**BRIEF COMMUNICATION**
Iran J Allergy Asthma Immunol
February 2019; 18(1):114-119

# Immunomodulatory Effects of Human Adipose Tissue-derived Mesenchymal Stem Cells on T Cell Subsets in Patients with Rheumatoid Arthritis

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***Received: 15 June 2018; Received in revised form: 23 September 2018; Accepted: 30 September 2018***

**ABSTRACT**

Adipose-derived mesenchymal stem cells (Ad-MSCs) have been reported to suppress the effector T cell responses and have beneficial effects on various immune disorders, like rheumatoid arthritis (RA). This study was designed to investigate the effects of co-cultured Ad-MSCs on peripheral blood mononuclear cells (PBMCs) of RA patients and healthy individuals, through assessing transcription factors of T cell subsets.

PBMCs from RA patients and healthy donors were co-cultured with Ad-MSCs with or without Phytohaemagglutinin (PHA). The quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure the expression of T-box 21 (T-bet), GATA-binding protein-3 (GATA3), retinoid-related orphan receptor γt (ROR-γt) and forkhead box P3 (Foxp3).

Based on the results, Ad-MSCs greatly upregulated Th2 and Treg cell transcription factors, i.e., GATA3 and Foxp3 ($p<0.05$), and downregulated Th1 and Th17 transcription factors, i.e., T-bet and RORγt ($p<0.05$).

These results demonstrate that Ad-MSCs can result in an immunosuppressive environment through inhibition of pro-inflammatory T cells and induction of T cells with a

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regulatory phenotype. Therefore, they might have important clinical implications for inflammatory and autoimmune diseases such as RA.

**Keywords:** Adipose tissue-derived mesenchymal stem cell; Foxp3; GATA3; Rheumatoid arthritis; ROR-γ; Regulatory T cells; T-bet; T helper 1; T helper 2; T helper 17

## INTRODUCTION

Mesenchymal stem cells (MSCs) are a heterogeneous population of fibroblast-like progenitor cells which can be isolated from bone marrow (BM), umbilical cord, fat, and other tissues. They have the ability to self-renew and differentiate into various cell lineages.[1] MSCs, isolated from adipose tissue (Ad-MSCs), are currently considered a good substitute for bone marrow-derived mesenchymal stromal cells (BM-MSC) in cellular therapy. Ad-MSCs can differentiate into mesodermal cells such as osteocytes, chondrocytes, and adipocytes.[2] In recent years, the biological properties of MSCs have attracted large attention of many researchers due to the potential use of these cells in the clinical treatment of some diseases.

Ad-MSCs are considered to indicate potent anti-inflammatory and immunomodulating properties that it has been shown to be associated with inhibition of effector T cell activation with or without a concomitant increase in the number of regulatory T (Treg) cells.[3]

More recently, the abilities of MSCs to home, promote tissue repair and counteract the inflammatory status have motivated researchers to investigate them in a variety of inflammatory immune-mediated disorders, such as rheumatoid arthritis (RA).[2]

RA is an autoimmune disease exhibiting chronic joint inflammation, synovial hyperplasia as well as bone and cartilage destruction. T cells are suspects in RA pathogenesis because numerous immune cells and their related components were identified in the joints of patients with RA.[4] Although RA is generally considered to depend on IFN-γ-producing Th1 cells, recent studies have indicated an important role for Th17 cells in RA development.[5] Natural Treg cells ($Foxp3^+CD4^+CD25^+$ regulatory T-cell subset) with impaired regulatory function have been detected in RA patients' synovium, especially in the synovial fluid.[6] Th1, Th2, Th17, and Treg cells express T-box 21(T-bet), GATA-binding protein-3 (GATA3), retinoid-related orphan receptor γt (ROR-γt) and forkhead box P3 (Foxp3), respectively.

According to the immunomodulatory effects of MSCs and the important roles of T cell subsets in RA pathogenesis, here we examined the effects of Ad-MSC presence on the expression of transcription factors associated with differentiated Th1/ Th2/ Th17/ regulatory T cells.

## MATERIALS AND METHODS

### Subjects

Fourteen RA patients with mean±SD age of 47±12 years (13 females and 1 male) that were confirmed based on American College of Rheumatology (ACR) diagnostic criteria by an internist using simple sampling, were selected in Motahari Hospital, Jahrom University of Medical Sciences, Iran. The study was approved by Jahrom University of Medical Sciences Ethics Committee. RA patients with other chronic diseases such as malignancies, coronary heart disease, thyroid disease, and also infectious diseases were excluded from this study. Blood samples from 11 healthy females with mean±SD age of 36±5 years without a history of malignancy or autoimmune disorder were also obtained for utilization as the control group.

### Co-culture of PBMCs and Ad-MSCs

PBMCs and Ad-MSCs were obtained according to the previous report.[5] MSC-PBMC co-cultures were prepared using different stimulation regimens in DMEM+10% FBS+antibiotics. Four experimental groups were considered for both patients and healthy groups. The main groups included Ad-MSCs, PBMCs, PHA, and Ad-MSCs with unstimulated PBMCs to eliminate PHA effects. The two negative control groups were as the following: 1) PBMCs with PHA and 2) PBMCs without PHA. To identify the effects of MSCs on PBMCs, the PBMC-containing groups were compared with PHA-stimulated and -unstimulated PBMC groups. First, Ad-MSCs with a cell density of



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$10^5$/mL were seeded onto a 12-well plate, followed by the addition of $10^6$ PBMCs/ml after 12 hours. For T cell stimulation, we used 5 μg/mL PHA (Gibco, USA). All culture sets were incubated in a saturated humidity incubator at 37°C and 5% $CO_2$ for 24 hours. PBMC suspensions were centrifuged for subsequent RNA isolation.

### RNA Isolation, cDNA Synthesis, and Quantitative Real-Time RT-PCR

Total RNA was extracted from blood cells. Then cDNA was synthesized total RNA.[7] The expression of GATA3, T-bet, Foxp3, and RORγt genes was analyzed using a Step One Plus Real-Time PCR system and SYBR Green PCR master mix kit (both from Applied Biosystems, Foster City, CA, USA). GAPDH was used as the reference gene.[8]

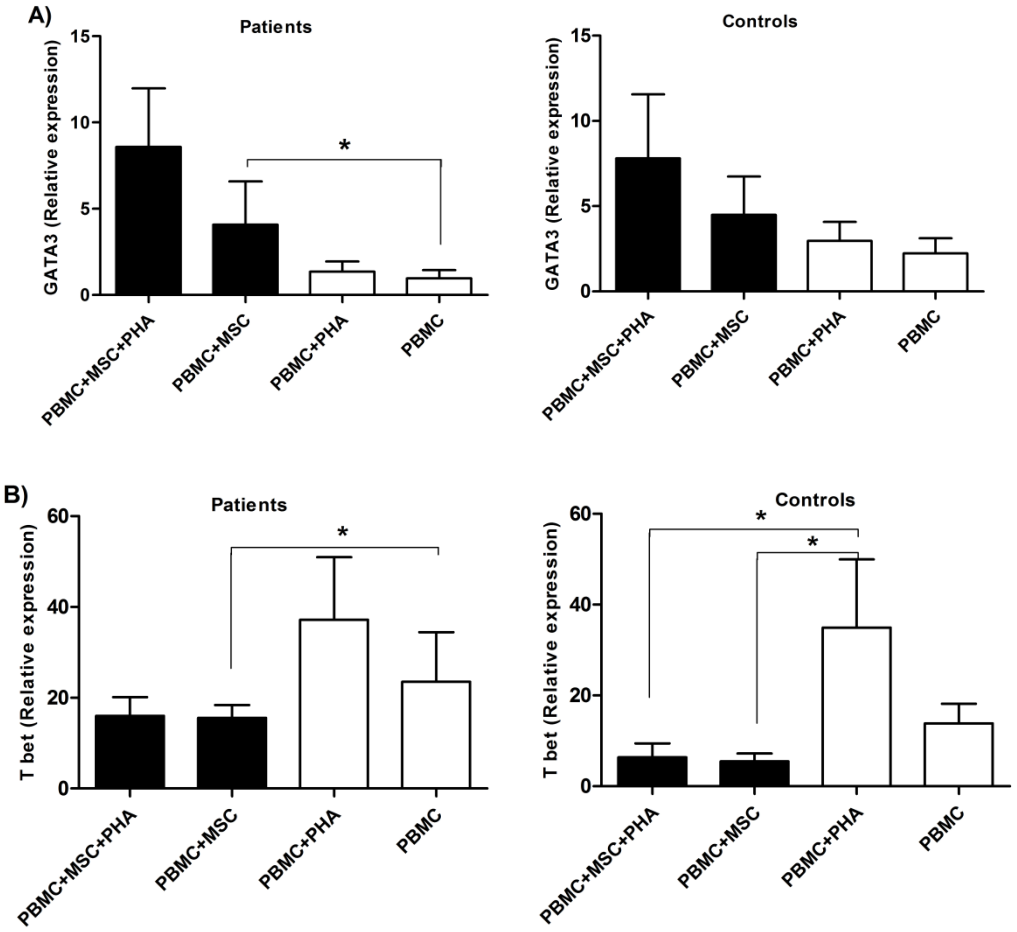
### Statistical Analysis

Data were analyzed based on nonparametric Kruskal-Wallis and Mann-Whitney *U* tests using Prism 5 software (Inc; San Diego CA, USA, 2003). $p<0.05$ was regarded as significant in all statistical analyses.

## RESULTS

### Immunomodulatory Effects of Ad-MSCs on the Gene Expression of Major Transcription Factors of T Cell Subsets

The expression of GATA3 in non-stimulated PBMCs cultured with Ad-MSC was significantly higher than that in PBMCs as the negative control groups in patients' category ($p=0.03$) (Figure 1A). Although the expression of GATA3 in PHA-stimulated PBMCs cultured with Ad-MSC was different from that in PHA-stimulated PBMCs, the difference was not statistically significant ($p=0.06$). Furthermore, no statistically significant difference in terms of GATA3 expression was observed among healthy groups.



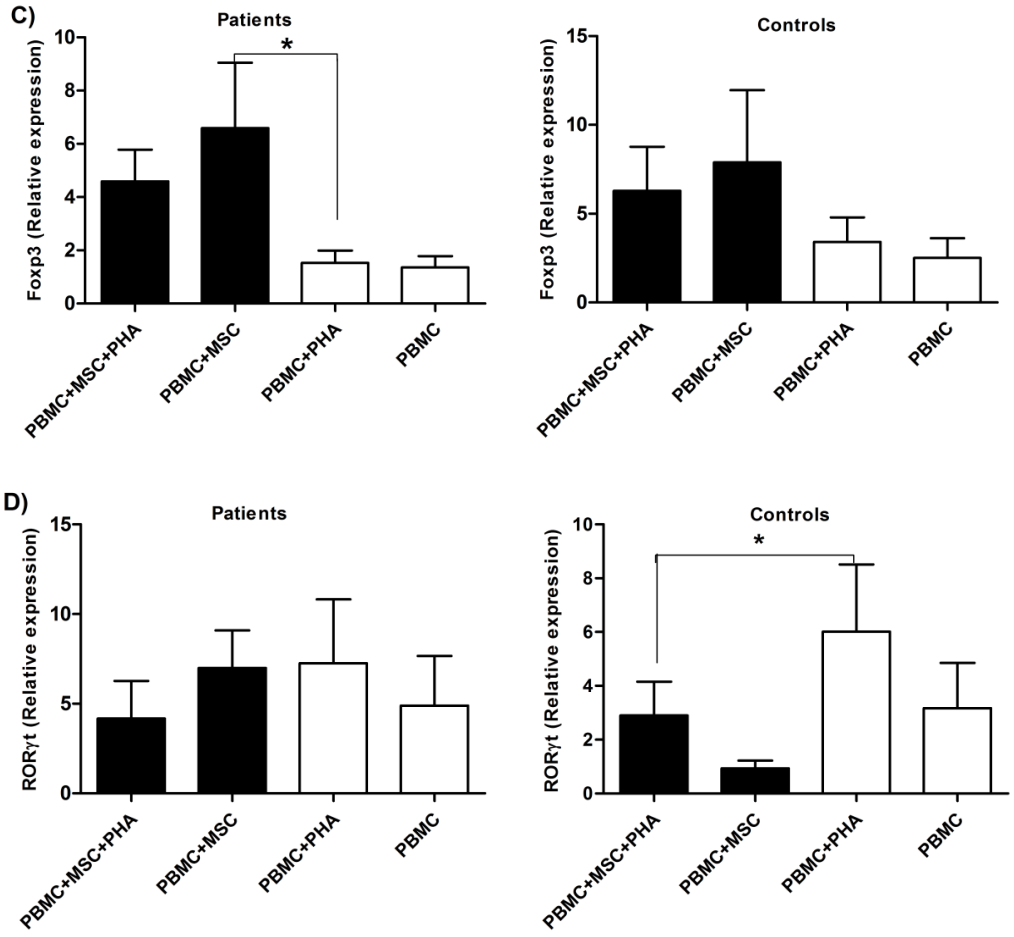



**Figure 1. Peripheral blood mononuclear cells differentiation co-cultured with Ad-MSCs in rheumatoid arthritis patients (n=14) and healthy individuals (n=11). MSCs-PBMCs co-cultures were established in different stimulation regimens in RA patients and healthy individuals. Figures are showing expression of GATA3 (A), T-bet (B), Foxp3 (C) and RORγt (D) in PBMCs under the indicated conditions. Data are showing mean±SEM of values; *=$p$<0.05; PBMCs: Peripheral blood mononuclear cells; Ad-MSCs: Adipose tissue-derived mesenchymal stem cells; PHA: Phytohaemagglutinin.**

As shown in Figure 1B, the non-stimulated PBMCs cultured with Ad-MSCs exhibited decreased expression of T-bet compared to PBMCs ($p$=0.05). In addition, the PHA-stimulated PBMCs cultured with Ad-MSC and non-stimulated PBMCs cultured with Ad-MSC were associated with decreased expression of T-bet, compared to the PHA-stimulated PBMCs in healthy controls ($p$=0.03 and 0.04, respectively).

Foxp3 expression in the PHA-stimulated PBMCs cultured with Ad-MSCs and non-stimulated PBMCs cultured with Ad-MSCs was different (but not significantly) with the PHA-stimulated PBMCs and PBMCs alone, respectively ($p$=0.06). In addition, Foxp3 expression was significantly higher in the non-stimulated PBMCs cultured with Ad-MSCs, compared to the PHA-stimulated PBMCs ($p$=0.04) (Figure 1C). Similar to GATA3 expression in PBMCs, Foxp3 expression of PBMCs in healthy volunteers was not influenced by the presence of Ad-MSCs.

RORγt expression of PBMCs in the patient group was not influenced by the presence of Ad-MSCs. But in healthy volunteers, the expression of this gene was lower in the PHA-stimulated PBMCs cultured with Ad-MSCs, compared to the PHA-stimulated PBMCs ($p$=0.05) (Figure 1D).

## DISCUSSION

MSCs play immunomodulatory and anti-inflammatory roles by modulating T and B cells and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inducing anti-inflammatory factors, which may enhance their regenerative ability.[9]

In our study, the treatment of PBMCs with Ad-MSCs significantly increased GATA3 expression in comparison to PBMCs in the patient group; however, this difference was not statistically significant in the control group. A few studies have demonstrated the effects of MSCs on immune-mediated diseases in which Th2 cell responses are effective. In accordance with our data, Aggarwal et al demonstrated that in MSC–Th2 co-culture systems, the amount of IL-4 secreted by Th2 cells is increased by MSCs.[10] In another study, MSCs altered T cell subset composition and the number of Th2 cells was significantly elevated.[11]

Based on our results, the expression of T-bet by Ad-MSC-treated PBMCs was significantly reduced. Consistently, Li et al showed that the amount of IFN-γ production by Th1 cells was reduced after treatment with Ad-MSCs.[12] MSCs have been reported to exert mainly suppressive effects on Th1 cell differentiation and effector function through indirect mechanisms.[13]

In this study, Ad-MSC-treated PBMCs showed an increased Foxp3 expression, which is a crucial transcription factor for Treg cells. Thus, MSCs can modulate immune responses via this mechanism, which play major roles in preserving self-tolerance and immune homeostasis. MSCs have been reported to induce the formation of Treg cells that are responsible for the inhibition of allogeneic lymphocyte proliferation.[14] Moreover, Aggarwal and Maccario have observed an increase in the population of Treg cells in mitogen-activated PBMCs cultures in the presence of MSCs.[10,15] According to the data reported by Bassi et al Ad-MSC therapy reduces Th1 immune response with the increased expansion/proliferation of Treg cells, consequently, the autoimmune diabetes pathogenesis is decreased in diabetic NOD/SCID mice and thus, the function of β-cells is preserved.[16]

RORγt is a transcription factor expressed by Th17 cells. The treatments applied in our study had no effect on the expression of RORγt in the patient group. However, in the control group, RORγt expression was significantly reduced in Ad-MSC-treated PBMCs. Several studies demonstrated that MSCs can inhibit Th17 cells in the treatment of some autoimmune diseases, in which Th17 cells are recognized as key players.[17,18] MSCs have been previously reported to change the development of naïve human $CD4^+$ T cells from the IL-17 producing cells into the expansion of a regulatory $CD4^+$ T cell phenotype characterized by the expression of Foxp3 and IL-10 production. Particularly in clinical applications, MSCs have been reported to be capable of directing fully differentiated Th17 cells towards Treg cells.[19,20]

Bai et al demonstrated that MSC administration in EAE mice favorably converts the balance between the pro-inflammatory Th1/ Th17 cell and anti-inflammatory Th2 cell responses.[21] In addition, Fiorina et al administrated allogeneic MSCs to NOD mice and observed a shift in Th1/ Th2 cell balance towards Th2 cells.[22] In agreement with previous studies, we found a Th2 shift in Ad-MSC-treated PBMCs with increased GATA3 expression and decreased T-bet expression.

Consistent with our findings, Crop et al showed that the immunosuppressive capacity of MSCs was enhanced strongly under inflammatory conditions. These changes related to the gene expression led to an increased immunosuppressive capacity of Ad-MSCs.[23] In accordance with some previous studies, our findings suggest that the more intensive immunosuppressive effects of Ad-MSCs on PBMCs of patients with RA is due to the inflammatory condition of patients with RA. Although investigation of gene expression of transcription factors showed effects of MSCs on PBMCs in RA patients, further studies of Th1, Th2, Th17 and Treg frequencies using flow-cytometry might be needed.

The capacity of human Ad-MSCs to regulate a wide spectrum of inflammatory mediators, together with the suppression of Th1 and Th17 responses, might offer therapeutic advantages along with conventional treatments using immunosuppressive drugs. The principal question is “What is the exact impact of MSCs on gene regulation in lymphocytes?” Further experiments are required to elucidate the downstream mechanisms and potential applications of this study.

## ACKNOWLEDGEMENTS

This work was financially supported by a grant from Iran National Science Foundation (INSF) (Grant No. 90005754) and Jahrom University of Medical Sciences.

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