UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCIÊNCIAS

BACHARELADO EM CIÊNCIAS BIOLÓGICAS

ALEXIA NEDEL SANT'ANA

REVISÃO BIBLIOGRÁFICA: EFEITO DE CÉLULAS ESTROMAIS MESENQUIMAIS VIÁVEIS, NÃO-VIÁVEIS, APOPTÓTICAS E SUAS PARTÍCULAS SUBCELULARES SOBRE MONÓCITOS E MACRÓFAGOS

Porto Alegre

2020

ALEXIA NEDEL SANT'ANA

REVISÃO BIBLIOGRÁFICA: EFEITO DE CÉLULAS ESTROMAIS MESENQUIMAIS VIÁVEIS, NÃO-VIÁVEIS, APOPTÓTICAS E SUAS PARTÍCULAS SUBCELULARES SOBRE MONÓCITOS E MACRÓFAGOS

Trabalho de Conclusão de curso apresentado como requisito parcial para obtenção do título de Bacharel em Ciências Biológicas na Universidade Federal do Rio Grande do Sul. Manuscrito formatado segundo as regras editoriais da Revista World Journal of Stem Cells.

Orientador(a): Profa. Dra. Ana Helena da Rosa Paz

Porto Alegre

2020

Name of journal: World Journal of Stem Cells

Manuscript type: Review

Effects of viable, non-viable, apoptotic MSCs and subcellular particles on monocytes and macrophages

Alexia N Sant'Ana, Ana H Paz

Alexia N Sant'Ana, Laboratório de Células Tecidos e Genes, Hospital de Clínicas de Porto Alegre, Brasil.

Ana H Paz, Laboratório de Células Tecidos e Genes, Hospital de Clínicas de Porto Alegre, Departamento de Ciências Morfológicas, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brasil.

Corresponding author: Ana H Paz, PhD, Professor, Laboratório de Células Tecidos e Genes, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, Porto Alegre, RS 90035-903, Brasil. ahpaz@hcpa.edu.br

Abstract

Mesenchymal stromal cells (MSCs) present great potential for cell therapy for autoimmune and inflammatory disorders due to its immunoregulatory and regenerative properties. MSCs modulate the inflammatory milieu by releasing soluble factors and acting through cell-to-cell mechanisms, reducing immune cell activation and function and hence inducing immunosuppression. *In vitro* as well as *in vivo* MSCs switch the classical inflammatory and M1 status of monocytes and macrophages towards a non-classical and anti-inflammatory M2 phenotype. This is characterized by increased anti-inflammatory cytokine secretion, decreased pro-inflammatory cytokine release, changes in cell membrane molecules expression and in metabolic pathways. Besides metabolically active MSCs and their secreted extracellular vesicles, non-viable and apoptotic MSCs or even MSC subcellular particles also exhibit immunosuppressive features that induce a regulatory phenotype in monocytes and macrophages. Indeed, the MSC modulation of monocyte and macrophage phenotype seems to be critical for therapy effectiveness in several disease models, since when these cells are depleted no immunosuppressive effects occur. Thus, here we review the effects of treatment with viable MSCs and MSC extracellular vesicles, further non-viable and apoptotic MSCs and MSC subcellular particles on macrophages and monocytes profile and its implications for immunoregulatory and reparative processes in different experimental models. Further, this work will include mechanisms of action exhibited in these different therapeutic approaches that induce anti-inflammatory properties in monocytes and macrophages.

Key words: Mesenchymal Stromal Cells, MSCs, Macrophage, Monocyte, Immunomodulation.

Core Tip

MSCs and their secreted extracellular vesicles possess immunoregulatory and regenerative properties that shift the classical activation of monocytes and macrophage towards an anti-inflammatory profile, marked by secretion of anti-inflammatory and reparative factors that guide the inflammation resolution and healing processes. Further, non-viable, apoptotic MSCs and subcellular particles without secretory abilities also induce a regulatory phenotype in macrophages and monocytes.

This review will comprise the effects of viable, non-viable, apoptotic MSCs and MSC extracellular vesicles and subcellular microparticles on monocytes and macrophages.

INTRODUCTION

Mesenchymal stromal cells (MSCs) are multipotent, self-renewal stem cells with immunoregulatory and regenerative properties. Found mainly in bone marrow stroma, these non-hematopoietic progenitor cells possess ability to differentiate in cells of mesenchymal origin, such as chondrocytes, osteoblasts, adipocytes, muscle, tendon, endothelial, stromal and neuron cells [1, 2]. In this way, MSCs can be easily isolated from several sources, such as bone marrow, adipose and muscle tissues, trabecular bone, articular cartilage, deciduous teeth and umbilical cord [1, 3]. In these tissues, MSCs maintain homeostasis through offering support to other resident cells [4].

MSCs have been targeted by several investigations due to their immunoregulatory and regenerative capacities. These cells secrete soluble factors like cytokines, chemokines, growth factors, and extracellular vesicles including exosomes and microvesicles that modulate immune cells such as T cells, B cells and monocytic cells which orchestrate inflammatory resolution and regenerative processes [2, 5–7]. Besides that, several published findings demonstrate that MSCs also support immune suppression through cell-to-cell contact [8, 9, 10, 11]. Further, MSCs express low levels of class I major histocompatibility complex (MHC-I) and do not express MHC-II, which make them cells with low immunogenicity and hence low rejection risk [12].

Due to these features, MSCs are great candidates for cell therapy for inflammatory, autoimmune disorders and other clinical conditions. The therapeutic potential of MSCs and their secreted extracellular vesicles has been demonstrated in several *in vitro* studies, animal models and clinical trials [5, 12]. Successful treatment with MSCs are observed in experimental models of lupus [13], colitis [14], diabetes [15], graft-versus-host disease (GvHD) [16], cardiovascular malignancies [17] and pulmonary diseases [18].

Moreover, until the moment of writing this manuscript, 691 ongoing MSC clinical trials at different phases were registered on US National Institutes of Health database, demonstrating therapy advances for GvHD [19], amyotrophic lateral sclerosis [20], rheumatoid arthritis [21], liver cirrhosis [22], acute respiratory distress syndrome [23], diabetes [24], acute myocardial infarction [25], lupus erythematosus [26], Crohn's disease [27], osteoarthritis [28], fibrosis [29], Parkinson's disease [30], cystic fibrosis [31], multiple

sclerosis [32], ulcerative colitis [33], organ transplant rejection [34], and the recent worldwide pandemic Covid-19 [35].

Since previous investigations have shown that MSCs are modulated by the inflammatory milieu and respond specifically to different stimuli, greater therapeutic potential is achieved through MSC priming [36]. MSC activation for improvement in its anti-inflammatory capacity happens through exposure to conditions commonly present at the inflammatory microenvironment, such as hypoxia treatment, which enhance angiogenic properties of MSC secreted extracellular vesicles [37]. Moreover, nutrient deprivation also improves MSC immunoregulation [38]. However, in general, MSC priming step is made *in vitro* before cell administration, with the inflammatory cytokines IFN-γ and TNF-α or Toll-Like Receptor 3 agonists [36, 39, 40]. This stimulation enhances MSC secretion of TSG-6, IL-6 and PGE-2 bioactive factors [40, 41].

Further, MSCs also can be activated by interaction with immune cells. MSC immunoregulatory potential is enhanced in response to macrophage-derived secretome, demonstrated by attenuation of macrophage proinflammatory activity [42, 43]. Besides that, since macrophages and monocytes are present at the inflammatory microenvironment and assume either a pro or anti-inflammatory profile, thereby orchestrating inflammation progression or resolution [44–46], studies investigating the crosstalk between these cells and MSCs are needed to elucidate mechanisms of action of MSC therapy.

In this perspective, biodistribution data further demonstrate that MSCs home to inflammatory sites, where they modulate macrophage and monocyte phenotype [47, 48]. However, after intravenous administration, a significant part of infused MSCs can get trapped in the lungs, leading to emboli formation [48, 49]. Thus, in an attempt to improve therapy efficacy and safety, several studies have explored the immunoregulatory features of MSC extracellular vesicles and microparticles as well as non-viable inactivated MSC as new therapeutic approaches and substitutes for entire and alive MSCs [6, 50–52].

Therefore, this review will focus on alive MSC and MSC extracellular vesicles besides to inactivated MSCs and MSC subcellular microparticles modulation of macrophage and monocyte immunophenotype, activation status and migration, as well as its

implication in inflammation resolution and healing processes in different diseases models. Further, this paper will include mechanisms of action exhibited in these different approaches that induce anti-inflammatory properties in monocytes and macrophages (Fig. 1).

VIABLE MSCs MODULATION ON MACROPHAGES AND MONOCYTES

Cytokine Profile in Monocytes and Macrophages

MSCs as well as their extracellular vesicles can switch the classical inflammatory and M1 status of monocytes and macrophages towards a non-classical anti-inflammatory and M2-like phenotype [53–56]. This monocytic tolerogenic phenotype is characterized by changes in cytokine expression, represented by an increase of anti-inflammatory IL-10 and TGF-β production [57–59] and decrease of inflammatory cytokines TNF-α, IL-1β and IL-6 levels [55, 56, 59, 60].

The shift in macrophage and monocyte cytokine production pattern, mainly marked by regulatory IL-10 upregulation, drives the inflammation resolution and alleviates injury in experimental models of allergic processes [61], colitis [59], and eye autoimmune and inflammatory disorders [57, 62]. Indeed, IL-10 derived from MSC exosomes-preconditioned macrophages induces an inhibitory effect on CD4+ T cells proliferation, indicating different ways in which MSCs exert immunosuppressive effects that include macrophage functions [61].

Furthermore, reducing production of inflammatory mediators like TNF-α and IL-1β have beneficial effects, since these cytokines promote maintenance of inflammation. These bioactive factors are involved in recruitment of inflammatory cells, induction of apoptosis and destructive enzymes release, such as metalloproteinases that lead to tissue degeneration. In addition, TNF-α facilitates autoimmunity by inhibiting T regulatory cells [63, 64]. Thus, MSC immunosuppressive action on monocytic cells contributes to tissue damage reduction.

Membrane molecules expression

MSC immunoregulatory effect on macrophages is also demonstrated by modulation of cell membrane protein expression. Mice and human macrophages in co-culture with MSCs diminished the expression of co-stimulatory molecule CD86 and increased mannose receptor CD206, well known markers of M1 and M2 polarization, respectively [65, 66]. These changes are also observed in *in vivo* mouse models of cutaneous wound healing, myocardial infarction, and diabetic cornea, since MSC transplantation decreased CD86+ macrophages, while increased anti-inflammatory CD163+ and CD206+ macrophages count [55, 67, 68]. Likewise, MSC exosomes and extracellular vesicles induce the same expression pattern of markers of M2 phenotype, both *in vitro* and *in vivo* [37, 55, 59, 69]. Further, M1 activated macrophages cultured with MSCs decreased the expression of cell surface molecules CD80, CD86, CD50, CD54, HLA-DR and HLA-ABC. This indicates immunosuppression through reduction in antigen presentation, since these membrane proteins are involved in this process [70].

Also noteworthy, co-culture of MSCs with the three human monocyte subsets, classical (CD14++CD16-), non-classical (CD14+CD16++) and intermediate monocytes (CD14++CD16+) reduces class II antigen presentation complex (HLA-DR) expression, while upregulates *MRC1*, *CD163*, *CD163L1*, *CD226*, *CD93*, *LILRB1* and *PTGER2* membrane receptors genes. *PTGER2* encodes EP2 receptor, activated through MSC-secreted PGE2, one of MSC bioactive factors. Meanwhile, there is upregulation of monocyte cytokines and growth factors genes, such as *IL-10*, *IGF1* and *VEGF-A.* Not by chance, IL-10 production is induced through MSC-derived PGE2, which results in reduced inflammation. This expression profile along with CD14 upregulation shows that MSC altered maturation of this monocyte subsets toward a M2 macrophage immunosuppressive phenotype [54].

Metabolic changes

The MSC induced M1-M2 phenotype switch is also accompanied by metabolic alterations. MSCs impair monocyte differentiation into antigen presenting dendritic cells through metabolic reprogramming. Monocytes, instead of assuming the antigen presentation profile, show a transcriptional and phenotypic profile of M2 macrophages

that induce a Th2 regulatory cytokine pattern in CD4+ T cells. In addition, these cells acquire higher spare respiratory capacity and more polarized mitochondrial membrane potential, resulting in better capacity of stimuli response in case of high energy demand [53]. In the same way, monocyte derived macrophages co-cultured with MSCs had increased mitochondrial function and ATP turnover, which allowed greater macrophage phagocytosis and antimicrobial ability. These results were demonstrated *in vitro* as well as *in vivo* using *E. coli* pneumonia mice, which when treated with MSCs showed ameliorated inflammatory parameters [18].

Importantly, macrophage conditioned with MSC or MSC-exosomes increased its oxygen consumption rate while decreased proton leak, indicating enhanced bioenergetics and mitochondrial coupling efficiency. In the same work, macrophage challenged with silica particles demonstrated homeostasis alterations highlighted by mitochondrial production of reactive oxygen species, which was reverted by MSC-exosomes [71]. On the other hand, *Salmonella* infected macrophages co-cultured with MSC had respiratory burst improvements. This was demonstrated by enhanced expression of NADPH oxidase subunits, concomitant with activation of antioxidant protection mechanisms like superoxide dismutase 2 (SOD2). These data, along with faster microbial clearance by macrophage promoted in MSC co-culture, indicates that these macrophage metabolic changes enhance its ability to respond to pathogens [70].

Besides improving macrophage and monocyte antimicrobial ability, MSC- induced metabolic changes modify macrophage energy generation pathways, while promotes transition towards M2 phenotype. Since M1 activated macrophages demand high energy, they have augmented expression of glucose transporter 1 (GLUT1), Hexokinase 2 (HK2) and mTOR, proteins needed in the glycolytic pathway. On the other hand, M2 macrophages exhibit preference for mitochondrial fatty acids β-oxidation, demonstrated by higher expression of Carnitine palmitoyl trasferase1α (CPT1α) enzyme and phosphorylated AMPKα (p-AMPKα). In this way, M1 macrophage-MSC co-culture reduced GLUT1 and HK2 expression and p-mTOR levels, while increased CPT1α expression and p-AMPKα levels, pointing to changes in energy metabolism underlying the MSC-induced M2 phenotype [70].

Regarding to amino acid metabolism, macrophage augment expression and proteins levels of arginase-1 and reduce inducible-nitric oxide synthase (iNOS) in response to

MSC or MSC exosomes [55, 68, 72, 73]. These enzymes are responsible for L-arginine metabolism, with arginase and iNOS enzymes competing for this substrate to convert into urea and ornithine or nitric oxide (NO), respectively. NO participate in macrophage anti microbicidal and effector functions, while ornithine is a polyamine necessary for cell proliferation and tissue remodelling action of M2 macrophages [44]. The balance between their activities indicates M1 or M2 polarization, and macrophage co-cultured with MSCs exhibit decreased NO production besides increased urea levels, which indicate the regenerative and resolutive phenotype typical of M2 polarization [73].

Migration and recruitment

MSCs also modulate macrophage and monocyte migratory behavior. *In vitro*, macrophage and monocyte actively migrate toward MSCs [74], and *in vivo*, they are recruited to lungs where they encounter MSCs after intravenous infusion [75]. In a mouse model of Coxsackievirus B3-induced myocarditis, MSCs recruit anti-inflammatory LyC6low monocytes to the inflammation site, whilst decrease proinflammatory LyC6high and LyC6middle monocytes levels. This regulation occurs through modulation of local chemokines, reducing levels of MCP-1 (CCL2), MCP-3 (CCL7) and CCL5 and abrogating ICAM-1 and VCAM-1 adhesion molecules expression, while increasing SDF-1α and CX3CL1 levels. The migration of anti-inflammatory monocyte subset helps tissue repair and leads to reduction of myocarditis severity [76]. Also noteworthy, MSC-exosomes intravenous infusion in experimental models of pulmonary fibrosis in mice also diminish proinflammatory Ly6Chigh monocytes recruitment, whereas increases alveolar macrophages and infiltrating anti-inflammatory monocytes. These changes are accompanied by reduction in fibrosis measurements, in agreement with the monocyte reparative profile [71, 77].

Moreover, administration of MSCs and MSCs conditioned medium in mice with angiotensin II-induced aortic aneurysm increased CD206+ M2 macrophages infiltration while diminished iNOS+ M1 cells at the injured site, which was concomitant with decreased levels of CCL5, CCL2, CCL3 and CXCL10 [60]. Accordingly, MCP-1 (CCL2) expression was reduced in pancreatic islets of type 2 diabetic mice infused

with MSCs compared to the non-treated group. This reduction did not alter macrophage amount, but induced M2 polarization and decreased the inflammation levels [42].

On the other hand, previous reports showed that MSC administration in mice increased MCP-1 (CCL2) levels, which recruited monocytic cells to lungs via CCL2-CCR2 axis. But consistently, after migration, monocyte and macrophage were modulated by MSCs and assumed an IL-10 producing phenotype [61, 62]. Similarly, in a mouse model of cardiotoxin-induced skeletal muscle injury, hypoxia-subjected MSC-extracellular vesicles treatment increased the expression of *MCP-1* (CCL2) and the CD206/Ly6c cells ratio when compared to normoxia-derived extracellular vesicles and control groups, indicating M2 polarization [37].

Furthermore, type 2 diabetic mice showed augmented M2 macrophage counts in liver, adipose tissue, skeletal muscle, pancreatic islands and spleen after MSC intravenous infusion. This, concomitant with a greater engraftment of administered MSC in spleen, brings up the possibility that MSC directly modulate macrophage and monocyte populations in immune organs, which could lead to systemic effects [47]. In fact, myocarditis mice treated with MSCs retain more pro-inflammatory monocytes in the spleen when compared to the control group, and recruit more anti-inflammatory monocytes to the heart, which improve healing processes and reduce inflammation [76].

In summary, despite the different triggered pathways and chemokine regulation for monocyte and macrophage recruitment, several investigations indicate that MSC treatment induces monocyte and macrophage migration to the inflammation site or to immune organs. Once there, MSCs modulate the cell activation status and profile, promoting a monocytic anti-inflammatory phenotype and hence a reparative milieu.

Mechanisms of action

The mechanisms underlying the MSC immunoregulatory capacities are still under investigation, but one of the most reported processes is induced-M2 polarization through secretion of soluble factors, like TSG-6, lactate, TGFβ, PGE-2 and IL-6 [40, 53,

[57, 65, 70]. Abrogation of several of these bioactive factors prevented the MSC induced macrophage M2 polarization and immunosuppressive effects [41, 42, 53, 70].

In addition to paracrine action, MSCs are phagocytized by monocytic cells in an active process. After phagocytosis, monocytes migrate to other body sites carrying the regulatory properties of MSCs, and macrophage acquire an immunosuppressive phenotype [74, 78].

Besides that, organelles transfer is another mechanism triggered by MSCs that enhances macrophage functions. *In vitro* and *in vivo* assays report that MSCs transfer mitochondria to macrophage through formation of a cytoplasmic bridge named tunnelling nanotubes and exosomes, which improves macrophage phagocytic ability and bioenergetics [18, 71].

Further, MSCs can exert immunomodulatory effects through microRNA transference [71]. The macrophage M2 phenotype promoted by MSC exosomes treatment are, at least in part, dependent of miR-182 and miR-181 post transcriptional control of TLR4, and subsequent downregulation of TLR4 downstream NF-κB signalling cascade [55, 79]. Besides, inhibition of TLR4/NF-κB activation is also triggered by MSC-exosome derived let-7b miRNA, while it induces STAT3/AKT signalling. The enhanced expression of the last molecules in the healing wound site demonstrate that regulation of these signalling pathways in macrophages promotes the M2 phenotype with reparative properties [80].

Also noteworthy, miR147 derived from MSC extracellular vesicles was found to decrease macrophage activation via diminishing HMBG-1 secretion [81]. Moreover, Bei Li et al (2019) reported that macrophage M2 polarization was associated with MSC exosome-derived miR-223 and consequently decreasing in pknox1 protein levels [82]. Interestingly, miR-223 also was shown to reduce levels of NLRP3, a protein of inflammasome complex, which activation leads to inflammatory cytokine release and exacerbation of inflammation in inflammatory bowel disease (IBD) [83].

In the same way, MSC treatment induced YAP and β-catenin association to negatively control XBP1-mediated NLRP3 activation, demonstrating that MSCs regulate macrophage M2 polarization through Hippo signalling and consequent repression of inflammasome activation [72]. Also, MSCs can suppress NLRP3 inflammasome-

mediated IL-1β production by macrophages through a feedback mechanism, where IL-1β may induce COX-2 signalling in MSCs [66].

NON-VIABLE MSCS AND SUBCELLULAR PARTICLES MODULATION ON MACROPHAGES AND MONOCYTES

Modulation of macrophage and monocyte by non-viable MSC or MSC subcellular particles that lack any secretory capacity is an emerging issue of interest to researchers, since these investigations can contribute to understanding MSC immunomodulatory mechanisms independent of its secreted soluble factors. In addition, despite some studies had shown that MSCs home to the injured site [47, 84], other experimental models of MSC infusion demonstrate that a great portion of MSCs get trapped in the lung capillaries and lose viability after 24 hours [48, 49, 84]. Nevertheless, the MSC immunoregulatory effect is maintained, raising questions about how MSCs still reduce local and systemic inflammation.

These questions bring up the hypothesis that MSCs transfer their immunomodulatory properties to other host cells that can act to decrease the inflammatory parameters. Besides, tracking studies demonstrate that the MSC signal found in inflammation site, organs and blood after intravenous administration is due to phagocytized MSC debris by immune cells such as monocytes, instead of viable MSCs [49, 74]. Thus, even inactivated or dead MSCs could trigger immunoregulation without being metabolically active. Further, since MSCs do not reach the inflammation site due to pulmonary barrier [49], MSC membrane particles treatment could overcome this issue.

In this way, studies have found that heat-inactivated MSC and MSC membrane microparticles without any cargo decrease the proportion of pro-inflammatory CD16+ monocytes by inducing its apoptosis [52, 85], and MSC membrane microparticles increase CD90+ and PDL1+ monocyte populations [52]. Moreover, monocytes conditioned with MSC membrane particles had enhanced indoleamine 2,3-dioxygenase (IDO) expression [52]. These molecules are anti-inflammatory factors, since programmed death ligand 1 (PDL1) is an immune checkpoint protein that inhibits activation and function of its target PD1 expressing immune cells, suppressing immune reactivity [86]. In addition, IDO is an enzyme that depletes the essential amino

acid tryptophan, generates kynurenine pathway metabolites, and these metabolic changes hence contribute to immune regulation [87]. Authors demonstrated that MSC membrane particles bind to monocyte membrane, which demonstrate the importance of cell surface molecules to MSC-induced immunosuppression [52]. Further, MSC membrane particles maintain CD73 enzymatic activity at the surface, which degrades AMP to adenosine, a molecule that possess immunoregulatory functions through P1 receptors activation [52, 88]. Importantly, activation of monocytes P1 receptors such as A_{2A} and A_{2B} inhibited TNF-α production [88].

Moreover, as living cells, secretome-deficient heat-inactivated MSCs also disappear after 24 hours of infusion in healthy mice and in an experimental model of kidney ischemia/reperfusion injury [51]. Despite the fast clearance, administration of heat inactivated MSCs still altered the expression levels of several cytokines and chemokines in serum and lungs, and reduced LPS-induced sepsis [51]. In line with that, *in vitro* assays demonstrated that secretome-deficient heat-inactivated MSCs modulate monocytes through reducing TNF-α production [51, 85]. This modulation occurs through phagocytosis of heat inactivated MSCs, and recognition of heat inactivated MSCs by monocytes were even more efficient than for control MSCs [85]. Besides, supernatant of LPS-stimulated macrophages that phagocytized dead MSCs improved hypoxic cardiomyocytes survival [89]. After phagocytosis macrophage augmented secretion of PGE-2, VEGF-α, KGF, IGF-1 and PDGF-BB reparative molecules, while decreased TNF-α, IFN-γ, IL-12, IL-6 production [89]. Together, these data suggest that for some disease models, monocytes that had phagocytized inactivated MSCs acquire their immunoregulatory properties and reduce inflammation.

Another therapeutic approach consists in administration of apoptotic MSCs, since it was demonstrated that to obtain therapy effectiveness in some diseases such as GvHD, there is need for inducing MSC cell death by host cytotoxic cells [90]. Thus, administration of *in vitro* produced apoptotic MSCs in GvHD mice induced IDO expression in recipient macrophages that had phagocytized the infused cells, which incited immunosuppression [90].

These new MSC-derived alternative therapies bring some advantages. Using non-viable MSCs ensure that the administered product is the same as it was before infusion, since once it is inside the target organism, these cells do not proliferate or

secrete any molecules in response to nonspecific host signals. Further, due to their small size, MSC membrane particles can pass the lung capillaries and reach other sites throughout the body, avoiding problems such as emboli formation, induced by administration of intact MSCs.

Effects of non-viable and apoptotic MSCs on macrophage and monocytes are still under investigation. Besides that, the mechanisms of action exhibited by these cells are not fully understood, but MSC phagocytosis by monocyte and macrophage seems to be essential for the systemic effects of inactivated and apoptotic MSC therapy [90]. Further, interaction between cell membranes may have an important role [52]. However, future studies will be necessary to reveal the possible interactions between non-viable MSCs and macrophage or monocytes *in vivo*, and its implication in treatment results.

CONCLUSION

Macrophage and monocyte interaction with either viable or non-viable MSCs seems to be critical for therapy effectiveness, since when these cells are depleted in several models of inflammatory diseases, or prevented to migrate into the local of inflammation, no immunosuppressive effects or benefits occur [61, 55, 62, 90]. As discussed, these immunosuppressive effects are mainly due to the induced shift toward M2 anti-inflammatory phenotype of monocytes and macrophages, by viable, non-viable, apoptotic MSCs and their subcellular particles.

This modulation of monocytes and macrophages by MSCs occurs through different ways and complex mechanisms, such as secreted soluble factors, mitochondria and micro-RNAs transfer and phagocytosis of MSC. In addition, emergence of different therapeutic approaches using non-viable MSCs and MSC membrane particles brings up the need for investigation of its immunomodulatory mechanisms. Nevertheless, MSC phagocytosis by monocyte and macrophage also is observed, and surface molecules interaction between MSC membrane particles and these monocytic cells seems to be important.

Here, we discussed the effects of viable, non-viable and apoptotic MSCs, as well as their secretome and subcellular particles on monocytes and macrophages (Fig. 1). In

summary, monocytes and macrophages can acquire the immunomodulatory features of MSCs, and this regulatory action seems to be crucial for therapy success in several clinical conditions.

REFERENCES

1. **Chen FH**, Rousche KT, Tuan RS. Technology Insight: adult stem cells in cartilage regeneration and tissue engineering. *Nat Clin Pract Rheumatol*. 2006; 2(7): 373–382. [PMID: 16932723 DOI: 10.1038/ncprheum0216]
2. **Samsonraj RM**, Raghunath M, Nurcombe V, Hui JH, van Wijnen AJ, Cool SM. Concise review: multifaceted characterization of human mesenchymal stem cells for use in regenerative medicine. *Stem Cells Transl Med*. 2017; 6(12): 2173–2185. [PMID: 29076267 DOI: 10.1002/sctm.17-0129]
3. **Romanov YA**, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells*. 2003; 21(1): 105–110. [PMID: 12529557 DOI: 10.1634/stemcells.21-1-105]
4. **Shi Y**, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. *Nat Rev Nephrol*. 2018; 14(8): 493–507. [PMID: 29895977 DOI: 10.1038/s41581-018-0023-5]
5. **Li N**, Hua J. Interactions between mesenchymal stem cells and the immune system. *Cell Mol Life Sci*. 2017; 74(13): 2345–2360. [PMID: 28214990 DOI: 10.1007/s00018-017-2473-5]
6. **Keshtkar S**, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. *Stem Cell Res Ther*. 2018; 9(1): 63. [PMID: 29523213 DOI: 10.1186/s13287-018-0791-7]
7. **Carreras-Planella L**, Monguió-Tortajada M, Borràs FE, Franquesa M. Immunomodulatory effect of MSC on B cells is independent of secreted extracellular vesicles. *Front Immunol*. 2019; 10:1288. [PMID: 31244839 DOI: 10.3389/fimmu.2019.01288]
8. **Luz-Crawford P**, Noël D, Fernandez X, Khoury M, Figueroa F, Carrión F, et al. Mesenchymal stem cells repress Th17 molecular program through the PD-1 pathway. *PLoS ONE*. 2012; 7(9): 45272. [PMID: 23028899 DOI: 10.1371/journal.pone.0045272]
9. **Duffy MM**, Pindjakova J, Hanley SA, McCarthy C, Weidhofer GA, Sweeney EM, et al. Mesenchymal stem cell inhibition of T-helper 17 cell- differentiation is triggered by cell-cell contact and mediated by prostaglandin E2 via the EP4 receptor. *Eur J Immunol*. 2011; 41(10): 2840–2851. [PMID: 21710489 DOI: 10.1002/eji.201141499]
10. **English K**, Ryan JM, Tobin L, Murphy MJ, Barry FP, Mahon BP. Cell contact, prostaglandin E(2) and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25(High) forkhead box P3+ regulatory T cells. *Clin Exp Immunol*. 2009; 156(1): 149–160. [PMID: 19210524 DOI: 10.1111/j.1365-2249.2009.03874.x]

11. **Li Y**, Zhang D, Xu L, Dong L, Zheng J, Lin Y, et al. Cell-cell contact with proinflammatory macrophages enhances the immunotherapeutic effect of mesenchymal stem cells in two abortion models. *Cell Mol Immunol*. 2019; 16(12): 908–920. [PMID: 30778166 DOI: 10.1038/s41423-019-0204-6]

12. **Squillaro T**, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. *Cell Transplant*. 2016; 25(5): 829–848. [PMID: 26423725 DOI: 10.3727/096368915X689622]

13. **Zhang Z**, Niu L, Tang X, Feng R, Yao G, Chen W, et al. Mesenchymal stem cells prevent podocyte injury in lupus-prone B6.MRL-Faslpr mice via polarizing macrophage into an anti-inflammatory phenotype. *Nephrol Dial Transplant*. 2019; 34(4): 597–605. [PMID: 29982691 DOI: 10.1093/ndt/gfy195]

14. **Alves VBF**, de Sousa BC, Fonseca MTC, Ogata H, Caliári-Oliveira C, Yaochite JNU, et al. A single administration of human adipose tissue-derived mesenchymal stromal cells (MSC) induces durable and sustained long-term regulation of inflammatory response in experimental colitis. *Clin Exp Immunol*. 2019; 196(2): 139–154. [PMID: 30663040 DOI: 10.1111/cei.13262]

15. **Sun X**, Hao H, Han Q, Song X, Liu J, Dong L, et al. Human umbilical cord-derived mesenchymal stem cells ameliorate insulin resistance by suppressing NLRP3 inflammasome-mediated inflammation in type 2 diabetes rats. *Stem Cell Res Ther*. 2017; 8(1): 241. [PMID: 29096724 DOI: 10.1186/s13287-017-0668-1]

16. **Fujii S**, Miura Y, Fujishiro A, Shindo T, Shimazu Y, Hirai H, et al. Graft-Versus-Host Disease Amelioration by Human Bone Marrow Mesenchymal Stromal/Stem Cell-Derived Extracellular Vesicles Is Associated with Peripheral Preservation of Naive T Cell Populations. *Stem Cells*. 2018; 36(3): 434–445. [PMID: 29239062 DOI: 10.1002/stem.2759]

17. **Huang P**, Wang L, Li Q, Xu J, Xu J, Xiong Y, et al. Combinatorial treatment of acute myocardial infarction using stem cells and their derived exosomes resulted in improved heart performance. *Stem Cell Res Ther*. 2019; 10(1): 300. [PMID: 31601262 DOI: 10.1186/s13287-019-1353-3]

18. **Jackson MV**, Morrison TJ, Doherty DF, McAuley DF, Matthay MA, Kissenpfennig A, et al. Mitochondrial transfer via tunneling nanotubes is an important mechanism by which mesenchymal stem cells enhance macrophage phagocytosis in the in vitro and in vivo models of ARDS. *Stem Cells*. 2016; 34(8): 2210–2223. [PMID: 27059413 DOI: 10.1002/stem.2372]

19. ClinicalTrials.gov [Internet]. National Institutes of Health Clinical Center, Bethesda, Maryland, United States of America: U. S. National Library of Medicine. 2015 March 5. Identifier NCT02379442. Early Treatment of Acute Graft Versus Host Disease With Bone Marrow-Derived Mesenchymal Stem Cells and Corticosteroids [cited 2020 Oct 20]. Available from: https://clinicaltrials.gov/ct2/show/NCT02379442?term=MSC+cell+therapy&recr

s=deh&draw=2

20. ClinicalTrials.gov [Internet]. Brainstorm-Cell Therapeutics, New York, United States of America: U. S. National Library of Medicine. 2013 December 23. Identifier NCT02017912. Phase 2, Randomized, Double Blind, Placebo Controlled Multicenter Study of Autologous MSC-NTF Cells in Patients With ALS [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT02017912?term=MSC+cell+therapy&recrs=deh&draw=5

21. ClinicalTrials.gov [Internet]. Kang Stem Biotech Co., Ltd, South Korea: U. S. National Library of Medicine. 2014 August 20. Identifier NCT02221258. Safety of FURESTEM-RA Inj. in Patients With Moderate to Severe Rheumatoid Arthritis(RA) [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT02221258?term=MSC+cell+therapy&recrs=deh&draw=3

22. ClinicalTrials.gov [Internet]. Beijing 302 Hospital, Beijing, China: U. S. National Library of Medicine. 2010 October 14. Identifier NCT01220492. Umbilical Cord Mesenchymal Stem Cells for Patients With Liver Cirrhosis [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT01220492?term=MSC+cell+therapy&recrs=deh&draw=2

23. ClinicalTrials.gov [Internet]. University of California, San Francisco, California, United States of America: U. S. National Library of Medicine. 2013 January 25. Identifier NCT01775774. Human Mesenchymal Stem Cells For Acute Respiratory Distress Syndrome [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT01775774?term=MSC+cell+therapy&recrs=deh&draw=3

24. ClinicalTrials.gov [Internet]. Royan Institute for Stem Cells Biology & Technology, Tehran, Iran: U. S. National Library of Medicine. 2019 September 6. Identifier NCT04078308. Mesenchymal Stem Cells Transplantation in Newly Diagnosed Type-1 Diabetes Patients [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT04078308?term=MSC+cell+therapy&recrs=deh&draw=2

25. ClinicalTrials.gov [Internet]. Yonsei University, Seoul, South Korea: U. S. National Library of Medicine. 2011 July 12. Identifier NCT01392105. Safety and Efficacy of Intracoronary Adult Human Mesenchymal Stem Cells After Acute Myocardial Infarction [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT01392105?term=MSC+cell+therapy&recrs=deh&draw=2&rank=23

26. ClinicalTrials.gov [Internet]. Yan'an Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China: U. S. National Library of Medicine. 2020 March 24. Identifier - NCT04318600. Allogeneic Amniotic Mesenchymal Stem

Cell Therapy for Lupus Nephritis [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT04318600?term=MSC+cell+therapy&recrs=deh&draw=5&rank=9

27. ClinicalTrials.gov [Internet]. Royal Perth Hospital, Perth, Australia: U. S. National Library of Medicine. 2010 March 23. Identifier NCT01090817. An Australian Study of Mesenchymal Stromal Cells for Crohn's Disease [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT01090817?term=MSC+cell+therapy&recrs=deh&draw=2&rank=15

28. ClinicalTrials.gov [Internet]. Institute of Tissular Regenerative Therapy, Barcelona, Spain: U. S. National Library of Medicine. 2010 October 35. Identifier NCT01227694. Adult Stem Cell Therapy for Repairing Articular Cartilage in Gonarthrosis [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT01227694?term=MSC+cell+therapy&recrs=deh&draw=2&rank=6

29. ClinicalTrials.gov [Internet]. Royan Institute, Tehran, Iran: U. S. National Library of Medicine. 2011 October 19. Identifier NCT01454336. Transplantation of Autologous Mesenchymal Stem Cell in Decompensate Cirrhotic Patients With Pioglitazone [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT01454336?term=MSC+cell+therapy&recrs=deh&draw=2

30. ClinicalTrials.gov [Internet]. The University of Texas Health Science Center, Houston, Texas, United States of America: U. S. National Library of Medicine. 2015 November 20. Identifier NCT02611167. Allogeneic Bone Marrow-Derived Mesenchymal Stem Cell Therapy for Idiopathic Parkinson's Disease [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT02611167?term=MSC+cell+therapy&recrs=deh&draw=2&rank=

31. ClinicalTrials.gov [Internet]. University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States of America: U. S. National Library of Medicine. 2016 August 15. Identifier NCT02866721. Safety and Tolerability Study of Allogeneic Mesenchymal Stem Cell Infusion in Adults With Cystic Fibrosis [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT02866721?term=MSC+cell+therapy&recrs=deh&draw=3

32. ClinicalTrials.gov [Internet]. University of Jordan, Amman, Jordan: U. S. National Library of Medicine. 2017 October 31. Identifier NCT03326505. Allogenic Mesenchymal Stem Cells And Physical Therapy for MS Treatment [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT03326505?term=MSC+cell+therapy&recrs=deh&draw=2&rank=4

33. ClinicalTrials.gov [Internet]. Children's National Research Institute, Washington, D.C., Michigan, United States of America: U. S. National Library of Medicine. 2014 May 30. Identifier NCT02150551. Safety and Tolerability Of Allogeneic Mesenchymal Stromal Cells in Pediatric Inflammatory Bowel Disease [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT02150551?term=MSC+cell+therapy&recrs=deh&draw=

34. ClinicalTrials.gov [Internet]. Leiden University Medical Center, Leiden, Netherlands: U. S. National Library of Medicine. 2014 February 7. Identifier NCT02057965. Mesenchymal Stromal Cell Therapy in Renal Recipients [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT02057965?term=MSC+cell+therapy&recrs=deh&draw=2&rank=2

35. ClinicalTrials.gov [Internet]. Beijing 302 Hospital, Beijing, China: U. S. National Library of Medicine. 2020 February 28. Identifier NCT04288102. Treatment With Human Umbilical Cord-derived Mesenchymal Stem Cells for Severe Corona Virus Disease 2019 (COVID-19) [cited 2020 Oct 21]. Available from: https://clinicaltrials.gov/ct2/show/NCT04288102?term=MSC+cell+therapy&recrs=deh&draw=2

36. **Rodriguez LA**, Mohammadipoor A, Alvarado L, Kamucheka RM, Asher AM, Cancio LC, et al. Preconditioning in an inflammatory milieu augments the immunotherapeutic function of mesenchymal stromal cells. *Cells*. 2019; 8(5): 462. [PMID: 31096722 DOI: 10.3390/cells8050462]

37. **Lo Sicco C**, Reverberi D, Balbi C, Ulivi V, Principi E, Pascucci L, et al. Mesenchymal Stem Cell-Derived Extracellular Vesicles as Mediators of Anti-Inflammatory Effects: Endorsement of Macrophage Polarization. *Stem Cells Transl Med*. 2017; 6(3): 1018–1028. [PMID: 28186708 DOI: 10.1002/sctm.16-0363]

38. **Furuhashi K**, Tsuboi N, Shimizu A, Katsuno T, Kim H, Saka Y, et al. Serum-starved adipose-derived stromal cells ameliorate crescentic GN by promoting immunoregulatory macrophages. *J Am Soc Nephrol*. 2013; 24(4): 587–603. [PMID: 23471196 DOI: 10.1681/ASN.2012030264]

39. **Monsel A**, Zhu Y, Gennai S, Hao Q, Hu S, Rouby J-J, et al. Therapeutic Effects of Human Mesenchymal Stem Cell-derived Microvesicles in Severe Pneumonia in Mice. *Am J Respir Crit Care Med*. 2015; 192(3): 324–336. [PMID: 26067592 DOI: 10.1164/rccm.201410-1765OC]

40. **Philipp D**, Suhr L, Wahlers T, Choi Y-H, Paunel-Görgülü A. Preconditioning of bone marrow-derived mesenchymal stem cells highly strengthens their potential to promote IL-6-dependent M2b polarization. *Stem Cell Res Ther*. 2018; 9(1): 286. [PMID: 30359316 DOI: 10.1186/s13287-018-1039-2]

41. **Song W-J**, Li Q, Ryu M-O, Ahn J-O, Ha Bhang D, Chan Jung Y, et al. TSG-6

Secreted by Human Adipose Tissue-derived Mesenchymal Stem Cells Ameliorates DSS-induced colitis by Inducing M2 Macrophage Polarization in Mice. *Sci Rep*. 2017; 7(1): 5187. [PMID: 28701721 DOI: 10.1038/s41598-017-04766-7]

42. **Yin Y**, Hao H, Cheng Y, Zang L, Liu J, Gao J, et al. Human umbilical cord-derived mesenchymal stem cells direct macrophage polarization to alleviate pancreatic islets dysfunction in type 2 diabetic mice. *Cell Death Dis*. 2018; 9(7): 760. [PMID: 29988034 DOI: 10.1038/s41419-018-0801-9]

43. **Saldaña L**, Bensiamar F, Vallés G, Mancebo FJ, García-Rey E, Vilaboa N. Immunoregulatory potential of mesenchymal stem cells following activation by macrophage-derived soluble factors. *Stem Cell Res Ther*. 2019; 10(1): 58. [PMID: 30760316 DOI: 10.1186/s13287-019-1156-6]

44. **Mantovani A**, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *J Pathol*. 2013; 229(2): 176–185. [PMID: 23096265 DOI: 10.1002/path.4133]

45. **Guilliams M**, Mildner A, Yona S. Developmental and functional heterogeneity of monocytes. *Immunity*. 2018; 49(4): 595–613. [PMID: 30332628 DOI: 10.1016/j.immuni.2018.10.005].

46. **Shapouri-Moghaddam A**, Mohammadian S, Vazini H, Taghadosi M, Esmaeili S-A, Mardani F, et al. Macrophage plasticity, polarization, and function in health and disease. *J Cell Physiol*. 2018; 233(9): 6425–6440. [PMID: 29319160 DOI: 10.1002/jcp.26429]

47. **Yin Y**, Hao H, Cheng Y, Gao J, Liu J, Xie Z, et al. The homing of human umbilical cord-derived mesenchymal stem cells and the subsequent modulation of macrophage polarization in type 2 diabetic mice. *Int Immunopharmacol*. 2018; 60: 235–245. [PMID: 29778021 DOI: 10.1016/j.intimp.2018.04.051]

48. **Leibacher J**, Henschler R. Biodistribution, migration and homing of systemically applied mesenchymal stem/stromal cells. *Stem Cell Res Ther*. 2016; 7:7. [PMID: 26753925 DOI: 10.1186/s13287-015-0271-2]

49. **Eggenhofer E**, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. *Front Immunol*. 2012; 3:297. [PMID: 23056000 DOI: 10.3389/fimmu.2012.00297]

50. **Riazifar M**, Mohammadi MR, Pone EJ, Yeri A, Lässer C, Segaliny AI, et al. Stem Cell-Derived Exosomes as Nanotherapeutics for Autoimmune and Neurodegenerative Disorders. *ACS Nano*. 2019; 13(6): 6670–6688. [PMID: 31117376 DOI: 10.1021/acsnano.9b01004]

51. **Luk F**, de Witte SFH, Korevaar SS, Roemeling-van Rhijn M, Franquesa M, Strini T, et al. Inactivated mesenchymal stem cells maintain immunomodulatory capacity. *Stem Cells Dev*. 2016; 25(18): 1342–1354. [PMID: 27349989 DOI:

10.1089/scd.2016.0068]

52. **Gonçalves F da C**, Luk F, Korevaar SS, Bouzid R, Paz AH, López-Iglesias C, et al. Membrane particles generated from mesenchymal stromal cells modulate immune responses by selective targeting of pro-inflammatory monocytes. *Sci Rep*. 2017; 7(1): 12100. [PMID: 28935974 DOI: 10.1038/s41598-017-12121-z]

53. **Selleri S**, Bifsha P, Civini S, Pacelli C, Dieng MM, Lemieux W, et al. Human mesenchymal stromal cell-secreted lactate induces M2-macrophage differentiation by metabolic reprogramming. *Oncotarget*. 2016; 7(21): 30193–30210. [PMID: 27070086 DOI: 10.18632/oncotarget.8623]

54. **Wise AF**, Williams TM, Rudd S, Wells CA, Kerr PG, Ricardo SD. Human mesenchymal stem cells alter the gene profile of monocytes from patients with Type 2 diabetes and end-stage renal disease. *Regen Med*. 2016; 11(2): 145–158. [PMID: 26544198 DOI: 10.2217/rme.15.74]

55. **Zhao J**, Li X, Hu J, Chen F, Qiao S, Sun X, et al. Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization. *Cardiovasc Res*. 2019; 115(7): 1205–1216.

56. **Jin L**, Deng Z, Zhang J, Yang C, Liu J, Han W, et al. Mesenchymal stem cells promote type 2 macrophage polarization to ameliorate the myocardial injury caused by diabetic cardiomyopathy. *J Transl Med*. 2019; 17(1): 251. [PMID: 31382970 DOI: 10.1186/s12967-019-1999-8]

57. **Ko JH**, Lee HJ, Jeong HJ, Kim MK, Wee WR, Yoon S-O, et al. Mesenchymal stem/stromal cells precondition lung monocytes/macrophages to produce tolerance against allo- and autoimmunity in the eye. *Proc Natl Acad Sci USA*. 2016; 113(1): 158–163. [PMID: 26699483 DOI: 10.1073/pnas.1522905113]

58. **Lohan P**, Murphy N, Treacy O, Lynch K, Morcos M, Chen B, et al. Third-Party Allogeneic Mesenchymal Stromal Cells Prevent Rejection in a Pre-sensitized High-Risk Model of Corneal Transplantation. *Front Immunol*. 2018; 9:2666. [PMID: 30515159 DOI: 10.3389/fimmu.2018.02666]

59. **Liu H**, Liang Z, Wang F, Zhou C, Zheng X, Hu T, et al. Exosomes from mesenchymal stromal cells reduce murine colonic inflammation via a macrophage-dependent mechanism. *JCI Insight*. 2019; 4(24): 131273. [PMID: 31689240 DOI: 10.1172/jci.insight.131273]

60. **Zhou Y-Z**, Cheng Z, Wu Y, Wu Q-Y, Liao X-B, Zhao Y, et al. Mesenchymal stem cell-derived conditioned medium attenuate angiotensin II-induced aortic aneurysm growth by modulating macrophage polarization. *J Cell Mol Med*. 2019; 23(12): 8233–8245. [PMID: 31583844 DOI: 10.1111/jcmm.14694]

61. **Takeda K**, Webb TL, Ning F, Shiraishi Y, Regan DP, Chow L, et al. Mesenchymal stem cells recruit CCR2+ monocytes to suppress allergic airway inflammation. *J Immunol*. 2018; 200(4): 1261–1269. [PMID: 29352000 DOI:

10.4049/jimmunol.1700562]

62. **Ko JH**, Lee HJ, Jeong HJ, Oh JY. Ly6Chi monocytes are required for mesenchymal stem/stromal cell-induced immune tolerance in mice with experimental autoimmune uveitis. *Biochem Biophys Res Commun*. 2017; 494(1–2): 6–12. [PMID: 29056505 DOI: 10.1016/j.bbrc.2017.10.097]

63. **Kalliolias GD**, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol*. 2016; 12(1): 49–62. [PMID: 26656660 DOI: 10.1038/nrrheum.2015.169]

64. **Gabay C**, Lamacchia C, Palmer G. IL-1 pathways in inflammation and human diseases. *Nat Rev Rheumatol*. 2010; 6(4): 232–241. [PMID: 20177398 DOI: 10.1038/nrrheum.2010.4]

65. **Liu F**, Qiu H, Xue M, Zhang S, Zhang X, Xu J, et al. MSC-secreted TGF-β regulates lipopolysaccharide-stimulated macrophage M2-like polarization via the Akt/FoxO1 pathway. *Stem Cell Res Ther*. 2019; 10(1): 345. [PMID: 31771622 DOI: 10.1186/s13287-019-1447-y]

66. **Shin T-H**, Kim H-S, Kang T-W, Lee B-C, Lee H-Y, Kim Y-J, et al. Human umbilical cord blood-stem cells direct macrophage polarization and block inflammasome activation to alleviate rheumatoid arthritis. *Cell Death Dis*. 2016; 7(12): 2524. [PMID: 28005072 DOI: 10.1038/cddis.2016.442]

67. **Zhang B**, Zhao N, Zhang J, Liu Y, Zhu D, Kong Y. Mesenchymal stem cells rejuvenate cardiac muscle through regulating macrophage polarization. *Aging (Albany NY)*. 2019; 11(12): 3900–3908. [PMID: 31212255 DOI: 10.18632/aging.102009]

68. **Di G**, Du X, Qi X, Zhao X, Duan H, Li S, et al. Mesenchymal Stem Cells Promote Diabetic Corneal Epithelial Wound Healing Through TSG-6-Dependent Stem Cell Activation and Macrophage Switch. *Invest Ophthalmol Vis Sci*. 2017; 58(10): 4344–4354. [PMID: 28810264 DOI: 10.1167/iovs.17-21506]

69. **Sun X**, Shan A, Wei Z, Xu B. Intravenous mesenchymal stem cell-derived exosomes ameliorate myocardial inflammation in the dilated cardiomyopathy. *Biochem Biophys Res Commun*. 2018; 503(4): 2611–2618. [PMID: 30126637 DOI: 10.1016/j.bbrc.2018.08.012]

70. **Vasandan AB**, Jahnavi S, Shashank C, Prasad P, Kumar A, Prasanna SJ. Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE2-dependent mechanism. *Sci Rep*. 2016; 6: 38308. [PMID: 27910911 DOI: 10.1038/srep38308]

71. **Phinney DG**, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, et al. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat Commun*. 2015; 6: 8472. [PMID: 26442449 DOI: 10.1038/ncomms9472]

72. **Li C**, Jin Y, Wei S, Sun Y, Jiang L, Zhu Q, et al. Hippo signaling controls NLR family pyrin domain containing 3 activation and governs immunoregulation of mesenchymal stem cells in mouse liver injury. *Hepatology*. 2019; 70(5): 1714–1731. [PMID: 31063235 DOI: 10.1002/hep.30700]

73. **Cho D-I**, Kim MR, Jeong H, Jeong HC, Jeong MH, Yoon SH, et al. Mesenchymal stem cells reciprocally regulate the M1/M2 balance in mouse bone marrow-derived macrophages. *Exp Mol Med*. 2014; 46: 70. [PMID: 24406319 DOI: 10.1038/emm.2013.135]

74. **de Witte SFH**, Luk F, Sierra Parraga JM, Gargesha M, Merino A, Korevaar SS, et al. Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells. *Stem Cells*. 2018; 36(4): 602–615. [PMID: 29341339 DOI: 10.1002/stem.2779]

75. **Hoogduijn MJ**, Roemeling-van Rhijn M, Engela AU, Korevaar SS, Mensah FKF, Franquesa M, et al. Mesenchymal stem cells induce an inflammatory response after intravenous infusion. *Stem Cells Dev*. 2013; 22(21): 2825–2835. [PMID: 23767885 DOI: 10.1089/scd.2013.0193]

76. **Miteva K**, Pappritz K, El-Shafeey M, Dong F, Ringe J, Tschöpe C, et al. Mesenchymal Stromal Cells Modulate Monocytes Trafficking in Coxsackievirus B3-Induced Myocarditis. *Stem Cells Transl Med*. 2017; 6(4): 1249–1261. [PMID: 28186704 DOI: 10.1002/sctm.16-0353]

77. **Mansouri N**, Willis GR, Fernandez-Gonzalez A, Reis M, Nassiri S, Mitsialis SA, et al. Mesenchymal stromal cell exosomes prevent and revert experimental pulmonary fibrosis through modulation of monocyte phenotypes. *JCI Insight*. 2019; 4(21): 128060. [PMID: 31581150 DOI: 10.1172/jci.insight.128060]

78. **Braza F**, Dirou S, Forest V, Sauzeau V, Hassoun D, Chesné J, et al. Mesenchymal stem cells induce suppressive macrophages through phagocytosis in a mouse model of asthma. *Stem Cells*. 2016; 34(7): 1836–1845. [PMID: 26891455 DOI: 10.1002/stem.2344]

79. **Li X**, Liu L, Yang J, Yu Y, Chai J, Wang L, et al. Exosome Derived From Human Umbilical Cord Mesenchymal Stem Cell Mediates MiR-181c Attenuating Burn-induced Excessive Inflammation. *EBioMedicine*. 2016; 8: 72–82. [PMID: 27428420 DOI: 10.1016/j.ebiom.2016.04.030]

80. **Ti D**, Hao H, Tong C, Liu J, Dong L, Zheng J, et al. LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. *J Transl Med*. 2015; 13:308. [PMID: 26386558 DOI: 10.1186/s12967-015-0642-6]

81. **Spinosa M**, Lu G, Su G, Bontha SV, Gehrau R, Salmon MD, et al. Human mesenchymal stromal cell-derived extracellular vesicles attenuate aortic aneurysm formation and macrophage activation via microRNA-147. *FASEB J*. 2018; fj201701138RR. [PMID: 29812968 DOI: 10.1096/fj.201701138RR]

82. **He X**, Dong Z, Cao Y, Wang H, Liu S, Liao L, et al. MSC-Derived Exosome Promotes M2 Polarization and Enhances Cutaneous Wound Healing. *Stem Cells Int*. 2019; 2019: 7132708. [PMID: 31582986 DOI: 10.1155/2019/7132708]

83. **Neudecker V**, Haneklaus M, Jensen O, Khailova L, Masterson JC, Tye H, et al. Myeloid-derived miR-223 regulates intestinal inflammation via repression of the NLRP3 inflammasome. *J Exp Med*. 2017; 214(6): 1737–1752. [PMID: 28487310 DOI: 10.1084/jem.20160462]

84. **Lee RH**, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell*. 2009; 5(1): 54–63. [PMID: 19570514 DOI: 10.1016/j.stem.2009.05.003]

85. **Weiss ARR**, Lee O, Eggenhofer E, Geissler E, Korevaar SS, Soeder Y, et al. Differential effects of heat-inactivated, secretome-deficient MSC and metabolically active MSC in sepsis and allogenic heart transplantation. *Stem Cells*. 2020; 38(6): 797–807. [PMID: 32101344 DOI: 10.1002/stem.3165]

86. **Francisco LM**, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev*. 2010; 236: 219–242. [PMID: 20636820 DOI: 10.1111/j.1600-065X.2010.00923.x]

87. **Munn DH**, Mellor AL. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. *Trends Immunol*. 2013; 34(3): 137–143. [PMID: 23103127 DOI: 10.1016/j.it.2012.10.001]

88. **Burnstock G**, Boeynaems J-M. Purinergic signalling and immune cells. *Purinergic Signal*. 2014; 10(4): 529–564. [PMID: 25352330 DOI: 10.1007/s11302-014-9427-2]

89. **Lu W**, Fu C, Song L, Yao Y, Zhang X, Chen Z, et al. Exposure to supernatants of macrophages that phagocytized dead mesenchymal stem cells improves hypoxic cardiomyocytes survival. *Int J Cardiol*. 2013; 165(2): 333–340. [PMID: 22475845 DOI: 10.1016/j.ijcard.2012.03.088]

90. **Galleu A**, Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS, et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. *Sci Transl Med*. 2017; 9(416). [PMID: 29141887 DOI: 10.1126/scitranslmed.aam7828]

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

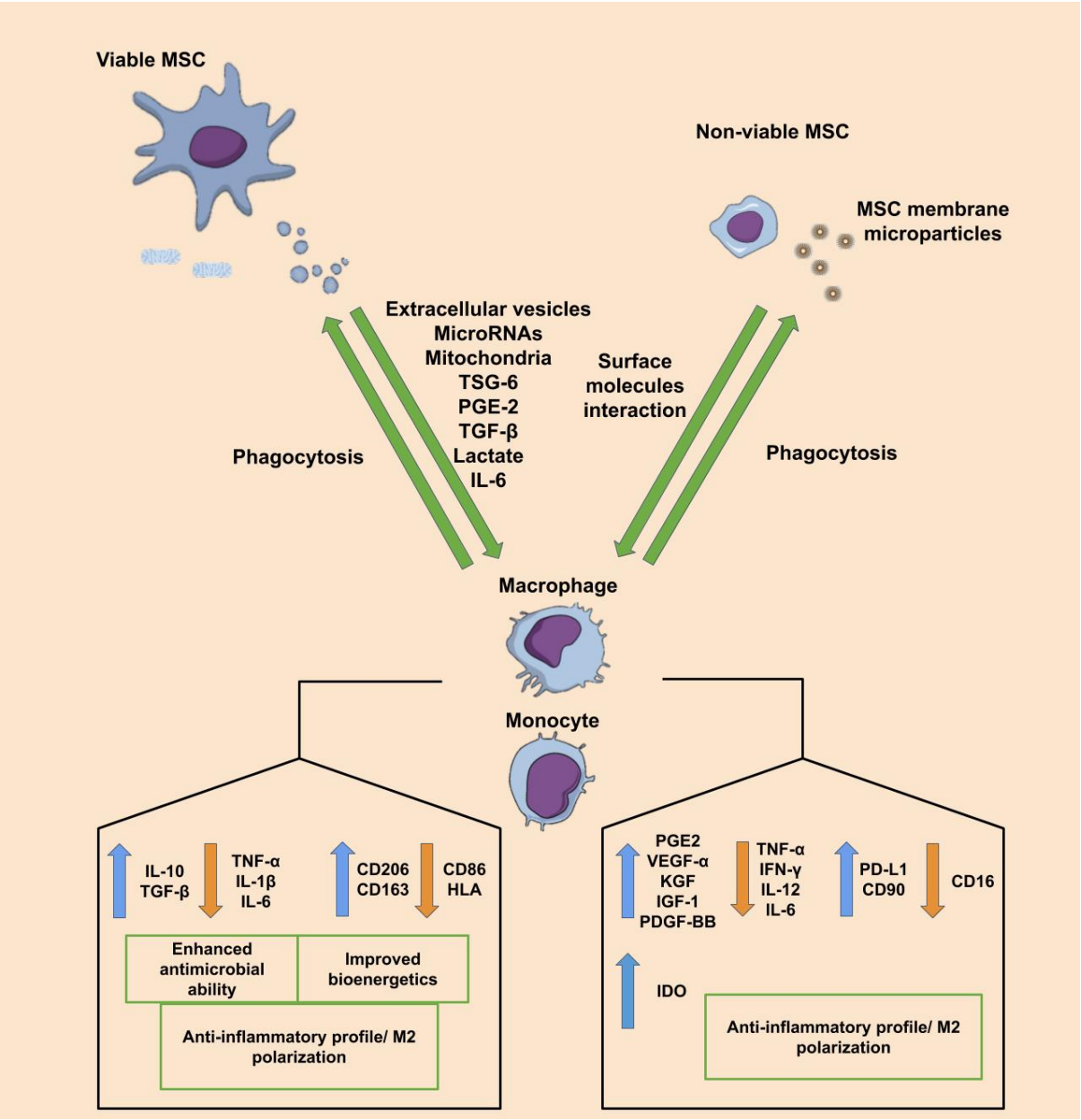

Figure 1 Main effects of viable, inactivated, apoptotic MSCs and MSC secretome and subcellular particles on monocytes and macrophages.

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL – UFRGS

INSTITUTO DE BIOCIÊNCIAS COMISSÃO

TERMO DE CONFIRMAÇÃO DE CORREÇÃO DE TRABALHO DE CONCLUSÃO DE CURSO

Informo que o trabalho de conclusão do aluno (a) Alexia Nedel Sant'Ana, intitulado: Effects of viable, non-viable, apoptotic MSCs and subcellular particles on monocytes and macrophages foi devidamente corrigido conforme as sugestões propostas pela banca examinadora.

Porto Alegre, 24 de novembro de 2020.

Assinatura do Orientador

Instituto de Biociências – Comissão de Graduação
Av. Bento Gonçalves, 9500, Bloco IV, Prédio 43423, Sala 202 - Campus do Vale – Bairro Agronomia
CEP 91501-970 – Porto Alegre – RS – Brasil
Fone 0 xx 51 – 33087674 Fax 0 xx 51 – 33087755 .
E-mail: gradbio@ufrgs.br