Research Paper

The Therapeutic Potential of Human Adipose and Bone-Marrow Derived Mesenchymal Stem Cells on Autoimmune Rheumatoid Arthritis

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How to cite this paper:
Tayebi Kamardi M, Hosseini S, Pourgholaminejad A, Asghari Vostikolaee MH, Sodeifi N, Sayahpour FA, et al. Lab-scale The Therapeutic Potential of Human Adipose and Bone-Marrow Derived Mesenchymal Stem Cells on Autoimmune Rheumatoid Arthritis. Advances in Skin Wound and Tissue Repair. 2026; 22(3):48-55.
doi 10.61882/aswtr.22.3.29

Article info
Received: 04/12/2025
Accepted: 20/01/2026
Published: 25/02/2026

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a systemic autoimmune syndrome which mainly affects joint spaces, and leads to the joint destruction. Mesenchymal stem cells (MSCs) possess potent immunosuppression functions that make them eligible candidates for RA cell-based immunomodulation therapy. This study aimed to compare the immunomodulatory effects of human bone marrow-derived MSCs (hBM-MSCs) and adipose-derived MSCs (hAD-MSCs) and evaluate their therapeutic potential in an experimental RA model.

Methods: The immunosuppressive effects of hBM-MSCs and hAD-MSCs on human and rat lymphocytes were first assessed in vitro using mixed lymphocyte reaction (MLR) assays. Subsequently, their therapeutic potential was evaluated in collagen-induced arthritis (CIA) rats that received daily MSC injections for 5 days. Clinical, radiographic, histopathological, and cytokine analyses were performed to assess treatment outcomes. Both hBM-MSCs and hAD-MSCs significantly inhibited rat and human lymphocyte proliferation in vitro compared with controls ($p < 0.001$).

Results: BM-MSCs and AD-MSCs similarly inhibited lymphocyte proliferation in-vitro. In-vivo administration of either MSC source significantly reduced arthritis severity compared with untreated groups ($p<0.001$). Additionally, both hAD- and BM-MSCs significantly inhibited the expression of gene coding of inflammatory cytokines tumor necrosis factor-α (TNF-α), interferon- γ (IFN-γ), interleukin-2 (IL-2), interleukin-6 (IL-6), and induced the expression of gene coding of anti-inflammatory cytokines transforming growth factor β (TGF-β) and interleukin-10 (IL-10) compared with untreated groups ($p<0.001$).

Conclusion: These results suggest that the hAD-MSCs could represent a valuable tool and a useful alternative to hBM-MSCs for stem cell-based therapy in chronic and immunologic inflammatory diseases.

Keywords: Rheumatoid Arthritis; Mesenchymal Stem Cells; Bone Marrow; Adipose Tissue; Pro-inflammatory Cytokine; MSC-mediated Immunomoregulation

Introduction

Rheumatoid Arthritis (RA) is an inflammatory autoimmune disease caused by loss of immunological self-tolerance against self-antigens, activation of auto-reactive T cells against joint components, followed by chronic inflammation and finally leading to cartilage destruction and bone erosion [1-3]. RA is characterized by hyper-proliferation and hyperplasia of synovial membrane together with massive infiltration of innate and adaptive immune cells into the synovia [2, 4-7]. Suppression of inflammatory processes would be a therapeutic strategy for development of novel immune-mediated therapies. Thus far, numerous disease-

[DOI: 10.61882/aswtr.22.3.29]

modifying anti-rheumatic biological drugs have been developed that target immune cells or block cytokine networks to regulate the pathologic consequences of the disease [8-11]. However, novel biologic therapies are subject to several limitations, notably their high cost and the potential for long-term side effects, including infections and malignancies. Reports indicate that approximately 50% of patients do not respond adequately to these treatments [9, 12-17]. Therefore, there is a need to establish safe and effective therapeutic strategies for RA, such as MSCs therapy.

MSCs are multipotent progenitor cells with the capacity to differentiate into mesodermal lineages including osteogenic, adipogenic and chondrogenic cells [17-19]. They are isolated from various sources including bone-marrow, adipose tissues and other tissues. Bone marrow-derived MSCs (BM-MSCs) are the most common cell source for cell-therapy approaches. Still their use in the clinic is limited due to the low numbers and the invasive method of aspiration [20-26]. In contrast, adipose tissue provides a large number of easily cultured and handled MSCs, making it a valuable source for cell therapy [27]. MSCs play crucial rules in regulating the proliferation, cytokine production and inflammatory function of almost all immune cells such as dendritic cells, NK, T and B cells, through cell-to-cell contact or the secretion of soluble mediators [28-35].

The immunomodulatory function of MSCs has been evaluated in many pre-clinical and clinical studies for the treatment of diseases such as graft-versus-host disease (GVHD) [36], Crohn's disease [37, 38], multiple sclerosis (MS) [39], diabetes [40] and rheumatoid arthritis [17, 20]. Although several studies have compared the immunomodulatory effects of hBM-MSCs and adipose tissue derived- MSCs (hAD-MSCs) *in-vitro* [41-43], there is little evidence comparing the therapeutic effects of hBM-MSCs and hAD-MSCs in collagen-induced arthritis (CIA) mice [17].

This study aims to evaluate the immunomodulatory and therapeutic potential of hAD- and BM-MSCs using the rat CIA model, a well-established experimental model of RA. Particular emphasis is placed on assessing the effects of xenogeneic MSC transplantation in an in-vivo setting.

Methods

Isolation and Culture of MSCs from Different Human Sources

Human BM- and AD-MSCs were obtained from Royan Cell Bank, Royan Institute, Tehran, Iran. Cells were cultured in α-MEM medium supplemented with heat-inactivated 10% fetal bovine serum (FBS, Gibco, USA), 100 IU/ml penicillin and 100 µg/ml streptomycin (Gibco, USA), 2mM L-glutamine (Gibco, USA), and 1% non-essential amino acids (NEAA; Sigma-Aldrich, USA). Culture plates were incubated at 37 °C in a humidified 5% CO_2. Passage 3 AD- and BM-MSCs were used in both *in vitro* and *in vivo* experiments.

Phenotypic Characterization of MSCs

hBM- and AD-MSCs were assessed for expression of surface markers, including CD34 (PE-conjugated Mouse Anti-Human CD34; BD Pharmingen), CD45 (PE-conjugated mouse Anti-Human CD45; BD Pharmingen), CD73 (PE-conjugated Mouse Anti-Human CD73; BD Pharmingen), CD90 (FITC-conjugated Mouse Anti-Human CD90; BD Pharmingen), and CD105 (PE-conjugated Mouse Anti-Human CD105; R&D Systems) using flow cytometry technique (FACS-Calibur™ cytometer, Becton Dickinson, San Jose, CA). All data were analyzed in comparison with appropriate isotype controls by Flow-Jo software, version 7.6.1 (Tree Star Inc., Palo Alto, CA, USA).

The differentiation capacity of MSCs toward mesodermal lineages was evaluated at 14 and 21 days after incubation in adipogenic, osteogenic, and chondrogenic induction media, as previously described [44].

Immunomodulatory Activity of hMSCs on Lymphocyte Proliferation

The suppressive effect of hBM- and hAD-MSCs on lymphocyte proliferation was assessed using a mixed lymphocyte reaction (MLR) assay. MLR was established by co-culturing the stimulator splenocytes of Sprague-Dawley (S; 1×10^5 cells) with responder lymphocytes of Wistar rats (R; 1×10^5 cells) in a 1:1 cell ratio for 3 days in α-MEM supplemented with 10% FBS, 100 IU/ml penicillin and 10 µg/ml streptomycin, and 2 mM L-glutamine. The stimulator cells were treated with 25 µg/ml mitomycin-C for 45 minutes. For fluorescent labeling of responder cells, the cells were labeled with 10 µM Carboxy-Fluorescein Succinimidyl Ester (CFSE) (Molecular Probes-Invitrogen), for 15 minutes according to the manufacturer's instructions. Cells were washed with PBS before being used in MLR. For R+S+MSC groups, 1×10^5 MSCs were co-cultured with responder and stimulator cells for 3 days. hBM- and AD-MSCs were already subjected to 10 µg/ml mitomycin-C (Sigma-Aldrich-USA) for 2 hours. A well map for this experiment included the following conditions: (R) wells, containing responder cells alone; no proliferative response was expected in these wells due to the absence of a stimulatory factor. (R+S) wells contained a co-culture of responder and stimulators cells and served as the positive control, in which the highest lymphocyte proliferative response was anticipated. (R+S+MSC) wells contained MSCs co-cultured with responder and stimulator cells. According to the hypothesis that MSCs exert anti-proliferative effect, a reduction in R cell proliferation is expected in these wells compared with the positive control group, indicating the inhibitory effect of MSCs on lymphocytes proliferation responses. The proliferation of CFSE-labeled responder cells was analyzed by flow cytometry (BD FACS-Calibur™

[DOI: 10.61882/aswtr.22.3.29]

cytometer) and Flow-Jo version 7.6.1 software. An independent MLR was also examined in the presence of human peripheral blood mononuclear cells (PBMCs), as a responder, and allogeneic PBMCs as stimulator cells with the hBM- and hAD-MSCs according to the previously described protocol [44].

Induction of Collagen-Induced Arthritis in Rats and Treatment Protocol

In the present study, 8-10-week-old male Wistar rats were used. Animals were housed in individual cages under 12-hour light/12-hour dark cycles with controlled temperature and humidity at the Royan animal facility. Collagen induced arthritis (CIA) was established according to a previously described protocol [45]. Briefly, chicken type II collagen (CII; Chondrex, Redmond, WA, USA) was dissolved at 2.0 mg/ml in 0.1 N acetic acid and emulsified in Freund's incomplete adjuvant (1:1, v/v; Chondrex) at a ratio of 1:1. The final concentration of collagen type II in the emulsion was 1 mg/ml. On day 0, rats were intradermally injected with the prepared emulsion at four evenly sites on the back (upper left, upper right, lower left, and lower right). The immunization dose for each rat was calculated by multiplying the body weight (kg) by 2. Seven days after the initial injection, all rats received an intradermal booster injection of 200 µg CII emulsified in incomplete Freund's adjuvant (1:1, v/v; Chondrex). A total of fifteen 8-week-old male Wistar rats with RA were randomly assigned to three experimental groups (n=5 per group): CIA rats treated with PBS as the control group, and CIA rats treated with either hAD-MSCs or BM-MSCs as treatment groups. The treatment protocol was initiated at the early stage of disease (day 11), when clinical sign of arthritis was evident (arthritis score>1). MSCs (2×10^6 cells per rat) suspended in 300 µl PBS were administered intraperitoneally once daily for five consecutive days to both treatment groups, while the control group received an equivalent volume of PBS. The five-day consecutive injection regimen was selected based on previously published studies demonstrating that repeated MSC administration over a short period effectively modulates immune responses and ameliorates disease severity in the CIA model (Ref). This regimen allows sufficient exposure to MSCs to exert their immunomodulatory effects while minimizing excessive handling and stress to the animals. Healthy rats were considered as a positive control.

Clinical Assessment of Collagen-Induced Arthritis

Rats were monitored daily after the boost injection for clinical symptoms of arthritis by three gold standard parameters including body weight, joint swelling, and arthritis scores, over a 30-day period. For assessment of joint swelling, the thickness of each hind paw was measured by a digital caliper (YAMAYO, Japan), and the diameter was expressed as an average for the inflamed hind paws per rat. Arthritis score was determined by using the macroscopic scoring system (score 0-5, Table 1) according to the criteria described previously [46].

A mean arthritic score value was calculated. Arthritic symptoms in each rat were graded blindly by a single observer. The body weight changes of each rat were also assessed with a balance with a precision of 0.1 g. At the end of the experiment (day 30), animals were scarified and the same animals were used for both histopathological and radiological analyses.

Table 1. Clinical scoring of CIA rat

Score	condition
0	no evidence of erythema and swelling,
1	slight swelling in the ankle or redness
2	progressive swelling, inflammation and redness from the ankle to the midfoot
3	swelling and inflammation of the entire foot, not including the toes
4	swelling and inflammation of the entire foot, including the toes
5	swelling and inflammation of the entire foot, with loss of mobility

Radiological Analysis

The ankle joint was radiographed with a radiograph equipped with a direct digital imaging system. The following radiographic scoring system was used: 0 = no swelling or bone damage, 1 = mild bone damage, 2 = moderate bone erosion, 3 = severe bone erosion. All experimental groups were repeated five times.

Histopathological Analysis

The hind paws were separated using a scalpel blade and fixed in 10% buffered formalin. The tissues were decalcified after complete fixation with 15% EDTA, dehydrated, and then embedded in paraffin. Sagittal sections (6-µm) of the whole knee joint were stained with hematoxylin and eosin (H&E). Histological sections were evaluated blindly for the presence of synovial hyperplasia, cartilage degradation, bone destruction, panuse formation, vascular proliferation, exuberant bone production, and Leukocyte infiltration. In this assessment, 4 score ranges were considered from 0 to 4 as follows: 0 = No leukocyte infiltration, synovial hyperplasia, joint erosion, cartilage degradation and exuberant bone production, 1= Minimal leukocyte infiltration, synovial hyperplasia, joint erosion, cartilage degradation and exuberant bone production, 2= Mild leukocyte infiltration, synovial hyperplasia, joint erosion, cartilage degradation and exuberant bone production, 3= Moderate leukocyte infiltration, synovial hyperplasia, joint erosion, cartilage degradation and exuberant bone production and 4= Severe leukocyte infiltration, synovial hyperplasia, joint erosion, cartilage degradation and exuberant bone production [47].

Real-time Quantitative PCR

The quantitative expression level of cytokines included tumor necrosis factor-α (TNFα), interleukin (IL)-2, interleukin-6 (IL-6), interferon gamma (IFN-γ), interleukin-2 (IL-2), interleukin-10 (IL-10), and

[DOI: 10.61882/aswtr.22.3.29]

transforming growth factor beta (TGF-β) were assessed in all experimental groups. After isolation of the Rat splenocytes, total RNA was isolated using the TRIZOL reagent (Sigma-Aldrich, T9424) in three biological repeats. The integrity and quality of extracted RNA were checked by RNA gel electrophoresis on 1% agarose gel, and its quantity was measured by Nanodrop spectrophotometer™ (Thermo Scientific). cDNA was synthesized from 1µg total RNA by the PrimeScriptTMRT reagent Kit (Takara Perfect Real Time) according to the manufacturer's instructions. Gene expression analysis was executed by quantitative RT–PCR with Applied Biosystems Life Technologies ABi Step One Plus system. All qRT-PCR reactions were performed in duplicate using SYBR Green Master Mix (Applied Biosystems Life Technologies, Inc., REF 4367659). β-actin was used as a reference gene, and all target genes were normalized relative to β-actin. Relative expression of all target genes was calculated by calibrating against the healthy group as a control group. Quantitative data analysis was calculated by the △△Ct method. The specific primers sequence for each cytokine are listed in Table 2.

Table 2. Specific primers for qRT- PCR analysis

Gene name	Primers	Length (bp)
TNF-α	FW 5′ GCCTCTTCTCATTCCTGCT 3 RW 5′ CTTGGTGGTTTGCTACGAC 3	201
IFN-γ	FW 5′ GAGCCAGATTATCTCTTTCTACCT 3 RW 5′ CGATGACACTTATGTTGTTGCTG 3	83
IL-2	FW 5′ TGAGAGGGATCGATAATTACAAGA 3 RW 5′ ATTTCCAGCGTCTTCCAAGT 3	182
IL-10	FW 5′ GACGCTGTCATCGATTTCTCC 3 RW 5′ TCACGTAGGCTTCTATGCAG 3	120
TGF-β	FW 5′ GCAACAACGCAATCTATGAC 3 RW 5′ TATTCCGTCTCCTTGGTTCAG 3	296
H-B2M	FW 5′ TCTGGTGCTTGTCTCTCTGG 3 RW 5′ ATTTGAGGTGGGTGGAACTG 3	138
IL-6	FW 5′ CTCTCCGCAAGAGACTTCCA 3 RW 5′ TCTGTTGTGGGTGGTATCCT 3	147

Measuring serum TNF-α and IL-10 in CIA rats

To measure IL-10 and TNF-α levels in serum, blood was collected and the supernatant was stored at -80°C for further analysis. The expression level of IL-10 and TNF-α was determined in the collected serum with a commercially available rat IL-10 and TNF-α ELISA kit (R&D Systems) according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism version 6.0 software. Two-tailed Student's t-test and one-way ANOVA with post hoc Tukey's Multiple Comparison tests were used for differences between two groups and more than two groups, respectively. P-value < 0.05 was considered significant. All data are reported here as Mean ± SEM of at least 5 independent experiments.

Results

Characterization of Human Bone Marrow and Adipose Derived Mesenchymal Stem Cells

Both hAD- and hBM-MSCs displayed long spindle-shaped morphology and plastic-adherent growth under standard culture conditions. Flow cytometry analysis of surface markers showed that both MSC populations were negative for hematopoietic markers of CD34, CD45 and positive for specific MSC markers of CD90, CD73, and CD105 (Fig. 1A). Both cell types exhibited adipogenic differentiation as evidenced by oil droplet formation; osteogenic differentiation indicated by calcium deposition; and chondrogenic differentiation, demonstrated by the deposition of cartilage-specific extracellular matrix detected by Toluidine Blue staining (Fig. 1B).

hAD- and hBM-MSCs have Similar Suppressive Effect on Proliferation of Human and Rat Lymphocytes in-Vitro

Given that the immunoregulatory effects of MSCs are primarily mediated through suppression of T-cell proliferation, lymphocytes proliferation was assessed using a CFSE-based MLR assay. The results demonstrated that stimulated rat responder lymphocytes (R) proliferated robustly in the presence of stimulator cells (S), whereas co-culture with either hAD-MSCs or hBM-MSCs for three days significantly suppressed lymphocyte proliferation ($n = 5$, $p < 0.001$) (Fig. 2A).

In the allogeneic MLR assay, CFSE-labeled human responder PBMCs were co-cultured with allogenic stimulator PBMCs in the presence or absence of hAD-MSCs or hBM-MSCs. Flow cytometry analysis after three days demonstrated a significant suppression of PBMC proliferation by both MSCs sources compared with the R+S control group (Fig. 2B). We observed that hAD-MSCs exhibited greater immunomodulatory efficacy than hBM-MSCs in inhibiting lymphocyte proliferation in the MLR assay; however, this difference did not reach statistical significance.

hAD-MSCs Suppress the Severity of CIA (Macroscopic)

The CIA model was induced by intradermal injections of collagen type II (CII) with adjuvant in 6–8

[DOI: 10.61882/aswtr.22.3.29]

week-old male rats at day 0 and a secondary boost at day 7. MSCs or PBS were injected intraperitoneally from day 11 for 5 consecutive days (Fig.3A). The appearance of CIA in rats started with joint inflammation after the boost injection (Fig.3B). Treatment with hBM-MSCs or hAD-MSCs significantly attenuated CIA progression compared to control groups. Joint swelling was significantly reduced from days 18 to day 30 in MSC-treated groups ($P< 0.001$) (Fig. 3C). Furthermore, hBM- or hAD-MSCs therapy significantly decreased the mean arthritis score in CIA rats between day 18 and 30 ($P< 0.001$) (Fig. 3D). The body weight graph showed a rapid weight loss after day 7 in both CIA rats and CIA+MSCs groups. However, rats treated with MSCs showed a gradual weight gain from day 14 until the end of the experiment (day30), whereas weight gain in the CIA rat group was observed from day 21 (Fig. 3E). There was a difference between the MSCs-treated group and CIA control groups, but this difference was not statistically significant.

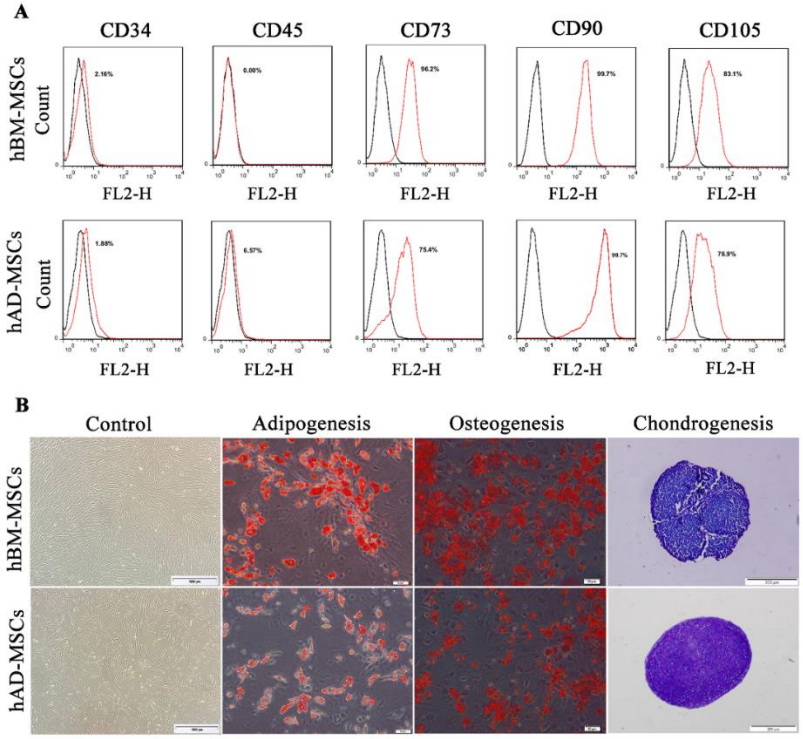

Figure 1. Characteristics of MSCs derived from hBM- or hAD-MSCs. (A) MSCs (hBM-MSCs and hAD-MSCs) were labeled with FITC- or PE-conjugated anti-MSC surface markers and analyzed by flow cytometry. (B) Differentiation capacity of hAD-MSCs and BM-MSCs into adipocytes, osteocytes and chondrocytes.

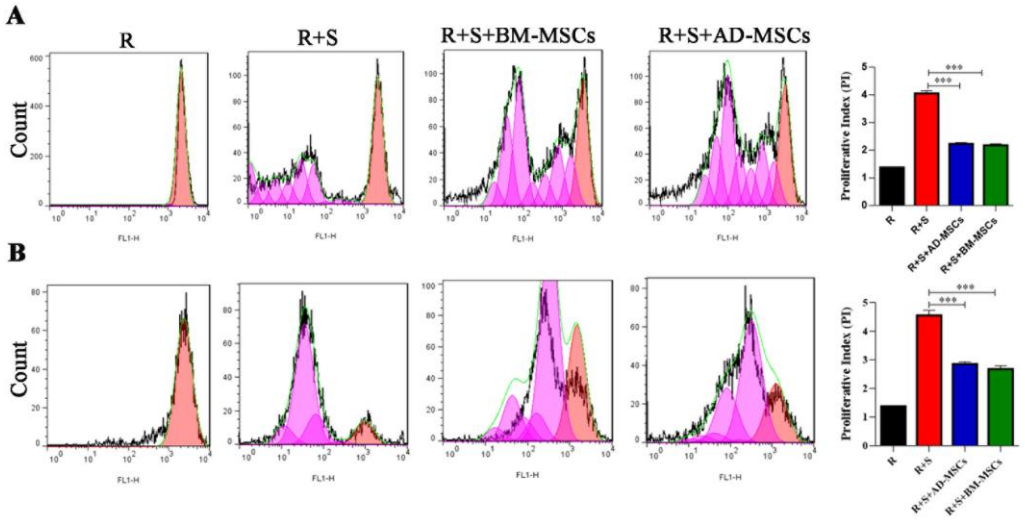

Figure 2. Immunomodulatory effect of human MSCs on human and rat lymphocytes proliferation in MLR. (A) Histogram data displays the intensity of CFSE in CFSE-labeled human PBMCs responder cells (R) when their proliferation is stimulated by allogeneic PBMCs (stimulator (S) cells), and co-cultured with hBM-MSCs and hAD-MSCs (B) Histogram data shows CFSE intensity in CFSE-labeled rat lymphocyte responder cells (R) when their

[DOI: 10.61882/aswtr.22.3.29]

proliferation is stimulated by allogeneic rat splenocytes, stimulator cells (S). Data is presented as average ± SEM of the proliferative index (PI) from at least five separate experiments.

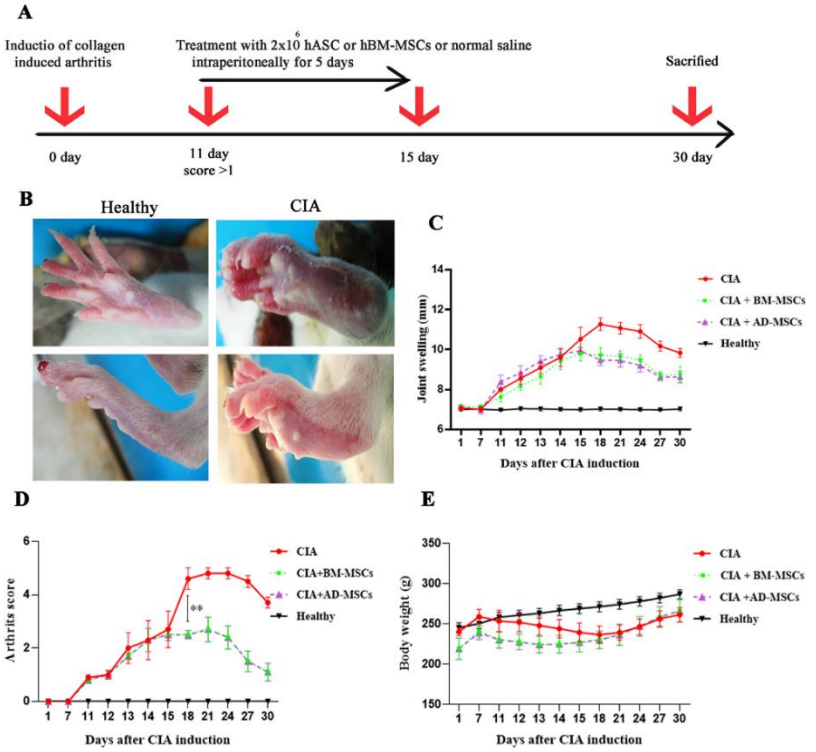

Figure 3. Effects of MSCs administration in clinical severity of arthritis in the CIA model. (A) Experimental schematic of the study design. CIA Rats with arthritis score >1 were treated with 2 x10^6 MSCs or normal saline intraperitoneally for 5 days. (B) Clinical manifestation of CIA in rats. The clinical severity of arthritis, assessed by (C) joint swelling, (D) the arthritis score and (E) the body weight.

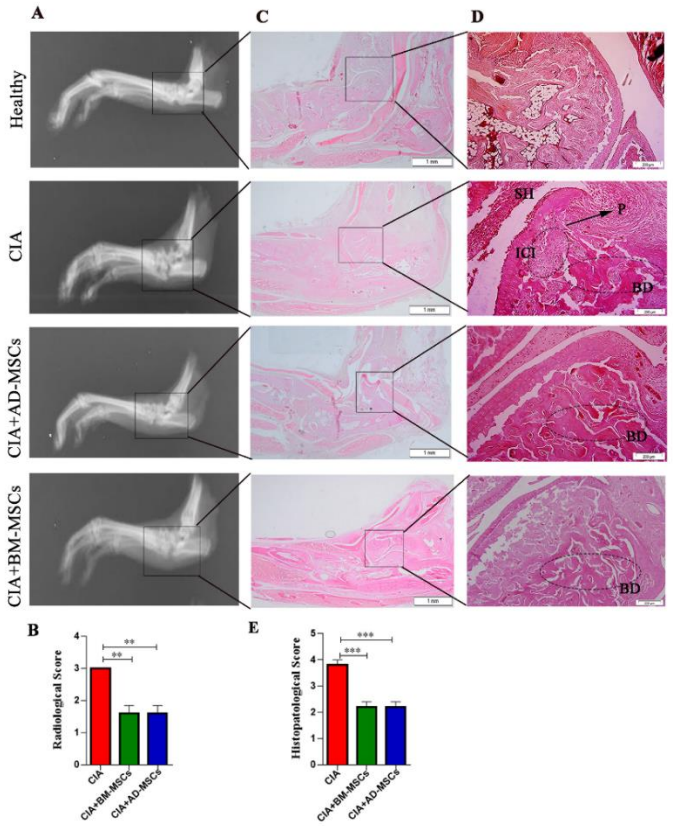

Figure 4. Effects of MSCs therapy in radiographs and histopathological scoring of the hind paws ~~of~~ in CIA rats. (A) The radiological images of the hind paws and (B) the related radiological scoring of the joints. (C) H&E -stained sections of the ankle joints. (D) H&E stained pictures of the talus bone

[DOI: 10.61882/aswtr.22.3.29]

region in the four groups with magnification 20x and (E) the histopathological scoring of the joints. SH: Synovial hyperplasia; ICI: Inflammatory cell infiltration; P: Panus formation; BD: Bone destruction.

Radiographic and Histopathological Analysis Revealed the Therapeutic Effect of MSC Therapy in the CIA Model

At day 30, X-ray analysis was performed to assess clinical severity of arthritis in CIA rats. Radiographs revealed distinct pathologic and clinical characteristics of severe arthritis, including soft tissue swelling, bone lysis, osteophyte formation, and periosteal reaction in CIA control rats. In contrast, the radiological score in MSCs treated groups was significantly improved in comparison with the CIA-untreated group ($p < 0.01$) (scored as 0-5) (Fig. 4A, B).

Histopathological examination of the tibiotarsal joints (navicular and talus) showed normal joint architecture in healthy rats. In contrast, CIA rats exhibited degenerative changes, including joint synovial hyperplasia, inflammatory cell infiltration, panus formation, vascular proliferation, exuberant bone, cartilage degradation, bone destruction, and leukocyte infiltration. Both hBM-MSCs and hAD-MSCs significantly reduced pathological scores compared to the CIA-untreated group (P value < 0.001) (Fig 4C, D, E).

Down-regulation of Splenocytes Cytokine Expression after MSC Therapy in CIA Rats

In the CIA, advancement of the autoimmune response requires the progress of autoreactive T cells and the production of their inflammatory mediators, entrance into the joint tissues, and their employment of inflammatory cells (50). We measured changes in the expression of pro- and anti-inflammatory cytokines in splenocytes by qRT-PCR analysis at day 30. The expression of IFN-ɣ, TNF-α, IL-6, and IL-2 (pro-inflammatory cytokines) in MSC- treated groups was significantly ($p<0.001$) lower than the CIA-untreated group. In contrast, the level of TGF-β and IL-10 as anti-inflammatory cytokines was robustly higher in MSCs-treated rats compared to the CIA-untreated group (Fig.5A).

MSCs Decreased Serum Levels of TNF-α and Increased Levels of IL-10

The inflammatory cytokine TNF-α was significantly decreased in CIA rats treated with MSCs compared to CIA-untreated groups ($p<0.001$) (Fig. 5B). In contrast, IL-10 in serum levels were significantly increased in CIA rats treated with hMSCs, but not in CIA-untreated groups ($p<0.001$). We observed that hAD-MSCs, with the same potency as hBM-MSCs, could downregulate TNF-α serum levels and increase the serum levels of the anti-inflammatory cytokine IL-10 in arthritis rats (Fig. 5B).

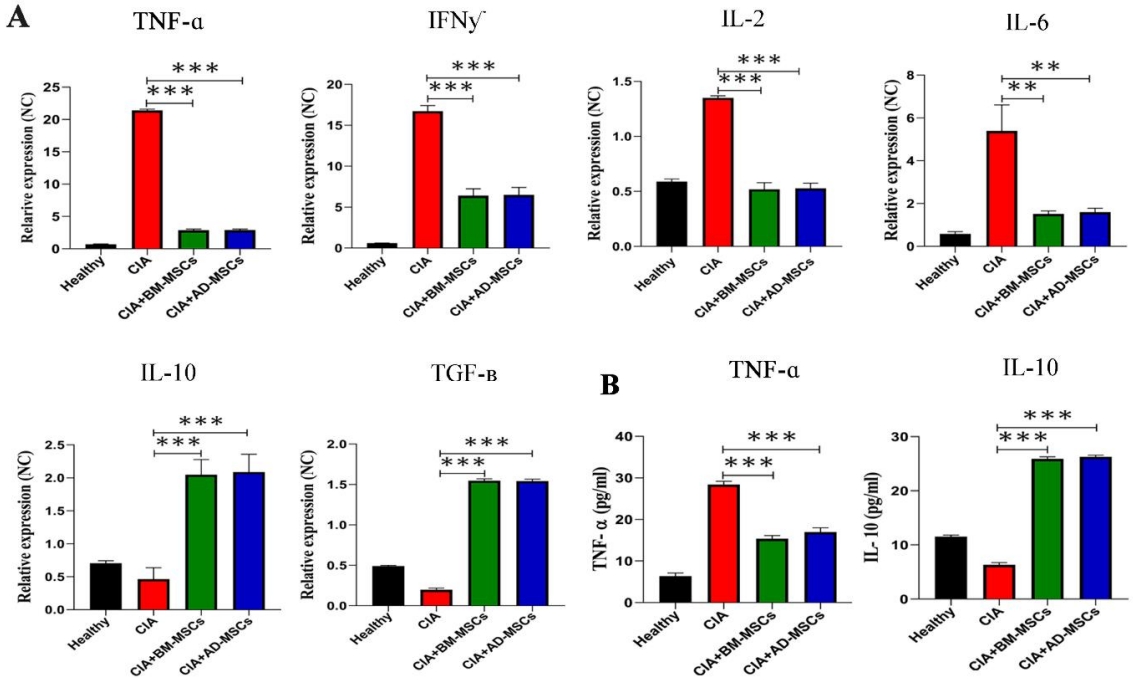

Figure 5. Effects of MSC administration on expression of cytokine profiles of splenic cells from CIA rat. (A) Real-time qPCR analysis of TNF-α, IFN-γ, IL-2, IL-6, IL-10 and TGF-β in splenocytes. (B) Cytokine production of TNF-α and IL-10 in the serum analyzed using ELISA. The results are presented as mean ± SEM. ***p <0.001, **p <0.01versus CIA controls. All experiments were performed at least five times.

[DOI: 10.61882/aswtr.22.3.29]

Discussion

MSCs are attractive cell sources for cellular therapies in degenerative diseases due to their unique properties, such as paracrine effects, multi-lineage differentiation, self-renewal potentials, and, more importantly, their immunomodulatory characteristics [48, 49]. MSCs can be isolated from a multitude of adult tissues, such as bone marrow, adipose tissue, inner organs, peripheral blood and neonatal tissues (e.g., umbilical cord, placenta, amniotic fluid, and amniotic membrane) [50]. However, hBM- and AD-MSCs appear to be the dominant sources for clinical translation in immune- and inflammation-mediated diseases [51]. There is a difference in the immunomodulatory ability of MSCs from different sources due to the amount of soluble cytokine receptors and the expression of paracrine factors among various MSCs [52, 53]. In the present study, we isolated, cultured and expanded MSCs from human adipose tissue and bone marrow and subsequently evaluated the immunosuppressive and therapeutic effects of both cell types under in vitro and in vivo conditions.

We first characterized isolated MSCs by immunophenotyping and by their functional differentiation capacity into three mesodermal lineages, and they were approved based on the criteria proposed by the International Society for Cell Therapy (ISCT) [54]. The immunomodulatory effects of hBM-MSCs and hAD-MSCs were evaluated using MLR systems by inhibiting human and rat lymphocyte activity and proliferation. Our results showed that both cells had the same suppressive and anti-inflammatory effects on the proliferation of human and rat T cells. The immunomodulatory properties of BM-MSCs [55] and AD-MSC [56] were previously investigated. Melief et al. reported that hAD-MSCs had a stronger immunomodulatory effect than BM-MSCs, which was associated with higher secretion of immunomodulatory cytokines, such as interleukin-6 and TGFβ [43]. Comparative studies have also reported similar inhibitory effects of hBM- or hAD-MSCs on T-cell proliferation and activation [57, 58]. The suppressive effect of MSCs on T-cells proliferation depended on several factors, such as the secretion of anti-inflammatory cytokines (TGF-β and IL-10), indoleamine 2,3-dioxygenase (IDO), interleukin-6 (IL-6), prostaglandin E2 (PGE2), and cell-cell interactions [58-66].

To find the therapeutic potential of MSCs in treating RA, we successfully established the CIA model in rats and subsequently injected the cells (hAD-MSCs or hBM-MSCs). The severity of RA was characterized and confirmed based on disease severity score, expression of pro- and anti-inflammatory cytokines, radiographic and histopathological analysis. MSCs exhibited comparable cell migration and therapeutic effects following either intravenous or intraperitoneal injection [67, 68]. In arthritis- affected joints, hyperplasia of the synovial membrane is a characteristic feature of rheumatoid arthritis pathology, identified by both hyperproliferation of synovial fibroblasts and massive infiltration of inflammatory immune cells, including CD4+T-cells and innate immune cells [4]. Based on our current data, injection of hAD-MSCs and hBM-MSCs decreased hyperplasia of the synovial tissue, leukocyte infiltrate, joint swelling and articular destruction in the CIA rat mode.

Since autoreactive T cell-mediated responses occur against collagen-rich joint antigens in the CIA model, autoreactive T cells and their cytokines play dominant pathological roles in the pathogenesis of RA [2, 3, 69]. Therefore, we assessed cytokine production by CD4+ T cells subsets in splenic cells. qRT-PCR analysis of the splenocytes showed significant suppression of TNF-α, IFN-γ, IL-6 and IL-2 from CIA rats treated with hAD-MSC or hBM-MSCs compared to the CIA-untreated group. Down-regulation of TNF-α, IFN-γ, IL-6, and IL-2 gene expression may be associated with MSCs-mediated suppression of autoreactive T cell proliferation [70]. In contrast, the expression of anti-inflammatory cytokines TGF-β and IL-10 from Treg cells increased in CIA-treated rats, which may be related to generation of Treg cells by MSCs [71]. TNF-α is identified as the dominant proinflammatory cytokine [20]. A prior investigation suggested that inhibiting TNF-α could effectively reduce the disease activity in patients with RA and improve the associated clinical signs [72]. IL-10 is also a target cytokine in RA, reducing of TNF-α secretion to induce RA improvement [73]. We therefore measured the effect of MSCs on IL-10 and TNF-α levels in serum. Our results showed that IP injection of hAD-MSCs could relieve TNF-α and increase IL-10 in CIA rat serum, similar to hBM-MSCs. The anti-inflammatory activity of MSCs in CIA rats has previously been reported to be achieved through the induction of antigen-specific Treg cells, which leads to strong inhibition of autoreactive T cells that produce anti-inflammatory cytokines [71]. Our findings agree with previous studies showing that hAD-MSCs [3] and hBM-MSCs [20] have therapeutic effects and reduce the severity of experimental arthritis. Based on our results, hAD-MSC showed similar therapeutic efficacy to hBM-MSC in CIA, as assessed by clinical scoring of arthritis, decreased expression of pro-inflammatory cytokines and heightened expression of anti-inflammatory cytokines. The ability of the hAD-MSC to reduce TNF-α and increase IL-10 levels in serum was similar to that of hBM-MSCs. Our findings is in agreement with other studies and revealed that human hAD-MSCs have functionality equal to hBM-MSCs in suppression of T-cell responses, inhibition of

[DOI: 10.61882/aswtr.22.3.29]

proinflammatory cytokines, and cartilage destruction [51, 66]

Conclusions

In summary, our results showed that hAD-MSCs and hBM-MSCs had immunomodulatory effects, suppressed the proliferation of human and rat lymphocytes in the mixed lymphocytes reaction and exerted immune regulatory effects in CIA rats. According to our results, hAD-MSCs had the same potency as hBM-MSCs, and improved the severity of arthritis in the hind paws of CIA rats by reducing bone/cartilage destruction and inflammatory cell infiltration, regulating the expression of pro-inflammatory mediators and upregulating anti-inflammatory cytokines. Therefore, hAD-MSCs could be an alternative to hBM-MSCs for cell therapy in chronic inflammatory disease, such as rheumatoid arthritis.

Acknowledgment

This study financially supported by Royan Institute for Stem Cell Biology and Technology. The authors express their gratitude to all members of the laboratories in the Department of Stem Cells and Developmental Biology at the Royan Institute for their generous assistance.

Funding/Supports

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author's Contributions

M.T. performed analysis, wrote the main manuscript and prepared the figures. S.H. supervised, reviewed and edited the manuscript. A.P. analyzed mixed lymphocyte reaction (MLR) assay. M-H. A, performed animal part of the study. N.S, performed histopathological analysis and scoring. F.A.S. designed primers, performed and analyzed the molecular experiments M.B.E. supervised, reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethical Considerations

All procedures were approved by the Animal Care and Ethics Committee at Royan Institute, Tehran, Iran (Ethical code: EC/92/1047).

Consent for Publication

All authors read and approved the final manuscript and consent for publication was obtained from all participants.

Data Availability

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare that they have no competing interests.

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