., 2019).

The MSCs have also beneficial effect on neonatal lung injury caused by hyperoxia in the mouse model by modulating macrophage phenotype and limiting pulmonary fibrosis (Al-Rubaie *et al.,* 2018). Findings such as this, prompted, recently, the idea to use the MSC therapies for the treatment of the acute respiratory distress syndrome (ARDS) in the lungs of COVID-19 patients. Because ARDS is caused by the overreactive immune response of lung macrophages and the MSCs are known to downregulate macrophage inflammatory response, they have been thought to improve or eliminate ARDS. Indeed, several animal studies and early clinical trials established already that MSC therapy is safe and beneficial for the treatment of ARDS (reviewed in Qin and Zhao, 2020). Consequently, there is increasing demand for compassionate MSC infusions in ICU patients with COVID-19-related ARDS (Can and Coskun, 2020).

Routes of information transfer between MSCs and macrophages

The MSC priming and immunomodulatory effects of MSCs on macrophages would not be possible without the transfer of signals and molecules/organelles between the participating cells. There are three main routes for this transfer: 1. paracrine, 2. vesicular, and 3. through the tunneling nanotubes (Fig. 2). The paracrine signaling is the most common type of signaling between the nearby cells and pertains to the exchange of molecules such as cytokines, chemokines, receptor ligands, and growth factors. The vesicular transfer is the transport of molecules, miRNA, or organelles within the membrane-bound extracellular vesicles (EVs) released from the donor cells and endocytosed by the recipient cells. The tunneling nanotubes (TNTs) are very long cell processes that extend between the adjacent cells and the preferred route for the transfer of organelles. Although the existence of the paracrine signaling between MSCs and macrophages has been known for decades, vesicular and nanotube transfers were discovered only recently (Kloc *et al.,* 2016; Kloc and Kubiak, 2017). The transfer of mitochondria from MSCs to macrophages, through the TNTs, in vitro, and in vivo, had been shown to improve mitochondrial functions, ATP turnover, and enhanced macrophage phagocytosis (Jackson *et al*., 2016). This study also showed the transfer of mitochondria via EVs, has a lower efficiency than via TNTs. Because the inflammatory M1 and the anti-inflammatory M2 macrophages differ in the energy production mode (aerobic glycolysis versus oxidative phosphorylation, respectively) and ADP/ATP homeostasis (Chen *et al.,* 2017) it is not hard to imagine that the mitochondrial transfer may, by changing the energy and ATP production/homeostasis pathways, modify the macrophage phenotype. Studies of Phinney *et al.,* (2015) showed that MSCs enclose depolarized mitochondria inside the EVs that are subsequently fused with the recipient macrophages, resulting in improved energy metabolism. They also showed that MSCs produce eEVs (exosomes) containing micro RNA (miRNA) that, suppresses, through the inhibition of the Toll-like receptor pathway, macrophage activation and makes macrophages tolerant

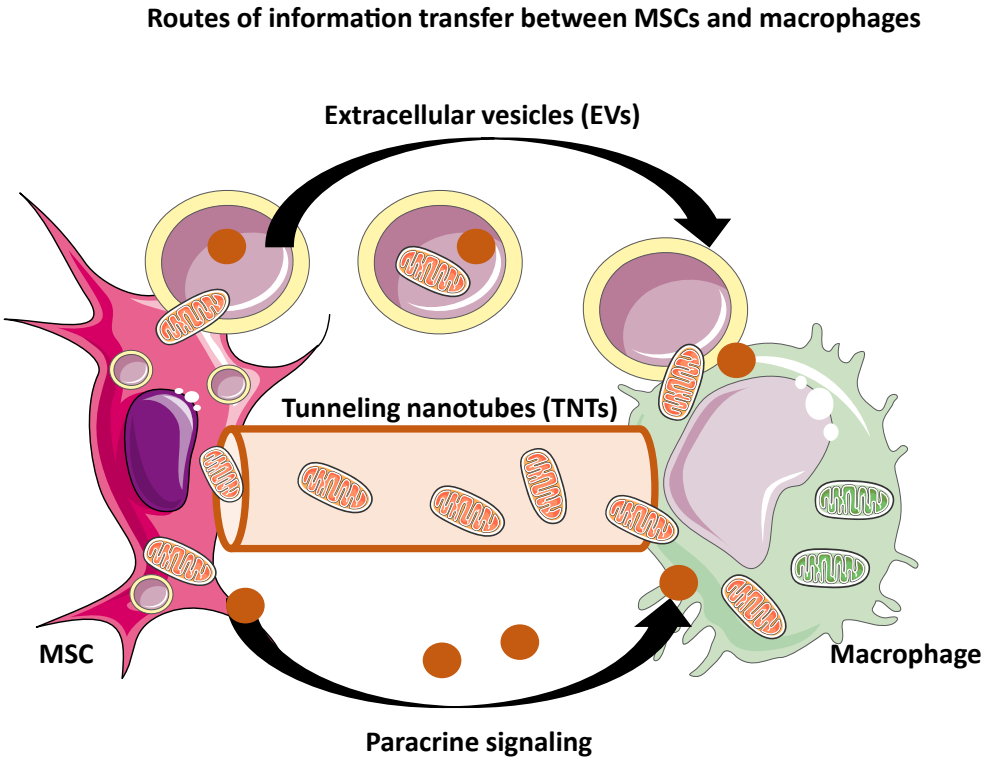

Fig. 2. The routes of information transfer between mesenchymal stem cells (MSCs) and macrophages. *The diagram shows three main routes of information transfer between MSCs and macrophages: 1. paracrine, 2. vesicular, and 3. through the tunneling nanotubes. The paracrine signaling exchanges molecules such as cytokines, chemokines, receptor ligands, and growth factors. The vesicular transport delivers molecules, miRNA, or organelles such as mitochondria within the membrane-bound extracellular vesicles (EVs). The tunneling nanotubes (TNTs) are the preferred routes for the transfer of organelles such as mitochondria.*

to the transferred mitochondria. Islam et. al. (2012) showed that EV mitochondrial transfer from MSCs to the alveolar epithelium protects the lungs from the injury.

It has been shown that the mitochondrial transfer from MSCs to damaged or abnormally functioning cells in the eye, brain, lung, heart, and kidney can repair the recipient cells and /or facilitate regeneration (Paliwal *et al.*, 2018). For example, the mitochondrial transfer via tunnel tubes or EVs, from the bone marrow-derived MSCs to the lung alveoli, bronchial and airway epithelial cells prevented or lessened lung injury, and the transfer to cardiomyocytes or cardiomyoblasts repaired ischemic damage of the myocardium (Paliwal *et al.*, 2018). One of the fascinating questions is what is the signal(s) which initiate the mitochondrial transfer between MSCs and macrophages or other recipient cells. Besides the reactive oxygen species, one of such signals released from the damaged cells is mtDNA. After the mtDNA is endocytosed by the MSCs, it induces replication and biogenesis of mitochondria, which can be subsequently donated to the recipient cells (Mahrouf-Yorgov *et al.,* 2017). The studies on the mitochondrial transfer to corneal epithelial cells showed that the tunneling nanotube formation is mediated by the oxidative inflammation-activated NF-κB/TNFαip2 signaling pathway and that the inhibitor of NF-κB, the SC-514, abrogates nanotube formation (Paliwal *et al.*, 2018; Jiang *et al.*, 2016). Besides the mitochondria, the tunneling nanotubes can also transfer extracellular vesicles containing the immunoregulatory molecules (Kolba *et al.*, 2019). In the murine allergic airway inflammation model, the MSC-derived EVs reduced inflammation of lung tissues. Proteomics analysis showed that the MSC-derived EVs contained 312 proteins known to be involved in the regulation of the inflammation and modulation of pulmonary macrophages (Fang *et al.,* 2020).

All these findings indicate that the transfer of information between the MSCs and the recipient cells can change the outcome of healing and regeneration of the damaged tissue/organs.

In summary, with the increasing demand for the use of stem cell therapies in the clinic, it is extremely important, before designing an efficient therapy for a particular disease, to understand and analyze all possible interactions between MSCs and macrophages and assess how they will affect the therapy outcome.

Acknowledgements

We acknowledge the support from the Polish Ministry of National Defense project "Kościuszko" # 5508/2017/DA to JZK. We also acknowledge that some of the images used to make figures were from the Servier Medical ART: SMART, smart.servier.com.

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