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# Mesenchymal stem cell therapy modulates macrophage dynamics in ARDS-associated liver injury in rats



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## ABSTRACT

Acute respiratory distress syndrome (ARDS) is a life-threatening pulmonary condition characterized by severe hypoxemia and respiratory failure. Beyond its devastating impact on the lungs, ARDS often triggers systemic responses affecting vital organs throughout the body. One such organ commonly affected is the liver, which experiences various degrees of injury during the course of ARDS. Pathophysiological changes in liver during ARDS, particularly polarization of Kupffer cells during the disease and its treatment, have drawn increasing attention.

**PURPOSE:** To explore the macrophage transformation in liver injury associated with ARDS and investigate the potential of multipotent mesenchymal stromal/stem cell (MMSCs) therapy as a means to modulate macrophage responses and mitigate liver injury.

**METHODS.** 72 mature male Wistar rats were randomly allocated to nine experimental groups as follows: the control group, groups assessed at 3 days, 7 days, and 28 days following intranasal lipopolysaccharide (LPS) administration, groups that received 24 hours of LPS followed by 2 days of human umbilical cord-derived multipotent mesenchymal stromal cells (hUC-MMSCs), groups exposed to 4 days of LPS and 3 days of hUC-MMSCs, groups subjected to 14 days of LPS and 14 days of hUC-MMSCs, groups treated with LPS 21 days and 7 days with hUC-MMSCs injection, and a control group assessed 3 days after hUC-MMSCs injection. For the administration of hUC-MMSCs, intraperitoneal injections were performed at a dose of  $1 \times 10^6$  cells/kg body weight. Immunohistochemistry was used to analyze macrophage subpopulations in liver tissue for CD68 as a pan-macrophage marker, CD86 for the identification of M1 macrophages, CD163 – for the identification of M2 macrophages.

**RESULTS.** Early ARDS stages showed increased M1 macrophages, indicating pro-inflammatory responses, while later stages showed M2 macrophage activation, suggestive of anti-inflammatory properties and tissue repair roles. hUC-MMSCs administration facilitated the transition from M1 to M2 macrophages, promoting an anti-inflammatory milieu.

**CONCLUSIONS.** hUC-MMSCs demonstrate the potential to modulate macrophage polarization into M2 anti-inflammatory phenotype. Such findings reflect one of the mechanisms of MMSCs action which holds practical significance for future ARDS therapies, aiming to mitigate excessive inflammation and enhance tissue repair.

**KEY WORDS:** acute respiratory distress syndrome; liver injury; multipotent mesenchymal stromal cells; macrophage polarization; inflammation, immunomodulation

Acute Respiratory Distress Syndrome (ARDS) is a life-threatening condition marked by severe inflammation and respiratory failure. It represents a critical challenge in the field of critical care medicine, given its complex pathophysiology and significant mortality rates. To gain deeper insights into this syndrome, it is imperative to unravel the intricate immune responses that take place during ARDS, particularly focusing on the pivotal role of macrophages. ARDS is characterized by overwhelming inflammation within the lungs, resulting in impaired gas exchange, profound hypoxia, and multiorgan dysfunction. Leading causes of ARDS encompass pneumonia, sepsis, trauma, and aspiration, all of which initiate robust inflammatory cascades [1-5]. In response to lung injury caused

by ARDS, the body initiates a systemic inflammatory response. This systemic inflammation can affect other organs, including the liver. In severe cases of ARDS, a cytokine storm can occur. This is an uncontrolled and excessive release of pro-inflammatory cytokines. These cytokines can affect liver function, leading to hepatocellular injury [6].

In ARDS, the lungs are unable to provide sufficient oxygen to the body. This leads to tissue hypoxia, which can impact liver cells. Hypoxia in the liver can lead to the production of free radicals and oxidative stress, damaging hepatocytes [7, 8]. ARDS patients are also prone to coagulopathy, which can lead to thrombosis or bleeding in the liver. These disturbances can further compromise liver function [9-11].

The use of multipotent mesenchymal stromal/stem cell (MMSCs) to treat liver injuries has emerged as a promising avenue for improving outcomes in both humans and animal models. Wang et al. compared the effects of human and mouse adipose-derived mesenchymal stem cells (AD-MMSCs) on mice with experimentally induced acute liver failure. Both human and mouse AD-MMSCs reduced the extent of liver injury, with mouse AD-MMSCs being more effective than human AD-MMSCs [12]. Zhang et al., while investigating the influence of human umbilical cord-derived multipotent mesenchymal stromal cells (hUC-MMSCs) on LPS-induced acute liver failure in rats, found that 48 h post-injection of hUC-MMSCs at passages 5 and 10 reduced the number of apoptotic hepatocytes and increased the number of proliferating cells. hUC-MMSCs at passage 5 exhibited more effective anti-apoptotic and regenerative effects compared to passage 10 [13]. The positive impact of MMSCs on liver structure regeneration is explained by their ability to differentiate into hepatocyte-like cells [14-16]. There is also evidence that transplanted allogeneic and even xenogeneic stem cells can restore the injured liver by merging with hepatocytes [17].

MMSCs secrete trophic molecules such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), which activate tissue regeneration through their paracrine proliferative effect [18-20]. It has been found that MMSC-derived exosomes contain HGF, which promotes hepatocyte proliferation. VEGF secreted by MMSCs is also capable of regenerating damaged liver tissue [21].

MMSCs reduce the levels of pro-inflammatory cytokines like IL-6 and TNF- $\alpha$  while increasing the levels of anti-inflammatory cytokines such as IL-10. The inflammation phase following an injury leads to fibrosis induction. Therefore, suppressing the release of pro-inflammatory cytokines and stimulating the secretion of anti-inflammatory cytokines can reduce liver injury and fibrosis. Liver fibrosis occurs due to synthesis of extracellular matrix which consists of collagen I, III, and IV. Hepatic stellate cells (HSCs) play a role in this process. Activated HSCs proliferate and transform into myofibroblasts, which synthesize the extracellular matrix and secrete tissue inhibitors of metalloproteinases, thus inhibiting the activity of interstitial collagenase and preventing extracellular matrix breakdown [22].

Kupffer cells, resident liver macrophages, play a role in activating HSCs. Activated Kupffer cells secrete oxidants, cytokines, and proteases that affect the proliferation, migration, and differentiation of HSCs [23, 24].

MMSCs can exhibit anti-fibrotic activity through their paracrine effects. By influencing signaling pathways, they can suppress the activation of HSCs and promote Kupffer cell mobilization. Exosomes, secretomes, and conditioned culture medium of MMSCs can also inhibit liver fibrosis [25-27].

Our previous research show that hUC-MMSCs have regenerative, anti-inflammatory, and antifibrotic effects on LPS-induced liver injury, reducing the degree of hepatocellular necrosis, liver structural damage, hepatocyte vacuolation, inflammation, signs of disseminated intravascular coagulation and fibrosis, as well as lowering the levels of liver markers in blood in rats with LPS-induced liver injury, although the immunomodulatory properties of MMSCs, particularly their impact on liver macrophage polarization, was not studied. [28].

Macrophages, versatile immune cells distributed throughout tissues, act as sentinels of the immune system. There are several distinct macrophage subpopulations. M0 macrophages, also known as naïve or unpolarized macrophages, represent a distinct subset of macrophages with versatile phenotypes and functions. Unlike polarized macrophages (M1 and M2), M0 macrophages do not possess specific pro-inflammatory (M1) or anti-inflammatory (M2) characteristics. Instead, they maintain a neutral or undifferentiated state, ready to respond dynamically to micro-environmental signals. During ARDS, unactivated macrophages respond to inflammatory signals by undergoing polarization into distinct phenotypes, with the primary classification being M1 and M2 macrophages. Classically activated M1 macrophages are typically activated in response to signals associated with microbial infections or inflammation, such as bacterial lipopolysaccharides. They exhibit pro-inflammatory charac-

teristics, releasing cytokines like interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). These macrophages are crucial in the initial defense against infections but, when overactivated or dysregulated, they can contribute to excessive inflammation and tissue damage. Alternatively activated macrophages (M2) are typically activated in response to signals related to tissue repair and wound healing. They possess anti-inflammatory properties and produce interleukin-10 (IL-10) and transforming growth factor- $\beta$  (TGF- $\beta$ ) [29-35].

Identification of different macrophage subpopulations is carried out using molecular markers. Among the most common pan-macrophage markers is the glycoprotein CD68, which is highly expressed by all types of macrophages [36]. Key markers for identifying M0 macrophages include CD14, CD68, CD11b, and CD16 (Fc $\gamma$ RIII). To identify M1 cells, CD86 is most commonly used, while M2 subpopulations are typically identified by the CD163 marker [36].

Understanding the nuanced equilibrium between M1 and M2 macrophage polarization during liver injury is pivotal for devising effective therapeutic interventions. Imbalance in this polarization can exacerbate inflammation and tissue injury. Therefore, therapeutic interventions that modulate macrophage polarization are an active area of research for improving outcomes in ARDS and its complications.

**THE PURPOSE.** In our study, we delve into the changes of macrophage polarization within the liver of rats subjected to ARDS modelling, with a particular focus on the impact of MMSC therapy. Through these investigations, we aim to contribute to the development of innovative strategies aimed at attenuating ARDS-associated inflammation, thus improving clinical outcomes.

## MATERIALS AND METHODS

**Ethical considerations.** Impeccable ethical standards and regulations were observed throughout the study to ensure the welfare and humane treatment of experimental animals. All experiments were conducted in strict accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental Purposes (Strasbourg, 1986; Oslo, 2018), as well as the Law of Ukraine #3447-IV "On Protection of Animals from Cruelty" (dated 21.02.2006). The study received ethical approval from the Bioethics Commission of Ternopil National Medical University, as indicated in protocol #60, dated 01.09.2020.

**Experimental animals.** Seventy-two mature male Wistar rats weighing 200-220 g were enrolled in this study. They were randomly divided into nine distinct groups, each serving a specific purpose within the experimental design:

1. Control group: rats were not subjected to any specific treatment.
2. 3 days post-LPS: rats were sacrificed 3 days after intranasal lipopolysaccharide (LPS) administration.
3. 7 days post-LPS: rats were sacrificed 7 days after intranasal LPS administration.
4. 28 days post-LPS: rats were sacrificed 28 days after intranasal LPS administration.
5. 24h of LPS + 2 days MMSCs: rats received LPS intranasally followed by MMSCs injection 24 hours later, and were sacrificed 2 days after MMSCs administration.
6. 4d of LPS + 3 days MMSCs: rats received LPS intranasally followed by MSC injection 4 days later, and were sacrificed 3 days after MMSCs administration.
7. 14d of LPS + 14 days MMSCs: rats received LPS intranasally and were subsequently treated with MMSCs 14 days later. Assessment occurred 14 days after MMSCs administration.
8. 21d of LPS + 7 days MMSCs: rats received LPS intranasally and were subsequently treated with MMSCs 21 days later. Assessment occurred 7 days after MMSCs administration.
9. Control-MMSCs group: intact rats were sacrificed 3 days after a MMSCs injection.

These time points were strategically chosen to correspond to different stages of ARDS (exudative, fibroplasia and fibrosis) [28]. In groups, treated with MMSCs, the animals were sacrificed at different time points following MMSCs injections to enable a comparative analysis with the corresponding groups that did not receive MMSC treatment. Time points in groups 7 and 8 were chosen to assess the differences in the impact of MMSCs administration at earlier as opposed to later stages.

**ARDS induction.** ARDS was induced by intranasal administration of LPS from *E. coli* (*Sigma-Aldrich*, USA) at a dosage of 5 mg/kg. This model was chosen for its capacity to closely mimic the inflammatory aspects of ARDS [28, 37-40]. During LPS administration animals were anaesthetized with ketamine (50 mg/kg).

**MMSCs administration.** hUC-MMSCs were delivered via intraperitoneal injection at a dose of  $1 \times 10^6$  cells/kg body weight. This route of administration was selected for its effectiveness in achieving a systemic therapeutic effect [28].

**Liver samples collection.** Terminal anesthesia was performed with intraperitoneal injection of lethal dose of sodium thiopental (150 mg/kg). Liver tissue samples were fixed in 10 % neutral buffered formalin, processed using a tissue processor LOGOS One (*Milestone Medical*, USA) and embedded in paraffin wax. 5  $\mu$ m thick sections were obtained using a rotary microtome AMR 400 (*Amos scientific*, Australia).

**Immunohistochemical study of macrophages.** To assess macrophage subpopulations within liver tissues, immunohistochemical staining was

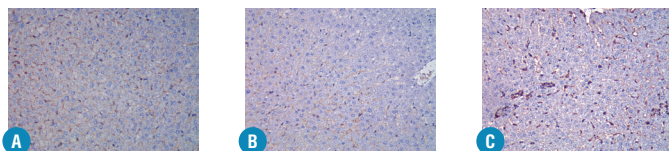
used. The following markers were utilized: CD68, a panmacrophage marker, CD86, used for the identification of M1 macrophages, CD163 – for the identification of M2 macrophages.

Paraffin-embedded tissue sections were deparaffinized, rehydrated, and subjected to heat-induced antigen retrieval. Endogenous peroxidase activity was blocked using 3 % hydrogen peroxide. Mouse anti-CD163 monoclonal antibodies (EDHu-1) (Cat. No. NB110-40686, *Novus Biologicals*, USA), mouse anti-CD86 monoclonal antibodies (C86/1146) (Cat. No. NBP2-44514, *Novus Biologicals*, USA) and rabbit polyclonal anti-CD68 antibodies (Cat. No. ab125212, *Abcam*, USA) were used, as well as a detection system Mouse/Rabbit PolyVue™ HRP/DAB (*Diagnostic BioSystems*, USA). The sections were counterstained with Mayer's hematoxylin. Liver samples were examined under a light microscope Eclipse Ci-E (*Nikon*, Japan) and documented using a camera M3CMOS 14000 (*Sigeta*, Ukraine) and a Toup View software (*ToupTek Photonics*, China).

**Quantitative data.** 540 fields of view (FOV) were analyzed at 100 $\times$  magnification (10 $\times$  objective, 10 $\times$  eyepiece). The acquired images were processed using the ImageJ software (*Wayne Rasband*, USA) and the "Analyze particles" command was used to calculate the percentage of the area occupied by macrophage cells. For the obtained data within each group, the values of the mean (M), standard error of the mean (m), and standard deviation ( $\sigma$ ) were calculated. The significance of the difference between the groups was determined using Student's t-test for normally distributed data. Differences were considered significant at  $p < 0.05$ .

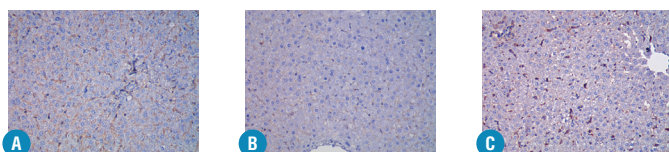
## RESULTS AND DISCUSSION

The immunohistochemical analysis of total CD68<sup>+</sup> macrophages within the liver tissue of intact rats demonstrated the presence of Kupffer cells located between hepatic plates within the sinusoidal capillary lumens. A prevalence of macrophages with the M2 immunophenotype CD163<sup>+</sup> was observed (Fig. 1).



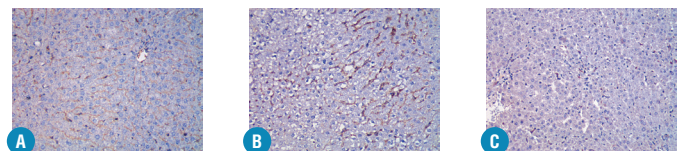
**Fig. 1.** Representative photomicrographs of rat liver sections stained for CD68 (A), CD86 (B) and CD163 (C) by immunohistochemistry from control group. CD68, CD86 and CD163 staining is brown. Light microscopy, 200 $\times$  magnification.

Macrophage distribution in the control-MMSCs group closely mirrored that of intact rats (Fig. 2).



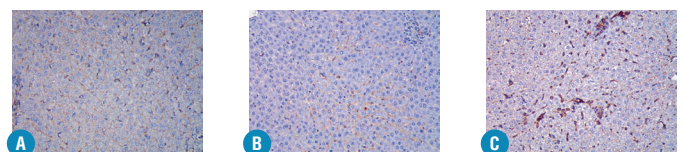
**Fig. 2.** Representative photomicrographs of rat liver sections stained for CD68 (A), CD86 (B) and CD163 (C) by immunohistochemistry from control-MMSCs group. CD68, CD86 and CD163 staining is brown. Light microscopy, 200 $\times$  magnification.

Upon assessing the liver tissue on the 3<sup>rd</sup> day of the experiment, a notable increase in macrophage count was observed, primarily attributed to the elevation of CD86<sup>+</sup> cells, characteristic of M1 macrophages, whereas M2 macrophages were scarcely detected (Fig. 3).



**Fig. 3.** Representative photomicrographs of rat liver sections stained for CD68 (A), CD86 (B) and CD163 (C) by immunohistochemistry from 3d of LPS group. CD68, CD86 and CD163 staining is a brown. Light microscopy, 200 $\times$  magnification.

In the animals of group 24h of LPS + 2d MMSCs, an increased amount of macrophages exhibited CD163 marker compared to the group 3d LPS, indicative of an emerging anti-inflammatory response (Fig. 4). This finding suggests that MMSCs administration initiated a shift in macrophage polarization towards the M2 phenotype.



**Fig. 4.** Representative photomicrographs of rat liver sections stained for CD68 (A), CD86 (B) and CD163 (C) by immunohistochemistry from 24h of LPS + 2d MMSCs group. CD68, CD86 and CD163 staining is brown. Light microscopy, 200 $\times$  magnification.

As the ARDS model progressed to the 7<sup>th</sup> day, a distinct shift occurred in the macrophage phenotype distribution within the liver. The proportion of M2 macrophages increased significantly, while M1 macrophages became less prominent (Fig. 5) comparing to the early stage of ARDS (3d LPS).

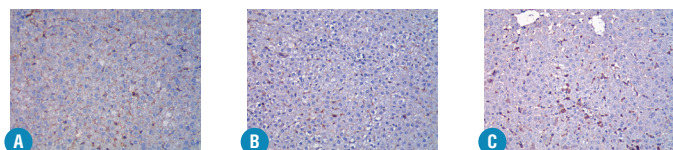


Fig. 5. Representative photomicrographs of rat liver sections stained for CD68 (A), CD86 (B) and CD163 (C) by immunohistochemistry from 7d of LPS group. CD68, CD86 and CD163 staining is brown. Light microscopy, 200× magnification.

In rats of the group 4d of LPS + 3d MMSCs the number of M2 macrophages was markedly elevated compared to untreated animals of the group 7d LPS (Fig. 6). This indicates that MMSCs play a pivotal role in fostering an anti-inflammatory milieu and reinforcing M2 macrophage activation, corroborating the established immunomodulatory properties of MMSC therapy.

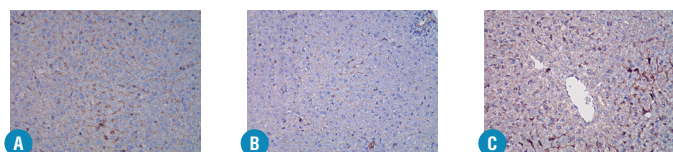


Fig. 6. Representative photomicrographs of rat liver sections stained for CD68 (A), CD86 (B) and CD163 (C) by immunohistochemistry from 4d of LPS + 3d MMSCs group. CD68, CD86 and CD163 staining is brown. Light microscopy, 200× magnification.

Upon reaching the 28<sup>th</sup> day of the experiment, the macrophage count exhibited a decrease, with approximately equal numbers of M1 and M2 macrophages present within the liver.

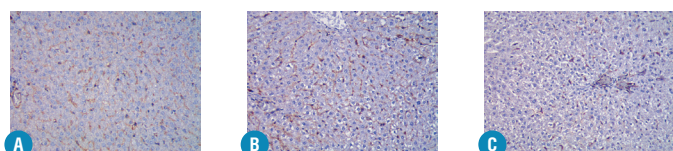


Fig. 7. Representative photomicrographs of rat liver sections stained for CD68 (A), CD86 (B) and CD163 (C) by immunohistochemistry from 28d of LPS group. CD68, CD86 and CD163 staining is brown. Light microscopy, 200× magnification.

In the group 14 days LPS + 14 days MMSCs, the distribution of macrophage subpopulations in the liver was similar to the group 28 days LPS (Fig. 8). Obviously, 14 days after MMSCs administration, there are no longer any signs of impact of stem cells on macrophage subpopulations.

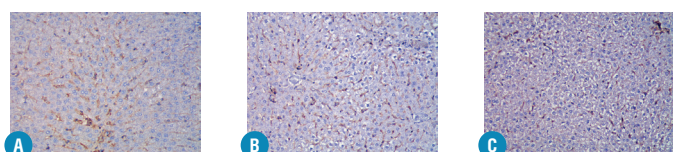


Fig. 8. Representative photomicrographs of rat liver sections stained for CD68 (A), CD86 (B) and CD163 (C) by immunohistochemistry from 14d of LPS + 14d MMSCs group. CD68, CD86 and CD163 staining is brown. Light microscopy, 200× magnification.

However, in group 21d of LPS + 7d MMSCs, a discernible shift towards the M2 immunophenotype was identified (Fig. 9).

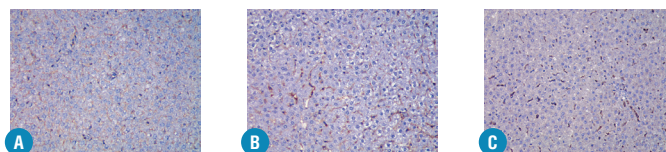


Fig. 9. Representative photomicrographs of rat liver sections stained for CD68 (A), CD86 (B) and CD163 (C) by immunohistochemistry from 21d of LPS + 7d MMSCs group. CD68, CD86 and CD163 staining is brown. Light microscopy, 200× magnification.

Quantitative analysis of the liver area occupied by different macrophage subpopulation showed statistically significant activation of pro-inflammatory M1 macrophages throughout the progression of ARDS. However, the groups treated with MMSCs contained statistically proven increase of anti-inflammatory M2 macrophage percentage, which signifies the prominent immunomodulatory MMSCs action (Table 1).

Table 1. Percentage of the liver area occupied by macrophages.

Group	Area of the liver FOV, occupied by macrophage cells, %		
	CD68+	CD86+	CD163+
Control	8.68 ± 0.32	2.01 ± 0.09	7.06 ± 0.19
Control-MMSCs	7.79 ± 0.27*	1.84 ± 0.06	7.25 ± 0.14
3 days post-LPS	9.03 ± 0.32	6.89 ± 0.27***	2.13 ± 0.15***
7 days post-LPS	8.50 ± 0.26	3.54 ± 0.10***	6.29 ± 0.23*
28 days post-LPS	7.88 ± 0.27	4.78 ± 0.15***	4.17 ± 0.18***
24h of LPS + 2 days MMSCs	9.06 ± 0.41	3.75 ± 0.12****	7.04 ± 0.41###
4d of LPS + 3 days MMSCs	8.14 ± 0.32	1.67 ± 0.07****	8.03 ± 0.22****
14d of LPS + 14 days MMSCs	9.12 ± 0.38	4.36 ± 0.16***	5.02 ± 0.23****
21d of LPS + 7 days MMSCs	8.76 ± 0.21	3.16 ± 0.11****	5.93 ± 0.19****

Note: \* – differences are significant between the group of intact animals and rats with ARDS model (\* –  $p < 0.05$ ; \*\* –  $p < 0.01$ ; \*\*\* –  $p < 0.001$ ); # – differences are significant between the experimental groups of animals without and with MMSC treatment at the respective observation times (# –  $p < 0.05$ ; ## –  $p < 0.01$ ; ### –  $p < 0.001$ ).

These findings collectively underscore the dynamic interplay between macrophage subpopulations in the liver during ARDS progression and the impact of MMSC therapy. The initial proinflammatory surge, marked by M1 macrophages, eventually gave way to an anti-inflammatory milieu characterized by M2 macrophages, with the extent and duration of MMSC treatment influencing the degree of immunomodulation.

The observed shifts in macrophage polarization reflect the intricate immunoregulatory mechanisms at play during ARDS and highlight the potential of MMSC therapy in mitigating inflammation and promoting tissue homeostasis.

The dynamic interplay between M1 and M2 macrophage phenotypes is pivotal in the pathophysiology of ARDS during its different phases. While M1 macrophages dominate the exudative phase with their pro-

inflammatory functions, the transition to M2 macrophages in the rehabilitation phase is essential for resolution and repair. In the fibrotic phase, the role of macrophages becomes more complex, and achieving the right balance is crucial to prevent excessive fibrosis and promote recovery [29, 30, 32, 35].

MMSCs exert a profound influence on macrophage polarization through the secretion of soluble factors. These factors, including cytokines and chemokines, create a paracrine signalling milieu that modulates macrophage behaviour [33, 41, 42].

Kwon et al. discovered that umbilical cord-derived mesenchymal stem cells exert their regulatory influence on the anti-inflammatory response through the secretion of decorin, a pivotal regulatory molecule. Decorin plays a crucial role in the transformation of inflammatory macrophages into anti-inflammatory macrophages [43].

MMSCs secrete exosomes, nano-sized vesicles containing bioactive molecules, representing an essential conduit for intercellular communication. These exosomes harbour a rich cargo of microRNAs, proteins, and lipids that can profoundly impact macrophage polarization [33, 44].

Another facet of MMSC-mediated macrophage regulation involves metabolic reprogramming. M1 macrophages rely on glycolysis for energy production, fostering a proinflammatory environment, while anti-inflammatory M2 macrophages favor oxidative phosphorylation. MMSCs can induce a shift in macrophage metabolism towards oxidative phosphorylation, thereby promoting an anti-inflammatory phenotype [33, 45-47].

MMSCs play a crucial role in regulating macrophage polarization through their apoptotic and efferocytosis effects. When MMSCs undergo

apoptosis, they release apoptotic vesicles with potent anti-inflammatory and immunomodulatory properties. These apoptotic MMSCs effectively reduce inflammation and vascular permeability in lung injury and alleviate pathological damage. Phagocytosis of apoptotic MMSCs by macrophages and monocytes triggers the production of anti-inflammatory mediators, demonstrating immunosuppressive effects [33, 48-51].

MMSCs also regulate macrophage polarization through mitochondrial transfer. This process involves the exchange of functional mitochondria between MMSCs and macrophages, influencing the metabolic and functional status of the latter. MMSCs, with their remarkable plasticity and immunomodulatory properties, have been found to engage in a unique form of intercellular communication by transferring their own healthy mitochondria to macrophages. This transfer of mitochondria can profoundly impact macrophage polarization. MMSCs, through mitochondrial transfer, can induce a shift in macrophage metabolism towards oxidative phosphorylation, favoring an anti-inflammatory phenotype [33, 52, 53].

The results of our research are consistent with previous studies on the influence of MMSCs on liver macrophages in the context of other pathological conditions. Fiore et al. suggest that MMSCs can induce a shift in hepatic macrophages from a pro-fibrotic to a resolutive phenotype, potentially contributing to the amelioration of liver fibrosis [54]. Li et al. demonstrate that MMSCs can induce the polarization of undifferentiated M0 macrophages into M2 phenotype through the activation of signal transducer and activator of transcription 6 (STAT6), which may have therapeutic effects in acute liver failure [55].

## CONCLUSION

***This study demonstrates that therapy with MMSCs has the potential to effectively modulate macrophage polarization. Specifically, hUC-MMSCs administration appears to enhance the transition from M1 to M2 macrophages, fostering a shift toward an anti-inflammatory milieu. The capacity of hUC-MMSCs to influence macrophage polarization holds great promise for future therapeutic interventions in ARDS. This research marks a significant step forward in our understanding of ARDS complications pathogenesis and the potential for innovative treatments that harness the immunomodulatory power of MMSCs.***

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