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The Effect of Exosomes Isolated from Poly (I:C) Treated Human Wharton's Jelly Mesenchymal Stem Cells on CD4+CD25+Foxp3+ Regulatory T Cells

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ABSTRACT

Mesenchymal stem cells (MSCs) are a potential cell therapy candidate for autoimmune and inflammatory diseases due to their multilineage capacity and immune modulating function. MSCs exert immunomodulatory effects on target cells through the secretion of exosomes. Inflammatory conditions such as Toll-like receptors (TLRs) engagement can change the biological functions and immunomodulatory activities of MSCs and the contents of exosomes derived from MSCs are changed. Regulatory T-cells (Treg) are crucial for maintaining immune cell homeostasis and self-tolerance. Our study aimed to investigate the impact of isolated exosomes from hWJ-MSCs that were treated with Poly (I:C) on regulatory CD4 CD25 Foxp3 T-cells.

MSCs were harvested from human umbilical cord Wharton's Jelly by explant method. Stem cells were treated by Polyinosinic-polycytidylic acid sodium salt (Poly (I:C)) for 48 hours. Exosomes were extracted from supernatant of cells and Scanning electron microscopy (SEM) and Dynamic light scattering (DLS) were performed for them. Peripheral blood mononuclear cells (PBMCs) isolated from the healthy donors were stimulated with PHA (Phytohemagglutinin) and co-cultured with Poly (I:C) treated hWJ-MSCs derived exosome and untreated hWJ-MSCs derived exosome or without hWJ-MSCs-derived exosome for 6 days. Then, frequency of CD4+CD25+ Foxp3+ regulatory T cells was measured by flow cytometry.

Our results showed that exosomes isolated from Poly (I:C) treated hWJ-MSCs significantly increased frequency of CD4+CD25+ Foxp3+ regulatory T cells compared to the untreated hWJ-MSCs derived exosome group and control group.

Stimulation by TLR3 improved the anti-inflammatory features of exosomes that were derived from hWJ-MSCs by increasing the frequency of Treg cells.

Keywords: Exosome; Mesenchymal stem cells; Regulatory T-cells; Toll-like receptor3

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INTRODUCTION

The differentiation and immunomodulatory capacities of MSCs make them interesting tools and potential cellular candidates for the treatment of undesirable inflammation in many diseases as well as various immunological disorders. Due to their immunomodulatory properties, MSCs can influence the immune system components, like Dendritic cells (DC), B lymphocytes, Natural killer cells (NK cells), and T-cells.[1–3] A number of problems and consequences are associated with cell therapy, including heterogeneity and donor-dependent variation, the possibility of transplant rejection due to MSCs immunogenicity, aging and the creation of epigenetic changes as a result of successive passages in vitro as well as the ability of tumor development due to differentiation and suppression power of the immune system. Therefore, researchers have proposed the use of MSCs secreted products in the culture medium.[4–6] Extracellular vehicles (EVs) are among the components secreted by MSCs, which are divided into subgroups based on size and cell origin. A subgroup of these vesicles having a diameter of 30–150 nm and an endosomal origin has been designated as exosomes.[7] Exosomes are secreted from the membrane of MSCs to the extracellular space. These vesicles express CD9, CD63, CD81 on their surface. These vesicles act as intercellular interfaces, which transmit a set of properties from the cells they are derived from, including lipids, miRNA, long noncoding RNA (LncRNA), mRNA and proteins) to other immune cells, changing their phenotype and functions. The results of most recent investigations have revealed that exosomes secreted from MSCs have a main role in the modulation of immune responses by modifying the function of immune cells. MSC-derived exosomes have the same immunosuppressive effect as MSCs.[8] Exosomes lead to the suppression of Th1/Th17 cells and increase the distinction of Th2/Treg cells, causing the suppression of immune responses and preventing inflammation.[9] Exosomes also induce apoptosis of T-cells due to the presence of molecules such as Fas ligand (FASL), Programmed death-ligand 1 (PD-L1) and trail on their surface.[9] as well as reducing the Zeta chain and CD3 to reduce the T-cell signal.[10] By reducing Chemokine receptor type 7 (CCR7) expression on dendritic cells, exosomes prevent them from migrating toward the accumulation of T lymphocytes, as along with reducing the expression of co-stimulatory molecules like CD80 and CD86 for preventing DCs presentation of antigens to T lymphocytes.[11,12] Treg cells, namely a subpopulation of T-cells modulating the immune system, keep the tolerance against self-antigens, avoiding an autoimmune disease. Immunosuppressive Treg cells normally downregulate or suppress the proliferation and induction of effector T-cells. Tregs exercise their activity by directly contacting other immune cells via inhibitory immune checkpoint receptors, such as Lymphocyte Activation Gene 3 (LAG3), PD-1, T cell immunoreceptor with Ig and ITIM domains (TIGIT), and Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and secretion of anti-inflammatory agents like Transforming growth factor beta (TGF-β), IL-10 and IL-35.[13,14] The immunosuppressive activity and various biological functions of MSCs have been modulated in previous studies using a strategy called priming. In this process, the cells were exposed to one or more inflammatory factors in the culture, simulating the inflammatory conditions in the body for them in the laboratory to elicit a more effective immunosuppressive response. Toll-like receptors (TLRs) can be mentioned as inflammatory factors that are utilized for this purpose.[15] TLRs are the primary sensors that are responsible for identifying pathogen-associated molecular patterns (PAMPs).[16] According to some pieces of evidence, a number of TLRs are abundantly detected on MSCs surface. Stimulating such receptors causes an increase in the immunosuppressive response of MSCs to change the multilineage potential, phenotype, and hematopoietic support, which can further influence their therapeutic potential.[17] TLR3 has an essential role in terms of augmenting the inhibitory response in MSCs. For instance, stimulation of TLR3 on the surface of MSCs isolated from tonsils (T-MSCs) increases the expression of PD-L1. Since PD-L1 is a key factor in Th17 inhibition by T-MSCs, an increase of this factor potentiates Th17 inhibition.[18] Wharton's jelly mesenchymal stem Cells (WJ-MSCs) improved atopic dermatitis in a mouse model after TLR3 stimulation and significantly decreased IL-13 and IL-17 lymph node secretion compared to non-primed cells. Additionally, WJ-MSCs diminished mast cells, lymphocytes, neutrophils, and eosinophils infiltrating the skin wound site. In other words, it was significantly more effective than non-primed cells in inhibiting Th2 and Th17 responses.[19]

In another study conducted on MSCs derived from the human umbilical cord, stimulation of TLR3 increased the suppression of miR-143 and improved the function of Transforming growth factor-β-activated kinase 1 (TAK1), which is a key factor in the signaling pathway of TLR3, causing the rise in the expression of cyclooxygenase-2 (COX-2) and Indoleamine 2,3-dioxygenase (IDO) and consequently reducing the inflammatory activity of macrophages. This research revealed that TLR3 stimulation in MSCs can prevent tissue damage because of increased inflammatory responses in sepsis.[20] A better understanding of the effect of TLR activation on the immunomodulatory characteristics of MSCs and their exosomes may help enhance cell-free therapy for numerous disorders. As a result, scientists have attempted to figure out how to enhance exosome functionality and make them more effective for the treatment of immune-related diseases.[21] In this study, we aimed to investigate the effect of exosomes that were separated from human wharton's jelly mesenchymal stem cells and treated by Poly (I:C) on the frequency of regulatory $CD4^+CD25^+Foxp3^+$ T-cells.

MATERIALS AND METHODS

WJ-MSCs Isolation and Culture

The collection of human umbilical cord (UC) was permitted by the Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Ethics NO: IR.AJUMS.MEDICINE.REC.1398.032). Human umbilical cord pieces with a length of 10-20 cm were harvested from full-term neonates that were delivered by cesarean section with obtaining informed consent of the mothers (N=10). Isolation of hWJ-MSCs was accomplished as previously described[22]. The remaining blood was completely washed away by phosphate-buffered saline (PBS, DNAbiotech, Iran). Umbilical vein and arteries were detached, and the residual tissue was cut into 1-2 mm pieces. Next, the pieces were seeded onto 25-cm^2 T-flask culture dishes (SPL, Korea) with Dulbecco's modified eagle medium that contained F-12 nutrient mixture (DMEM-F12; Gibco, USA), which was supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco, USA), 100 µg/mL streptomycin (Sigma, Germany), 100 U/mL penicillin (Sigma, Germany) and 2.5 µg/mL amphotericin B (Sigma, Germany) at 37°C, 95% humidity and 5% (v/v) CO_2. For removing non-adherent cells, the medium was replaced twice a week. After 10 days, the pieces were removed and when the adherent cells reached 80% confluence, they were trypsinized using 0.025% trypsin that included 0.02% EDTA (Gibco, USA) and subsequently passaged into a new flask. In this study, the cells from the third passage were utilized.

Differentiation of WJ-MSCs

WJ-MSCs were cultured at 30,000 cells/ml within a 6-well plate and then incubated in DMEM-12 supplemented with 10% FBS (Gibco, USA). Upon reaching 80%–90% confluency, the cell medium was substituted with an adipogenic and osteogenic differentiation medium. To achieve osteogenic differentiation, we incubated the cells in DMEM-F12 that was supplemented with 10 mM b-glycerol phosphate (Sigma, Germany), 10 nM dexamethasone (Sigma, Germany), and 50 µg/ml ascorbic acid 2-phosphate (Sigma, Germany). For adipogenic differentiation, the cells were incubated with DMEM-F12 that contained 100 nM dexamethasone (Sigma, Germany) and 50 µg/ml indomethacin (Sigma, Germany). After a period of 21 days, the cells were washed using PBS, fixed using 4% paraformaldehyde and stained with Alizarin Red for assessing osteogenic differentiation as well as with Oil Red for detecting differentiation towards adipocytes.

Flow Cytometry Analysis

To confirm the mesenchymal nature of cells investigated in this study, approximately 5×10^4 cells were stained on ice using the monoclonal antibodies listed below based on suggestions of the manufacturer: FITC-conjugated mouse anti-human CD31, FITC-conjugated mouse anti-human CD34, FITC-conjugated mouse anti-human CD45, FITC-conjugated mouse anti-human CD73, FITC-conjugated mouse anti-human CD90 and FITC-conjugated mouse anti-human CD105 antibodies and incubated at 4°C for 30 min. After incubation, we washed the cell suspension with PBS to eliminate any antibodies that were not labelled. FITC-conjugated mouse IgG1 was utilized as isotype control, given that all the antibodies were purchased from eBioscience, USA. The cells were analyzed using BD FACSCalibur flow cytometry (Becton Dickinson, CA, USA). Each sample was evaluated by at least 20,000 events, and the data were analyzed using FlowJo™ software. The fluorescence intensity of the negative control group was indicative of the positive expression gate.

Poly (I: C)-priming of WJ-MSCs

The starvation method was used to obtain pure WJ-MSCs-derived exosomes and remove FBS from them. MSCs were cultured in T75 flasks (SPL, Korea) until reaching 50% confluency. After that, we diminished the FBS level from 10 to 0% over 4 days and allowed the cells to gradually adjust to FBS reduction conditions. For priming, (Poly (I:C); P1530, Sigma, Germany) was developed as suggested by the instruction manual and added into the WJ-MSCs culture medium at a final stimulation concentration of 50 μg/mL together with 0% FBS for 48 hours. After 48 hours, the cell culture medium containing Poly (I:C) was discarded and MSCs were rinsed twice with PBS before adding fresh medium that was replaced with 0% FBS. Then, for exosome isolation, the culture medium was collected after at least 48 hours. All of the above steps except for adding Poly (I:C) were carried out for the control group.

Exosome Extraction

MSC-derived exosomes were separated using a kit meant for exosome isolation (Exospin, Cell Guidance Systems, LLC, MO, USA) according the manufacturer's instructions. Briefly, first, supernatant of cells passage 3 transferred to a microcentrifuge tube and spin at 300×g for 10 minutes to remove cells. Then, supernatant transferred to a new microcentrifuge tube and add Exo-spin™ Buffer in a 2:1 ratio and mixed well by inverting the tube and incubate at 4°C for at least 1 hour. Carefully, discarded the supernatant and resuspended the exosome-containing pellet in 100 μL of PBS. The protein content of exosomes derived from WJ-MSCs was evaluated using the BCA Protein Assay kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions.

Exosome Morphology

Exosome morphology was observed using scanning electron microscopy (SEM) (KYKY-EM 3200). This was accomplished by first fixing the exosome suspension with 2% paraformaldehyde and then diluting it with distilled water. Next, 1-5 μL of the sample was transferred to silicon chips and placed in acetone for 5 minutes to dehydrate and dry. Then, it was washed and dried with water. Afterwards, an SEM microscope was used to capture the images.

Dynamic Light Scattering

The size distribution of exosomes was assessed using a well-known dynamic light scattering (DLS) technique (Malvern Instruments, Malvern, UK).

Peripheral Blood Mononuclear Cells Isolation

We isolated peripheral blood mononuclear cells (PBMCs) from 10 mL of blood samples from healthy individuals by Ficoll-hypaque density gradient solution (density=1.077±0.002) (Sigma, Germany). Briefly, first, we diluted whole blood with sterile PBS at a 1:1 v/v ratio. We gently overlaid the diluted blood onto the Ficoll and taked care not to mix the layers and centrifuged at 450×g for 30 min. After that, carefully harvested the PBMCs layer via gentle aspiration. Then, the isolated mononuclear cells were twice rinsed with PBS. To determine the effect of exosomes separated from Poly (I:C) primed and non-primed WJ-MSCs on PBMCs, 1 ml of RPMI-1640 (Sigma, Germany) culture medium supplemented with 10% FBS containing 1×10^6 PBMCs was seeded into a 24-well plate and divided into three groups as follows: (1) PBMCs that were only subject to 10 μg/mL phytohemagglutinin (PHA; Sigma, Germany) as control group; (2) PBMCs treated with 10 μg/mL PHA and 50 μg/mL exosomes isolated from Poly (I:C) primed WJ-MSCs; (3) PBMCs exposed to 10 μg/ml PHA and 50 μg/mL exosomes isolated from non-primed WJ-MSCs. The medium was changed three days later. After six days, PBMCs were removed and identified by flow cytometry to notice alteration in Tregs ratio. The test was done in triplicate.

Flow Cytometry Analysis for $CD4^+$ $CD25^+$ $Foxp3^+$ Regulatory T-cells

After six days, PBMCs were harvested and washed with PBS. Treg cells were stained in this experiment following the manufacturer's instructions (Human Regulatory T Cell Staining Kit #2, cat. No. 8998-88, eBioscience, USA). The conjugated antibodies were anti-human CD4-FITC, CD25-PE Cocktail (cat. No. 22-8425, eBioscience, USA), and anti-human Foxp3- APC (cat. No. 17-4776, eBioscience, USA). Rat IgG2a Isotype Control-APC (cat. No.77-4321, eBioscience, USA) was used as a control for the detection of non-specific binding signal of anti-human Foxp3-APC. Rat FCR blocker solution isolated from mouse serum (Normal Rat Serum, cat. No. 5555-24, eBioscience, USA) was also used. After staining, the ratio of Treg cells was evaluated using BD FACSCalibur flow

cytometry (Becton Dickinson, CA, USA). A minimum of 100,000 events were recorded for each sample, and the flow cytometry data were analyzed using FlowJoTM software (version 10). The cells to be analyzed were gated for lymphocytes via forward and side scatter, $CD4^+$ cells were subsequently gated, the CD25 gate was drawn against Foxp3, and the cells positive for CD25 and Foxp3 marker were selected. Positive expression was based on fluorescent minus one (FMO) control.

Statistical Analysis

Statistical analysis was done using GraphPad Prism (version: 8.3, GraphPad Software Inc., San Diego, CA). One-way ANOVA and t-tests were employed to distinguish the differences between groups, and *p* values>0.05 were regarded as statistically significant. Data was presented as the mean±standard deviation (SD).

RESULTS

Description of WJ-MSCs

Two weeks after plating and removing tissue fragments from the culture dish, spindle and star- shaped cells along with fibroblast-like ones appeared in the dish. After four weeks, WJ-MSCs started to develop colonies and gradually became confluent, and after 2-3 passages, they had often constant fibroblast-like shape (Figure 1A). The flow cytometry analysis revealed that WJ-MSCs were positive for CD105, CD73, CD90 but negative for CD45, CD34, CD31, indicating MSCs not originating from endothelial or hematopoietic cells (Figure 1D).

Adipogenic and Osteogenic Differentiation

To confirm the mesodermal origin of cells, the culture of cells was done under osteogenic and adipogenic differentiation conditions for three weeks. After three weeks, WJ-MSCs showed several lipid vacuoles visualized by oil red O (Figure 1B), and massive deposition of calcium was observed after Alizarin red staining (Figure 1C). These things showed adipogenic and osteogenic differentiation of WJ-MSC, respectively.

Exosomes Characterization

Exosomes separated from WJ-MSCs were categorized in terms of size using DLS and shape by using SEM. Particles' diameter in the exosome suspension isolated from WJ-MSCs was measured using t DLS technique. The size of exosomes ranged from 50 to 200 nm. The average size of exosomes was 132 nm, and 93% of purified exosomes had a diameter of <132 nm (Figure 2B). The results in this image are based on the value of e, which has been reported to be approximately equal to 2.71. SEM images revealed that the exosome membrane integrity was preserved and that its spherical structure was not damaged (Figure 2A)

Effects of Exosomes derived from Poly (I:C) Primed WJ-MSCs on Peripheral Blood $CD4^+CD25+Foxp3^+$ Treg Cells

PBMCs harvested from the blood circulation of healthy individuals were cultured for 6 days with exosomes isolated from Poly (I: C) primed and non-primed WJ-MSCs, and the capacity to induce $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells was assessed by flow cytometry. Flow cytometry data showed that the number of $CD4^+CD25^+Foxp3^+$ Treg cells was considerably increased in the exosomes isolated from the Poly (I:C) primed WJ-MSCs group (Figure 3C) compared to those isolated from none-primed WJ-MSCs group ($p<0.05$) (Figure 3B) and control group (i.e., PBMCs that were only treated with PHA) ($p<0.05$) (Figure 3A). Nevertheless, no significant difference was observed between the exosomes isolated from non-primed WJ-MSCs and control group in the frequency of induced $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells ($p<0.10$) (Figure 3F).

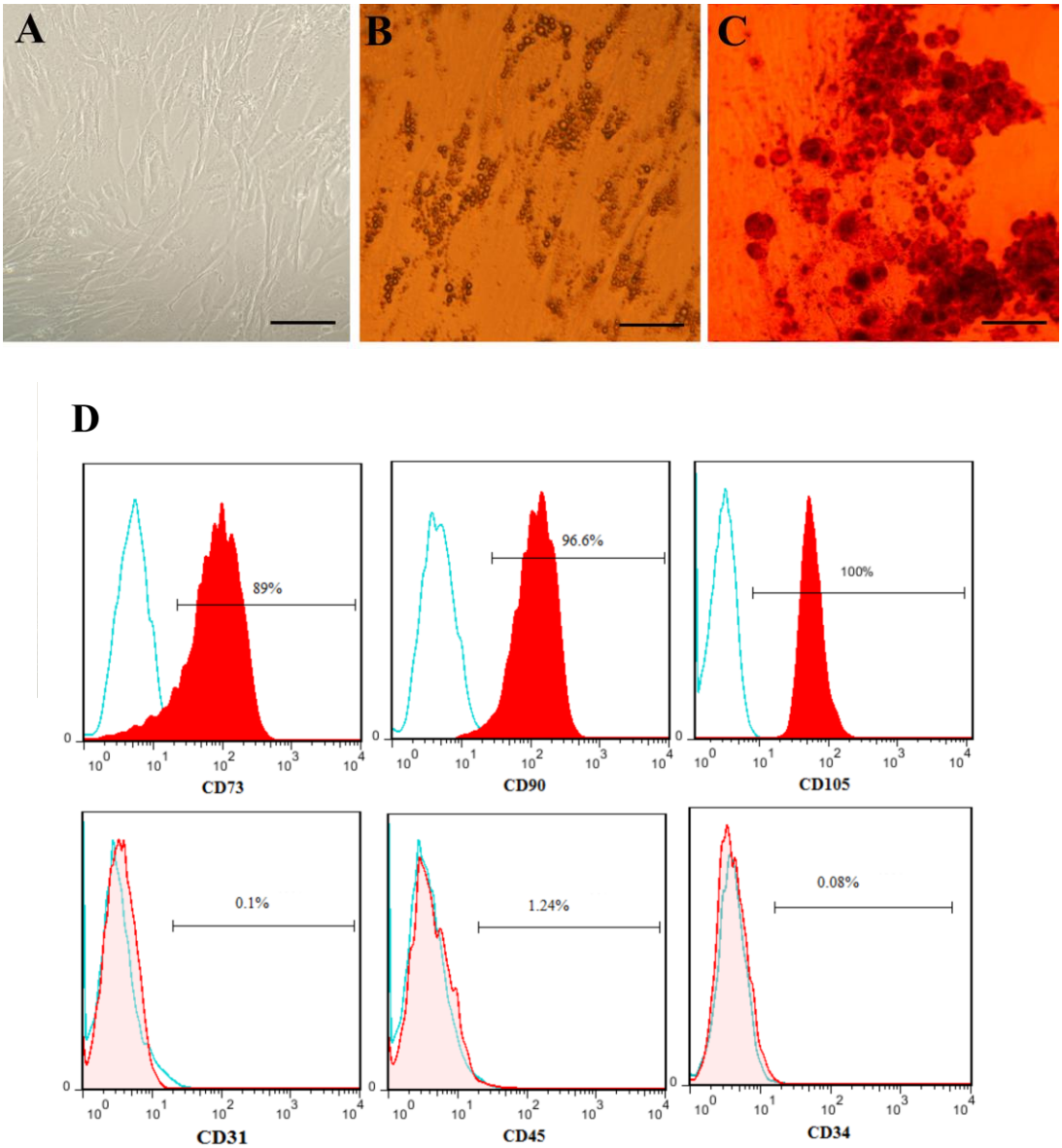
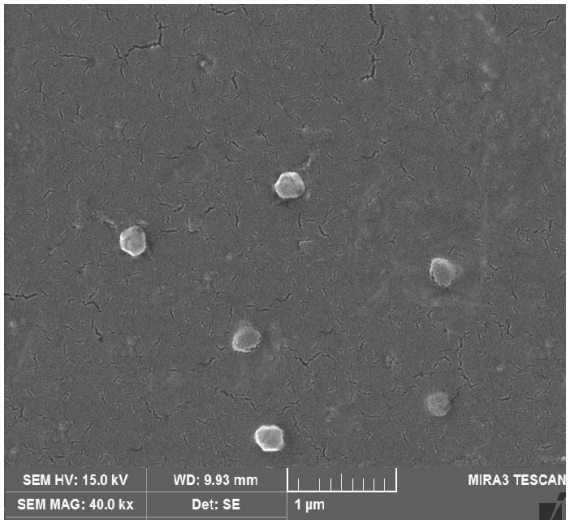


Figure 1. Isolation and characterization of human Wharton's Jelly Mesenchymal Stem Cells. (A) Morphology of WJ-MSCs after about four weeks (B) Oil Red O staining of WJ-MSCs, intracellular lipid accumulation was stained bright red in adipocytes at day 21 (C) After 21 days, osteogenic differentiation was assessed by Alizarin Red S staining, calcium deposition was stained bright orange-red. (D) Flow cytometry analysis of surface markers showed that WJ-MSC were positive for CD73, CD90 and CD105 but negative for CD34, CD31 and CD45. Positive expression was based on isotype control (blue curve). Original magnifications=40×, bar=200 µm (A-C).



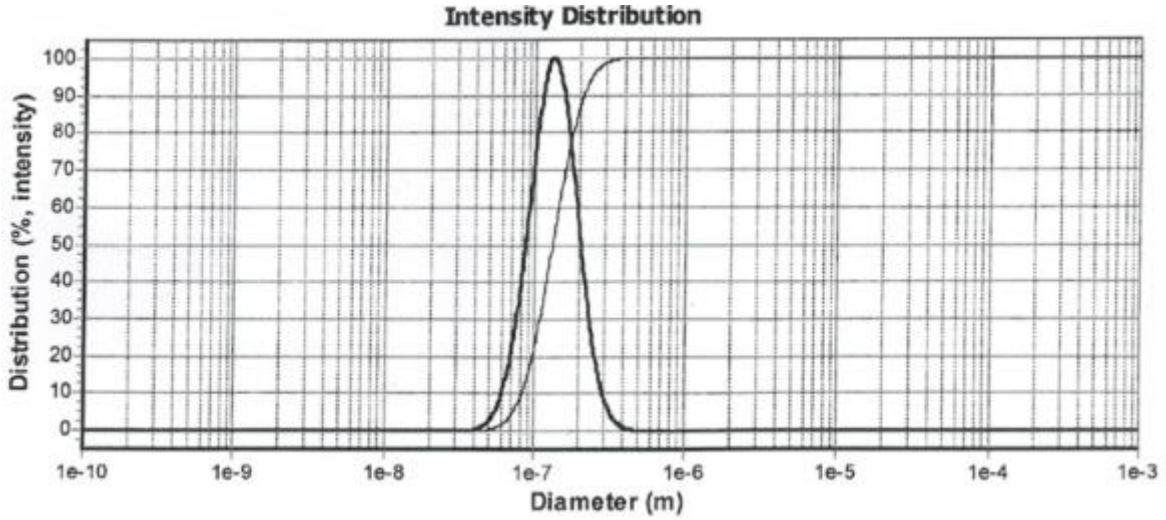


Figure 2. Characterization of exosomes isolated from human Wharton's Jelly Mesenchymal Stem Cells. (A) Scanning electron microscopy (SEM) images indicated that exosomes had a uniform spherical shape with no significant deformities. (B) Dynamic light scattering (DLS) indicated that the mean size of isolated exosomes was 132 nm.

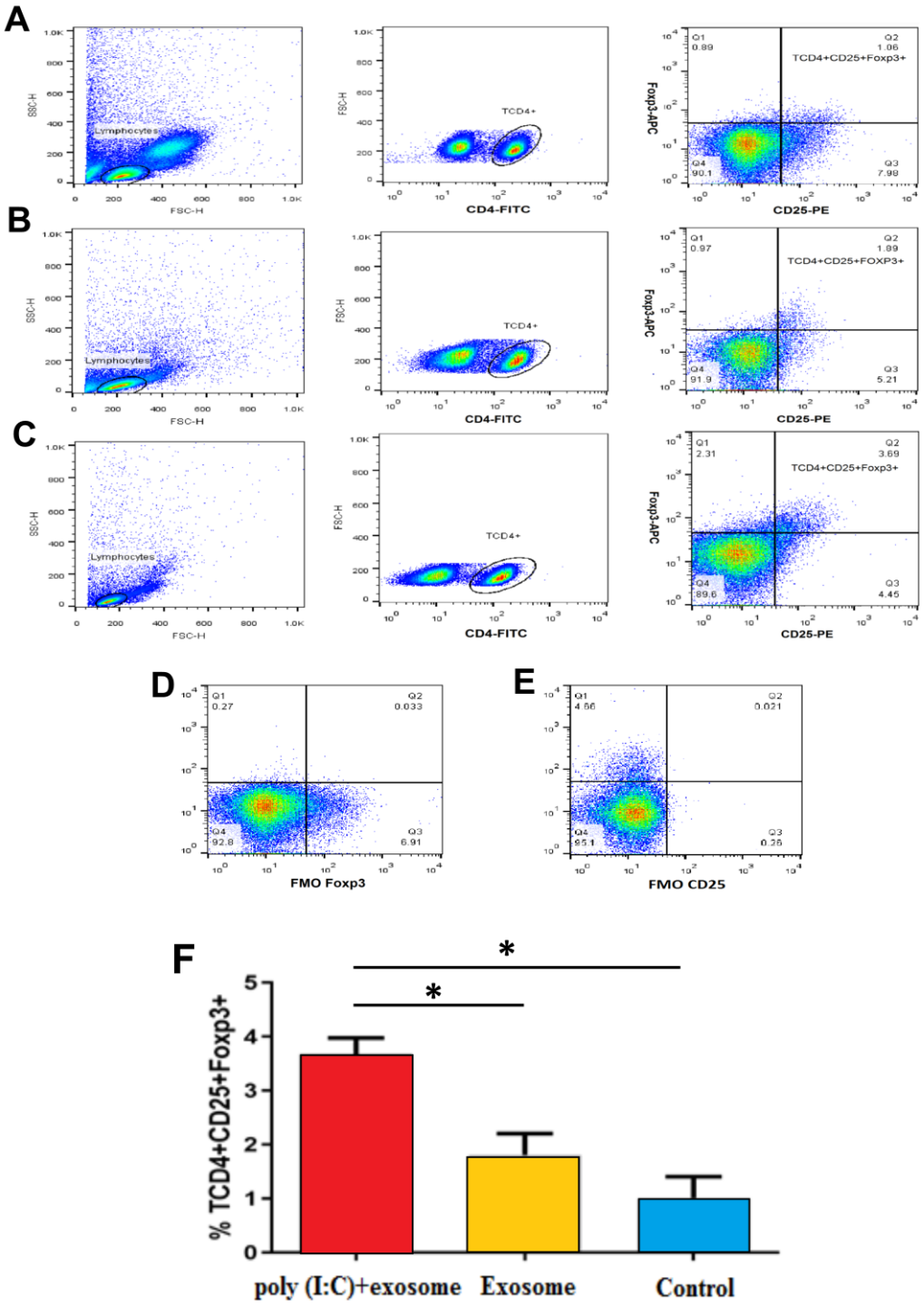


Figure 3. The effects of exosomes isolated from human Wharton's Jelly Mesenchymal Stem Cellsin different groups on CD4+CD25+Foxp3+ Treg cells by flow cytometry (A) Control group, PBMCs stimulated just with PHA (B) Exosomes isolated from non-primed WJ-MSCs group. (C) Exosomes isolated from Poly(I:C) primed WJ-MSCs group (D) FMO/Foxp3, (E) FMO/CD25 (F) Comparison of the effects of exosomes isolated from WJ-MSCs in different groups on CD4+CD25+Foxp3+ Treg cells. The frequency of CD4+CD25+Foxp3+ Treg cells was significantly increased in the exosomes isolated from Poly (I:C) primed WJ-MSCs group compared to exosomes isolated from none-primed WJ-MSCs group (*p<0.05*) and control group (*p<0.05*). The data were obtained from 3 independent experiments and were presented as the mean ± standard deviation (SD).

DISCUSSION

Mesenchymal stem cells (MSCs) have been considered an auspicious alternative therapy for various immune disorders given their exclusive biomedical potentials, including immune system regulatory properties as well as the ability to regenerate different tissues.[2,3] Nonetheless, in spite of several years of preclinical research in this field, the results of clinical trials employing these cells have been highly contradictory. These differences are caused by several factors such as weak engraftment, low survival, aging in vitro, functional shutdown after MSCs use, and donor-dependent cell diversity. Increasing the stability and effectiveness of mesenchymal stem cells is still a challenge in order to overcome the problems in mesenchymal stem cell-based therapy and subsequently attain a better therapeutic consequence.[4–6,23] Priming is a major component of functional improvement strategies. We can mention agonists of innate immune receptors among these stimulating factors that have been proposed to preserve mesenchymal stem cells and increase the therapeutic effect after injection. Priming with innate immune receptor agonists can increase the therapeutic power of MSCs as a non-specific or non-selective strategy for priming. MSCs expressing TLR receptors on their surface can detect "danger" signals. TLR3 is among the dominant targets for improving the cellular function of MSCs. After ligand binding to TLR3 and activity of downstream responses, Poly(I:C) alters the paracrine pattern of MSCs, which involves an increase in the Notch signaling pathway and immune regulatory activity such as increased Treg number and impaired proliferation.[15] In addition to cell-to-cell contact and the release of mediators in a paracrine manner, the immune system regulatory property of MSCs is dependent on the production and release of exosomes outside the cells. In recent years, exosomes as a unique subset of small extracellular vesicles have attracted great interest concerning mesenchymal stem cell research. Studies have confirmed that MSC-derived exosomes keep the immunosuppressive phenotype and mimic the therapeutic benefits of stem cells. [8,24-26] Using exosomes instead of mesenchymal stem cells is preferable because they are not living cells, so exosomes are safer and easier to store, transport and administer. [24,27-29] It has been observed that priming of MSCs with inflammatory cytokines increases the immunosuppressive power of their derived EVs. For example, in splenic mononuclear cell cultures, exosome-like EVs derived from MSCs and primed with IL-1β expressed remarkably greater levels of TGF-β and IL-10 than EVs derived from unprimed MSCs.[30] Exosomes originating from mesenchymal stem cells and pretreated with TGF-β/IFN-γ were more efficient in converting mononuclear cells to Treg.[31] In this research, we investigated the induction of human peripheral blood Treg by MSCs-derived exosomes that were taken from human umbilical cord Wharton jelly exposed to Poly(I:C). We showed that the stimulation of WJ-MSCs with 50 µg concentration of Poly(I:C) as an inflammatory stimulus increased the ratio of Treg cells in human peripheral blood cultures. Qiu et al, conducted a study focusing on the activity of TLR3 on the differentiation of MSCs originating from umbilical cord blood in mouse models of colitis. The outcome of these studies revealed that the stimulation of TLR3 leads to the reduction of inflammatory responses in mice. It was also noticed that IL-10 released by mesenchymal stem cells stimulates TLR3, preventing the differentiation of Th17 and Th1 cells and increasing the differentiation of regulatory T-cells.[32] This finding was in line with the result of our study. Moeller et al, investigated the immunomodulatory effects of murine adipose tissue-derived mesenchymal stem cells (AD-MSC) with different combinations of TLR3/4 agonists, including Poly (I:C), LPS, and the TLR4-antagonist TAK242. The results of these investigations showed that AD-MSCs stimulated with Poly (I:C) have the highest efficiency in inhibiting the proliferation of lymphocytes; in other words, short-term stimulation by TLR3 agonist enhances the immune modulatory effect of AD-MSCs both in vitro and in vivo, which can induce the production of Tregs and the expression of FGL2 (fibrinogen-like protein 2) as a molecule that affects Treg increases.[33] This finding was in agreement with our study. Fuenzalida et al, injected UCMSCs stimulated with Poly (I:C) into a murine-induced colitis model and found that the level of IDO secretion in these cells increased and that the anti-inflammatory responses were enhanced. The presence and increase of IDO leads to a change in the phenotype of the immune system towards an anti-inflammatory phenotype, namely an increase in Treg.[34] Lim et al, in a study regarding the impact effect of stromal mesenchymal cells on the treatment of mice colitis found that MSC stimulation with IFN-γ and Poly (I:C) increased IDO secretion, which has a better

therapeutic effect on mouse colitis. In other words, in mice that received MSCs treated with IFN-γ and Poly (I:C), the disease activity index was reduced and epithelial regeneration was stimulated, while enterocyte proliferation and Treg levels increased.[35] This finding was in line with the result of our study. There is certain limitation that should be acknowledged. Our research was conducted to measure only the percentage of Treg after priming with exosomes and we did not checked the level of IL-10, TGF-β and IL-35 which is the main cytokines secreted by Treg. This limitation will underscore the need for more comprehensive studies in the future.

STATEMENT OF ETHICS

The study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Ethics NO: IR.AJUMS.MEDICINE.REC.1398.032).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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