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A Significant Decrease of BAFF, APRIL, and BAFF Receptors Following Mesenchymal Stem Cell Transplantation in Patients with Refractory Rheumatoid Arthritis

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Abstract

In the present study, we aimed to evaluate effects of autologous mesenchymal stem cells (MSCs) intravenous administration on the response of B cells, BAFF, APRIL, and their receptors on the surface of B cells at 1, 6, and 12 month follow-up periods in refractory rheumatoid arthritis (RA) patients. Thirteen patients with refractory RA received autologous MSCs. Plasma levels of BAFF and APRIL were measured employing ELISA method, followed by estimating B cell population and BAFFRs evaluation by flow cytometry technique. Gene expression of BAFF, APRIL, and their receptors on B cell surface in PBMCs was evaluated by SYBR Green real-time PCR technique. Plasma concentration of BAFF significantly decreased 1 and 6 months after the MSCT

(MSCs Transplantation). Plasma concentration of APRIL significantly decreased 1 month after the MSCT. Percentages of CD19 + B cells in the PBMC population significantly decreased 12 months after the MSCT. Percentages of BR3 + CD19 + B cells and BCMA + CD19 + B cells significantly decreased at the 12th month after the MSCT. The gene expression of BAFF in the PBMC population significantly decreased during 6, and 12 months after the MSCT. The gene expression of APRIL significantly decreased on month 6 after the MSCT. The gene expression of BR3 significantly decreased during 1, 6, and 12 months after the MSCT. The MSCT seems to decrease B cells response because of the reduced production of BAFF and APRIL cytokines and decrease the expression of their receptors on the surface of B cells.

Keywords: Rheumatoid arthritis, Mesenchymal Stem Cell Transplantation, B-Lymphocytes, BAFF Ligand, APRIL protein, BAFF Receptors.

Introduction

Rheumatoid Arthritis (RA) is a common autoimmune disease affecting 0.5 to 1% of the world's population. This health problem leads to a symmetrical polyarthritis in synovial joints with systemic inflammation and joint degeneration. Ultimately, it results in an increased risk of atherosclerosis that can result in cardiovascular problems as the most important cause of mortality in these patients (Ferri, 2009; Wei et al., 2015). B cells have key functions in the pathogenesis of autoimmune diseases owing to production of autoantibodies and cytokines that can affect natural killer cells, macrophages, and T cells (Dörner et al., 2009; Yap et al., 2018). According to the previous studies, approximately 80% of RA patients have at least one of the two following

autoantibodies: anti-cyclic citrullinated peptide (anti-CCP) or rheumatoid factor (RF)(Nell et al., 2005).

B-cell activating factor (BAFF) and A proliferation-inducing ligand (APRIL) are two cytokines from a large family of TNF promoting differentiation and survival of B cells. The excessive expression of BAFF is accompanied by the polyclonal development of B cells and the development of hyperglobulinemia and autoimmunity (Shabgah et al., 2019). In patients with RA, an increase in the APRIL and BAFF levels in serum, synovial fluid, and saliva (only BAFF) is noticed. This increase represents the function of these cytokines in the pathogenesis of autoimmune diseases simultaneous with high activity of B cells in these patients (Gaugler et al., 2013). In addition, concentrations of these cytokines in the synovial fluid are more than those of serum, thereby causing survival of B cells in the joint (Shabgah et al., 2019).

Neutrophils, as dominant cells in the joint, are the sources for a large part of the BAFF secretion. A large amount of BAFF is produced from dendritic cells in the initial phase of this disease, resulting in increased B cell proliferation. Furthermore, macrophages are considered a significant source of BAFF production in the established phase of the disease (Ancuta et al., 2017). APRILs are mainly secreted by monocytes, dendritic cells, macrophages, eosinophils, B cells, and activated T cells (Roth et al., 2001; Roschke et al., 2002). In the presence of BAFF, T cells are differentiated into TH17 and TH1 cells, while B cells are proliferated and differentiated to plasma cells and thus producing antibodies (Lam et al., 2008). In B cells, BAFF along with IL-6 causes the gene re-expression of recombination activating gene 1 (RAG-1) producing autoantibodies (Rochas et al., 2009). The excessive expression of APRIL (as BAFF homologs) enhances survival signals of T lymphocytes in vivo and results in infiltration of plasma cells in RA patients' joints. APRIL increases the secretion of inflammatory cytokines such as TNF-α, IL-1, IL-6, and APRIL (in

autocrine action) that eventually multiplies fibroblast-like synoviocytes (FLS) in joints (Zhao et al., 2014). In addition to structural and functional similarities, BAFF and APRIL also overlap with their dedicated receptors; i.e., Transmembrane Activator and CAML Interactor Protein (TACI, TNFRSF13B) and B-Cell Maturation Antigen (BCMA, TNFRSF17). BAFF can alone bonds with a third receptor, which is a B Cell Activation Factor Receptor (BR3, TNFRSF13C) (Shabgah et al., 2019).

In RA, the treatment is often carried out by anti-inflammatory agents such as DMARDs and biological agents, including anti-TNFα. It has been shown that in patients receiving these drugs, joint damage is visible and progressive in radiography even in the recovery phase, and the use of these therapeutic approaches is not effective in 30% of patients (Eseonu and De Bari, 2014; De Bari, 2015). Recently, regenerative medicine and stem cell investigation have been the subjects of intense research (Ratajczak et al., 2014). This new therapeutic procedure includes preparation of functionalized biomaterials, as well as production of effective pharmaceutical agents based on cellular therapy (Wang et al., 2012; Kim and Cho, 2013). According to previous studies, BM-derived stem cells circulate in peripheral blood (PB) at a relatively low concentration under steady-state conditions (Lapidot and Kollet, 2010). This circulation facilitates maintaining the spread of pool of stem cells in BM to bones existing in the distant parts of the body. Another important function of this circulation might be several types of such circulating stem cells that play a major role in “patrolling” peripheral tissues to prevent tissue damage and infections (Massberg et al., 2007; Marycz et al., 2016).

MSCs as multipotent stromal cells can migrate to inflamed and damaged tissues and stimulate tissue repair due to the ability to be differentiated into various cell types, including osteoblasts, chondrocytes, and myocytes cartilage. Because of their immunomodulatory effects, these cells can

regulate functions of many immune cells, including T, B, and NK cells, dendritic cells, and neutrophils. Hence, they can be used as alternative therapies in refractory RA (DelaRosa et al., 2012; Sohni and Verfaillie, 2013; Cislo-Pakuluk and Marycz, 2017). Owing to their straightforward expansion protocols and accessibility, MSCs are considered promising candidates for cellular therapy (Kornicka et al., 2017).

Accordingly, the present study aimed to investigate the effects of intravenous administration of autologous mesenchymal stem cells on the response of B cells, the plasma level of APRIL and BAFF cytokines, and the expression of their receptors on B cells surface (BR3-TACI-BCMA) 1, 6, and 12 months after MSCT in patients with refractory RA (Figure. 1).

Methods

Study Population

Based on the ACR/EULAR 2010 rheumatoid arthritis classification criteria, 15 patients (all women) with refractory RA were selected from the rheumatology department of Imam Reza Hospital (Mashhad University of Medical Sciences, Mashhad, Iran) and after obtaining written consent, enrolled in the project. Two patients were reluctant to participate in the clinical trials due to the personal reasons, so just 13 patients finished the trial. All patients continued to receive their conventional therapy including prednisolone (< 10-15 mg/day), and/or sulfasalazine (< 1-2 g/day), and/or hydroxychloroquine (< 400 mg/day), and/or methotrexate (7.5–25 mg/ week) without change in dosage and type of the medicines during 12 months follow-up.

Approval of the Ethics Committee of Mashhad University of Medical Sciences is IR.MUMS.REC.1395.548. Additionally, our research project was registered in the IRCT and ClinicalTrials.gov, which their codes mentioned in a previously study (Ghoryani et al., 2019).

Clinical and Para clinical assessments were performed based on patient visits by a rheumatologist at 1, 6, and 12 months after the mesenchymal stem cell administration.

MSCs Preparation and Blood Collection

Aspiration of patients' bone marrow was performed to obtain autologous MSCs. The isolation, culture and administration of MSCs are described in detail in the previous published study by our team (Ghoryani et al., 2019). In short, after receiving 50 ml of bone marrow from the patient's Iliac crest, the mononuclear cells were isolated using Ficoll density gradient centrifugation (Cedarlane, Canada) and cultured in 75-cm2 flasks (SPL, South Korea) with alpha minimum essential medium (alpha-MEM, Caisson, USA) containing 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (Caisson) in a humidified incubator at 37 °C under 5% CO2 for separation of MSCs of bone marrow. The supernatant was changed every 3 days and all cells were harvested within 3 to 4 weeks after reaching the confluence of 80%. All patients received once $1*10^6$ MSCs per kilogram of body weight through intravenous administration.

10 ml of blood samples were collected in anticoagulant EDTA tubes to isolate plasma and PBMCs before the MSCT and during the follow-ups of 1, 6, and 12 months after the MSCT.

Cell isolation and flow cytometry

The isolation of PBMCs from fresh blood containing EDTA was done by Ficoll Hypaque density gradient centrifugation method, and cells were stained using anti-human monoclonal antibodies, namely FITC-CD19, Per CP5.5-BR3, PE-TACI, APC-BCMA and relevant isotypes (Biolegend, USA). Flow cytometric staining was carried out according to the company instructions for cell surface receptors. In short, the presence of these receptors on the surface of B cell was done by staining PBMCs ($1* 10^6$/100 mL) by antibody cocktails; and staining was performed after the incubation for 30 minutes in the dark at 4 °C, and then the stained cells were read by FACSCalibur

flow cytometer (BD Biosciences, USA). The obtained data were analyzed using FlowJo 7.6.2 (TreeStar, USA).

Enzyme-linked immunosorbent assay

Plasma was collected to measure levels of BAFF and APRIL by the Enzyme-linked immunosorbent assay (ELISA), and kits were kept at -20 °C until use. ELISA kits for measuring plasma levels of BAFF (SEB686Hu) and APRIL (SEB750Hu) were prepared from cloud clone Company according to the manufacturer's instructions (http://www.cloud-clone.com/). Enzyme activities were determined at an optical density of 450 nm.

RNA extraction, cDNA synthesis, and Real-Time PCR analysis

RNA was extracted from PBMC cells and converted into cDNA (according to instructions of kit Yekta Tajhiz Azma kits) to measure gene expression of BAFF, APRIL and their receptors (BR3, TACI, BCMA). Quality and quantity of extracted RNA and cDNA were evaluated by NanoDrop ONEC model from Thermofisher Company. Primer3Plus free online software (http://primer3plus.com/), beacon designer 7.9 (premier biosoft international, USA) and NCBI Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast) were used to design and determine the specificity of primers for each gene. Forward and reverse primers for all target genes and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene were designed in house and presented in table 1.

The SYBR green real-time PCR was performed in the RotorGene 6000 thermal cycler (QIAGEN, Germany) machine in the volume of 10 µl containing 5µl of SYBR® Premix EX Taq II (2X) (Takara Bio Inc., Japan), 4µl of cDNA, 0.4µl of each primer pairs and 0.2 µl molecular water. The PCR condition was as follows: initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 10 s; annealing at 60°C for 30 s and extension at 72°C for 20 s. To

confirm the purity and specificity of the reaction, a melting curve analysis was performed at the end of the PCR by slowly increasing (0.1°C/s) the temperature of the reaction from 65 to 95°C. The expression was analyzed according to the ΔCT method so that higher ΔCt value represents higher expression of target genes at the mRNA level.

Statistical analysis

Data analysis performed using Generalized Estimating Equations (GEE) by IBM SPSS Statistics 21 (IBM Corp, USA). The results are expressed as mean ± standard error of mean (mean ± SEM), $P<0.05$ was considered to indicate a statistically significant difference. The GraphPad Prism software 8 was used for generating graphs.

Results

Demographic data of patients

In the present study, 15 patients with refractory RA participated in the project, and only 13 of them continued follow-ups of 1, 6, and 12 months. All female patients have the age range= 33-58, mean ± SD: 44 ± 7.50 years, and disease duration= 12.16 ± 4.08. VAS score, DAS28ESR, ESR and CRP were measured and analyzed for all 13 patients (Table 2). VAS score was significantly decreased at month 6 and 12 compared to the pre-injection (P-value < 0.01, 0.001, respectively) (Figure 2). RF decreased during follow-ups of 1, 6 and 12 months after the MSCT, and the reduction was significant at month 1, 6 and 12 compared to the base line (P-value <0.05, <0.01, <0.05, respectively). Additionally, RF was significantly reduced at month 6 compared to the 1 month after the MSCT (P-value<0.05) (Figure 3, Table 3). Anti-CCP was decreased at the 12 months after the MSCT, but the reduction was not significant (Table 3).

Evaluation of plasma levels of BAFF and APRIL Before and after the MSCT

Plasma concentration of BAFF significantly decreased 1 and 6 months after the MSCT (P-value <0.001, P-value< 0.01 respectively), but the plasma concentrations of this cytokine significantly increased at months 12 after the MSCT compared with months 1 and 6 (P-value < 0.01, P-value<0.05 respectively) (Table 4, Figure 4a).

Plasma concentration of APRIL significantly decreased 1 month after the MSCT (P-value<0.05), but plasma concentrations of this cytokine significantly increased at months 6 and 12 after the MSCT compared to the 1 month (P-value <0.05) (Table 4, Figure 4b).

Evaluation of cell surface expression of BR3, TACI and BCMA in B cells of patients with refractory RA before and after MSCT

Geometric mean fluorescence intensity (GMFI) and percentages of B cells in the PBMC population, as well as expression of BR3, TACI, and BCMA on the B cell surface were determined before, 1, 6 and 12 months after the MSCT. The gating strategy for immunophenotyping of B cells, expression of BR3, TACI, and BCMA on the B cell surface is shown in Figure 5.

Percentages of CD19+ B cells significantly decreased in the PBMCs population at month 12 compared to before the MSCT and also 1 month after the MSCT (P-value< 0.01 and P-value< 0.05 respectively) (Table 5, Figure 6).

GMFI for BR3+ CD19+ B cells significantly decreased at month 12 after the MSCT compared to the before MSCT and 1 and 6 months after the MSCT (P-value <0.001), but this reduction was insignificant at months 1 and 6 after the MSCT (Table 5, Figure 7a). Furthermore, percentages of BR3+ CD19+ B cells decreased during follow-ups at 1, 6 and 12 months after the MSCT, but the reduction was significant only at month 12 compared to before the MSCT (P-value< 0.01) (Table 5, Figure 7b).

GMFI for TACI+ CD19+ B cells significantly decreased at the 1st and 12th month after the MSCT (P-value< 0.05 and P-value< 0.01 respectively), but the expression of the receptor had an insignificant increase on CD19+ B cells surface at the month 6 (Table 5, Figure 8a). The changes in percentages of TACI+ CD19+ B cells also followed the same pattern during the time periods of follow-ups, but none of changes were significant (Table 5, Figure 8b).

GMFI for BCMA+ CD19+ B cells decreased at months 1, 6 and 12 after the MSCT, but the reduction was insignificant (Table 5, Figure 9a), while percentages of BCMA+ CD19+ B cells significantly decreases at the 12th month after the MSCT (P-value< 0.05) (Table 5, Figure 9b).

Evaluation of gene expression of BAFF, APRIL and their cell surface receptors (BR3, TACI, BCMA) in the PBMCs population of patients with refractory RA before and after the MSCT

The gene expression of BAFF decreased in the PBMCs population at months 1, 6 and 12 after the MSCT (Table 6), but this reduction was only significant at months 6 and 12 (P-value < 0.01 and P-value< 0.05, respectively) (Figure 10a).

The gene expression of APRIL slightly and insignificantly increased 1 month after the MSCT, but it significantly decreased at the 6 months after the MSCT compared to before MSCT and 1 month after the MSCT (P-value<0.05 and P-value<0.01 respectively) and slightly and insignificantly increased at the 12th month after the MSCT (Table 6, Figure 10b).

The gene expression of BR3 significantly decreased 1, 6, and 12 months after the MSCT (P-value<0.05, P-value<0.01 and P-value<0.01, respectively) (Table 6, Figure 11a). The gene expression of TACI and BCMA slightly and insignificantly decreased 1, 6, and 12 months after the MSCT (Figure 11b,c).

Discussion

In the present study, we aimed to evaluate effects of autologous MSCs intravenous administration on B cells population, the plasma level of BAFF and APRIL cytokines, as well as B cell surface expression of BR3, TACI, and BCMA as receptors for BAFF and APRIL in patients with refractory RA.

The results of the present study revealed that the percentage of CD19+ B cells decreased after the MSCT in 1, 6, and 12 months follow-ups. Therefore, MSCs therapy may have decreasing effects on the proliferation of B cells, and this decreasing trend significantly continues for one year following the intervention with MSCs. The production of rheumatoid factor autoantibody also decreased during follow-ups of 1, 6, and 12 months after MSCT, and the reduction was significant in month 6 compared to before MSCT and 1 month after MSCT. Additionally, the Anti-CCP significantly decreased in month 12 after MSCT.

In patients with RA, central and peripheral checkpoints are defective and lead to production of autoreactive B cells such that in untreated RA patients, the number of autoreactive B-cells is 3.4-fold higher than in non-RA patients (Samuels et al., 2004). In one study by Béatrice Gaugler et al. on peripheral blood B cells in RA patients compared to healthy controls, it was found that the number of CD19+ B cells significantly decreased in RA patients, and the population of naive and transmission B-cells was significantly impaired in these patients (Gaugler et al., 2013). Andrea Fekete et al. showed a decrease in naive B-cells of RA patients and an increase in memory B cells (IgD-CD27+) (Fekete et al., 2007). Results of several previous studies are consistent with our findings and indicate that the MSCs could inhibit the proliferation and differentiation toward plasma cells (Smith et al., 2000; Craxton et al., 2003; Li et al., 2007; Asari et al., 2009; Koarada and Tada, 2011; Akiyama et al., 2012; Koarada and Tada, 2012; Fan et al., 2016). On the contrary, other studies indicated that MSCs could increase the proliferation and differentiation of B cells to

plasma cells (Klinker and Lundy, 2012; Menard et al., 2013; Brandau et al., 2014; Fan et al., 2016). Asari et al. reported that MSCs could inhibit the proliferation of LPS-stimulated B cells via reduction of B lymphocyte-induced maturation protein-1 (Blimp1) mRNA, and there is an association between the level of inhibition with ratios of B cells and MSCs in the medium. Moreover, they showed that the ratio of IgM and IgG secreting plasma cells was decreased and increased, respectively (Asari et al., 2009). Corcione et al. indicated that Bone Marrow Mesenchymal Stem Cells (BM-MSCs) inhibited the B cell proliferation by blocking the G0/G1 phase of the cell cycle. This study revealed that production of IgM, IgG and IgA from plasma cells was downregulated following treatment of cultured B-cells with MSCs (Corcione et al., 2006). Another study on peripheral blood lymphocytes revealed that MSCs could inhibit the proliferation of B cells, differentiation to plasma cells, and production of immunoglobulins in the co-culture of MSCs-B cells-peripheral blood lymphocytes (PBLs), but this inhibitory function disappeared by removing CD3+ cells and thus caused an increase in the production of immunoglobulin and proliferation of B cells (Rosado et al., 2014). According to another work, the inhibition of B cell proliferation occurred only in the presence of TCD4+ cells, but the inhibition of differentiation of these cells to plasma cells was not related to the presence of TCD4. However, the results of in vitro studies demonstrated that proliferation of B cells was increased in the presence of MSCs. This result is probably due to the dissimilarities between the in vitro and in vivo conditions as the influence of various immune cells and their interaction with MSCs are different between these two conditions (Franquesa et al., 2015). According to one study by Xiaolei Ma (2013), the co-culture of BM-MSCs with B cells in an environment with optimum conditions to set up functions of most B cells dramatically reduced B-cells and production of immunoglobulin. The MSCT on MRL/lpr

mice also indicated the reduction in marginal zones (MZs), T1, T2, activated B cells, and plasma cells after 8 weeks (Ma et al., 2013).

In the present study, we investigated the impact of MSCs administration on the expression of BAFF receptors on the surface of B cells. The surface expression of BR3 and BCMA receptors decreased on the B cell surface 1, 6, and 12 months after the administration, and the reduction for BR3 was significant only in month 12 compared to before MSCT. Expression of TACI receptor on the B cell surface significantly decreased 1 and 12 months after MSCT. Our results revealed that the expression of TACI insignificantly increased on the B cell surface in month 6. In addition, the gene expression of BR3 receptor in PBMCs significantly decreased 1, 6, and 12 months after MSCT. Furthermore, the gene expression of TACI and BCMA receptors slightly but insignificantly decreased 1, 6, and 12 months after MSCT.

The expression of BAFF receptors was disrupted on the surface of B cells in autoimmune diseases like RA, indicating that abnormalities in BAFF/APRIL and their receptors might interfere with the homeostasis of B cells (Vincent et al., 2013). BAFF and BAFF receptors are extensively expressed in the synovium of individuals with severe RA (Woo et al., 2011). Rita A. Moura showed that BR3 expression significantly raised by the progression of RA disease, while TACI expression increased from the first week of onset of the disease, and BCMA expression did not differ in RA patients and the healthy group (Moura et al., 2013). Elsaeed et al. (2017) reported that BR3 expression on B Lymphocytes in established RA (ERA) patients was significantly higher than that in early RA patients, but there was no significant alteration between early RA patients and the healthy group (Elsaeed et al., 2017). Slyer et al. reported that the level of BR3 expression in rheumatoid synovitis tissue samples was much greater than that in TACI (Seyler et al., 2005). In patients with RA, the

expression of BCMA increased on the surface of memory and naïve B cells, while the expression of TACI decreased on the surface of memory B cells (Gaugler et al., 2013).

We found that the MSCT could reduce the production of BAFF and APRIL by affecting various immune cells. Our results indicated that the BAFF plasma concentration significantly decreased 1 and 6 months after the MSCs administration compared to before MSCT, but it was significantly increased 12 months after the administration compared to 1 and 6 months after it. However, the increase was not significant compared to the time point zero. Administration of MSCs could reduce the secretion of BAFF cytokine, but the effect decreased 12 months after the administration, suggesting the requirement for the re-administration of MSCs. Results of BAFF gene indicated that the expression of this gene decreased during follow-up periods 1, 6, and 12 months after the MSCs therapy. Nevertheless, the reduction was significant only on months 6 and 12 compared to before the intervention.

Regardless of a decrease in the gene expression of BAFF in PBMCs in month 12, the plasma level of this cytokine significantly increased. The controversy between the results of the gene expression and protein levels of BAFF might be owing to the difference between the cell sources involved in producing BAFF, as neutrophils are able to produce this cytokine in addition to PBMCs (Shabgah et al., 2019). The co-culture of BM-MSCs with DC cells showed that MSCs could reduce the production of BAFF from DCs and inhibit the additional activity of B cells (Yan et al., 2014). It is possible that MSCs in our study could employ the above mechanism to reduce the production of BAFF from DCs and decreased the expression of BAFF and APRIL receptors on the surface of B cells by affecting B-cells.

BAFF and APRIL concentrations were greater in the synovial fluid than in serum, which probably plays a role in maintaining B cells in joints (Shabgah et al., 2019). Therefore, the inhibition of the

activity of these cytokines can improve symptoms of the disease and delay its progress in mice models and clinical trials. According to one study by Mitchell Thorn on induced arthritis by collagen in rats, the production of autoantibodies was associated with the level of BAFF (Thorn et al., 2010). Furthermore, the increased concentrations of BAFF and APRIL in the plasma of seropositive patients in terms of Anti-CCP and RF were higher than in seronegative patients. This result may suggest that increased plasma levels of these cytokines contribute to the autoantibody production and synovitis and development of RA (Bosello et al., 2008; Zhao et al., 2014). According to the results obtained by J Zhao et al., the serum level of APRIL was significantly higher in patients with RA than in healthy individuals, whereas no change occurred in the level of BAFF. Moreover, the ratio of BAFF to the number of B cells (BAFF/ B cell number) in the peripheral blood of RA patients was higher than in healthy individuals (Zhao et al., 2014). MSCs can lead to reduction of B cells proliferation and their differentiation to plasma cells by reducing the production of BAFF from DCs and inhibiting the secretion of IFN-γ from T cells (Fan et al., 2016). In addition, Pérez-Simon J. A. et al. reported that the mechanism of increasing the regulation of immune response of Bregs by MSCs was related to reduction of BAFF serum levels and an increased ratio of naive and memory B-cells (Pérez-Simon et al., 2011). In one study on balb/c mice, it was found that BM-MSCs could inhibit the proliferation of B-cells by inhibiting the production of BAFF from DCs. The co-culture of MSC-DC-B-cell also confirmed this finding. In one study on MRL/lpr mice under treatment with BM-MSCs, it was found that levels of TGF-β increased after 8 weeks of decline in the serum levels of BAFF and IL-10. BM-MSC cells could reduce the production of BAFF and subsequently inhibit additional responses of B cells by affecting DCs. The co-culture of BM-MSCs from MRL/lpr mice with DC cells in vitro proved that MSCs could reduce the production of BAFF from DCs and inhibit the additional activity of

activated B cells. MSCs reduced the expression and secretion of IFN-γ and IL10 and increased the level of TGF-β, leading to a reduction in the serum level of BAFF. Decreased BAFF down-regulates T1, T2, and mature B cells and plays a role in reducing serum levels of autoantibodies (Ma et al., 2013). In general, MSCs had inhibitory effects on T cells and could regulate responses of B cells by affecting BAFF cytokines (Ma et al., 2013).

Our results showed that plasma concentrations of APRIL significantly increased 6 and 12 months after MSCT compared to 1 month after it, but the increase was not statistically significant compared to the pre-intervention. Plasma concentrations of APRIL in months 6 and 12 returned to their initial levels before starting the intervention. It seems that the impact of MSCT on the APRIL production had been probably lower than that 6 months after it. The gene expression of APRIL in PBMCs significantly decreased 6 months after MSCT compared to the time point zero as well as 1 month after MSCT. Furthermore, the gene expression of APRIL increased in month 12 following MSCT. The discrepancy between the gene expression and plasma levels of APRIL in our results might be for several reasons. MSCs can perform their regulatory functions through microRNAs. According to one study in 2016, the MSCT improved the experimental model of RA probably through suppressing miR-548e-mediated IκB inhibition (Yan et al., 2016). Therefore, increased gene expression despite the decreased plasma levels of APRIL might be either owing to the reduction of cytokine production from other cell sources like eosinophils rather than PBMCs or owing to the regulatory functions of MSCs through microRNAs. This result demonstrates the need for further research on the influence of the candidate microRNAs on MSCT and their interactions on the immune system. Additionally, the previous report indicated that the protein levels and mRNA levels were not in agreement with each other in less than 40% of items (Kendrick, 2012).

Conclusion

Results of the present study provided a new perspective on the influence of MSCT on B-cells. The MSCT inhibited B cells by reducing the production of BAFF and APRIL cytokines as well as reducing the expression of their receptors on the B-cell surface. Our results demonstrate a significant decrease in the plasma levels of BAFF and APRIL following MSCT, suggesting the remarkable effects of MSCs on humoral responses. Employing this knowledge, we conclude that BAFF might be a good target for further studies on the immunopathogenesis of RA. Overall, we can conclude that APRIL, BAFF, and BAFF receptors play a major role in the pathogenesis of RA, and MSCT seems to be a good choice to inhibit these immunological factors.

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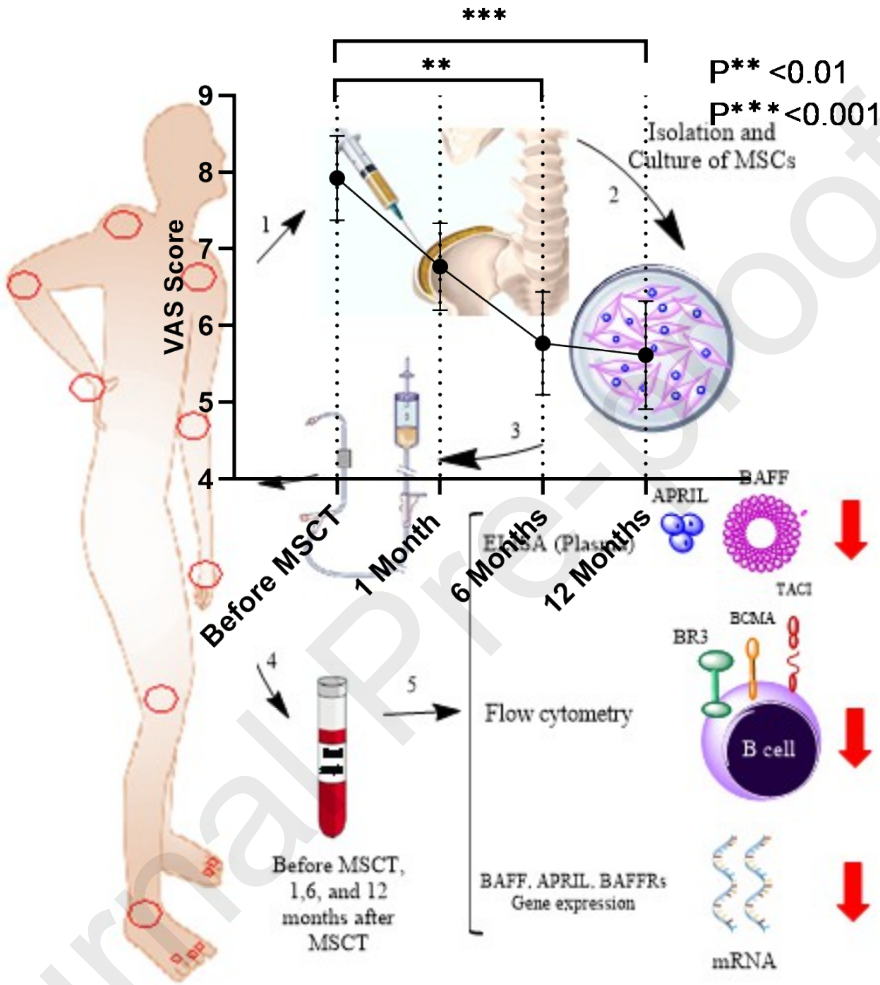
Conflict of interest statement

There is no conflict of interest to be stated.

Figure1: Schematic illustration of MSCs preparation, culture, and intravenous administration to evaluate the response of B cells, BAFF, APRIL, and their receptors on the surface of B cells in



RA patients.

Figure 2: VAS score for patients with refractory RA before and after the mesenchymal stem cell transplantation. Data are presented as mean ± SEM.

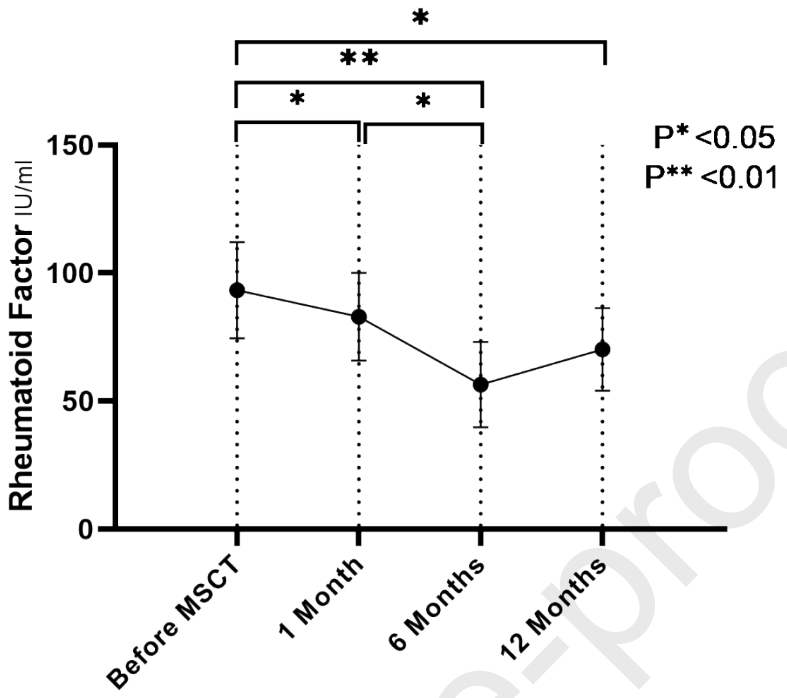


Figure 3: Rheumatoid Factor (RF) for patients with refractory RA before and after the mesenchymal stem cell transplantation. Data are presented as mean ± SEM.

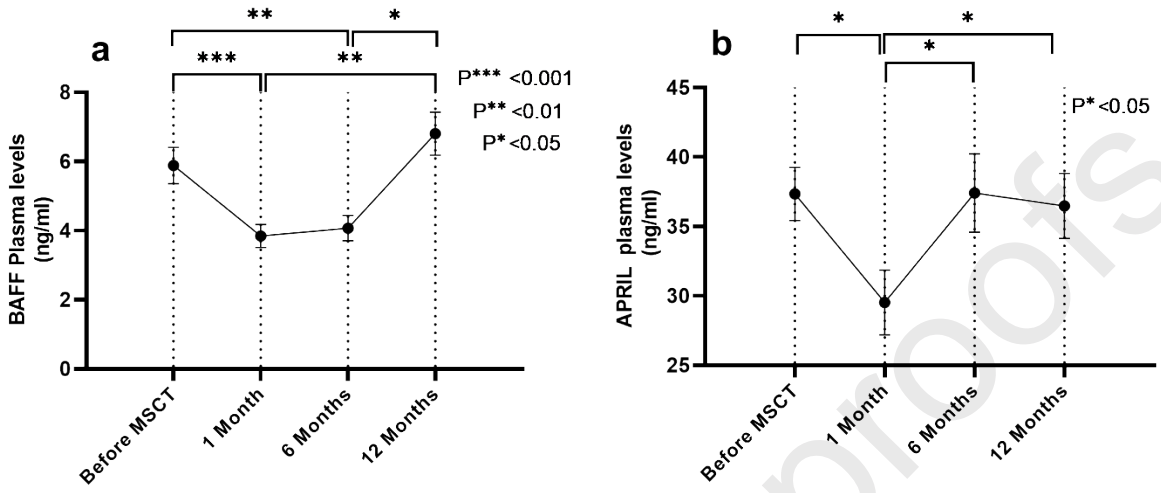


Figure 4: Plasma levels of BAFF and APRIL before and after the mesenchymal stem cell transplantation. Data are presented as mean ± SEM.

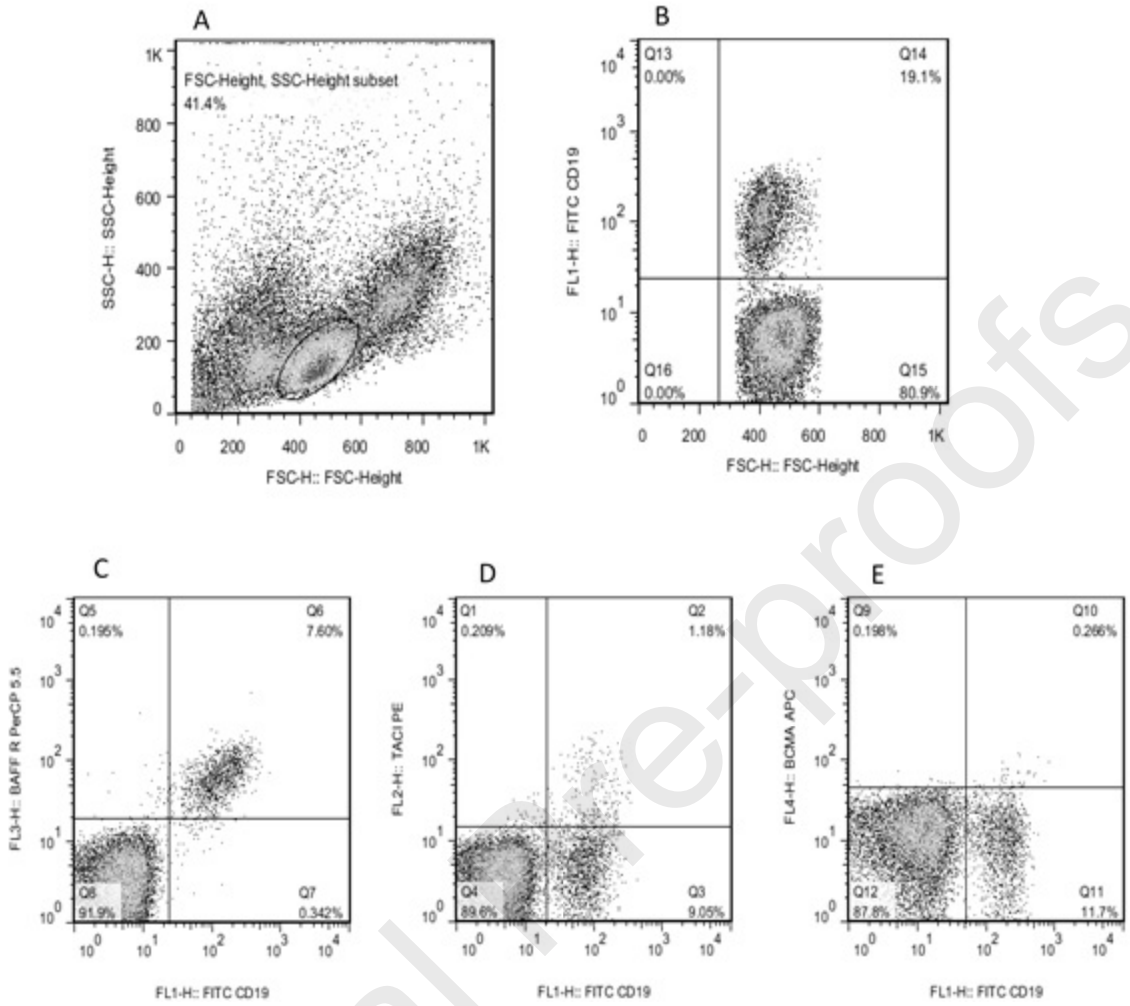


Figure 5: B-cell activating factor receptor 3 (BR3), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA) expressions on B-cell subsets. At first, to evaluate the BR3, TACI, and BCMA expression on the surface of B cells, the PBMCs are gated (A). Then, in the PBMCs series, CD19+ B cells are selected (B). Subsequently, the receptors (BR3, TACI, and BCMA) are evaluated in CD19+ B cells. Expression of BR3 (C), TACI (D) and BCMA (E) on CD19+ B cells was shown in refractory RA before and after the mesenchymal stem cell transplantation.

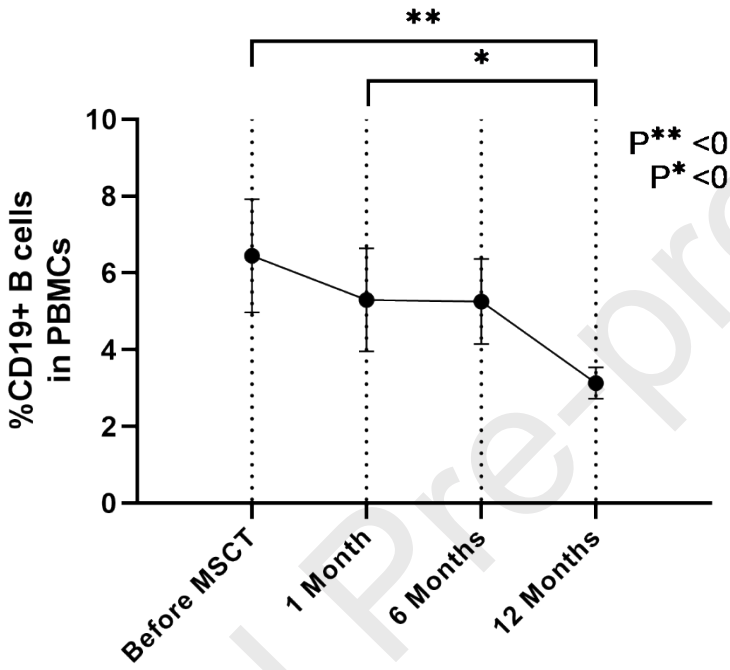


Figure 6: Flow cytometric findings in patients with refractory RA before and after the mesenchymal stem cell transplantation. Percentage of CD19+ B cells in PBMCs. Data are presented as mean ± SEM.

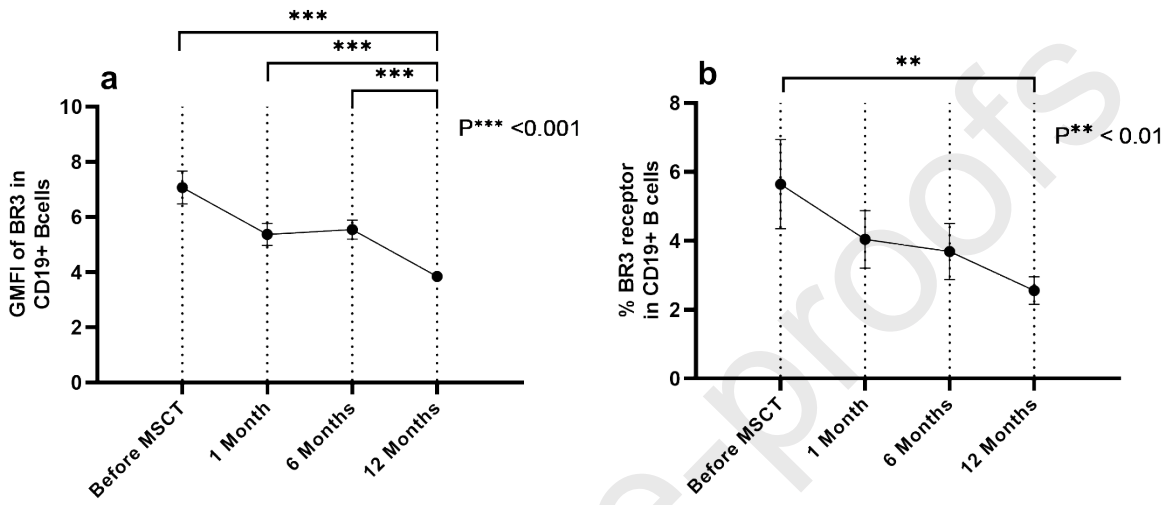


Figure 7: Flow cytometric findings in patients with refractory RA before and after the mesenchymal stem cell transplantation. (a) GMFI of BR3+ CD19+ B cells in PBMCs. (b) Percentage of BR3+ CD19+ B cells in PBMCs. Data are presented as mean ± SEM.

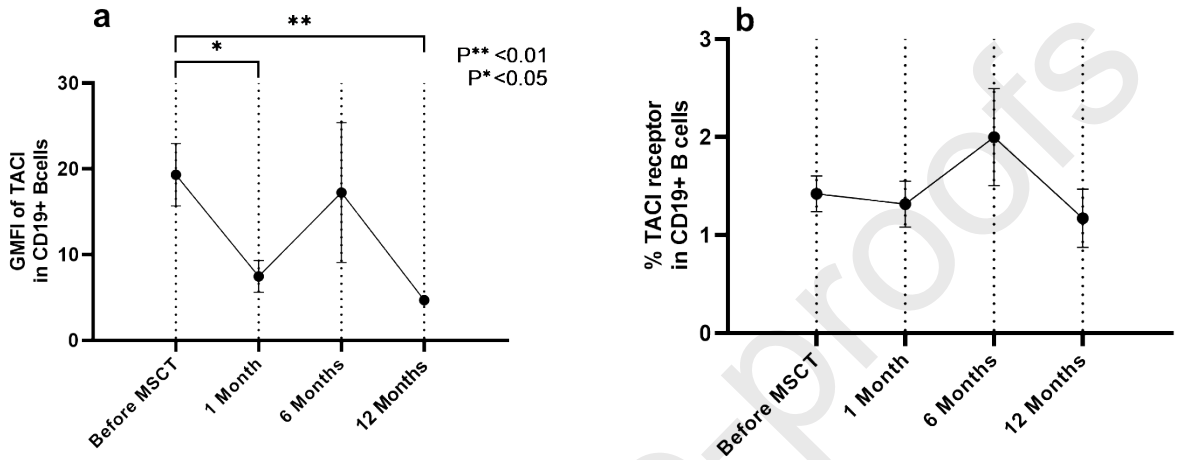


Figure 8: Flow cytometric findings in patients with refractory RA before and after the mesenchymal stem cell transplantation. (a) GMFI of TACI+ CD19+ B cells in PBMCs. (b) Percentage of TACI+ CD19+ B cells in PBMCs. Data are presented as mean ± SEM.

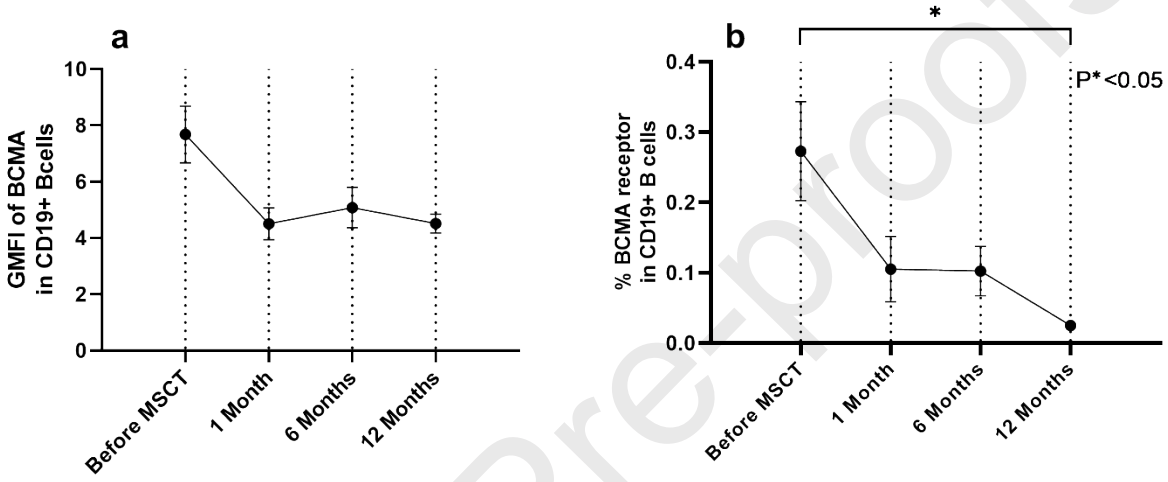


Figure 9: Flow cytometric findings in patients with refractory RA before and after the mesenchymal stem cell transplantation. (a) GMFI of BCMA+ CD19+ B cells in PBMCs. (b) Percentage of BCMA+ CD19+ B cells in PBMCs. Data are presented as mean ± SEM.

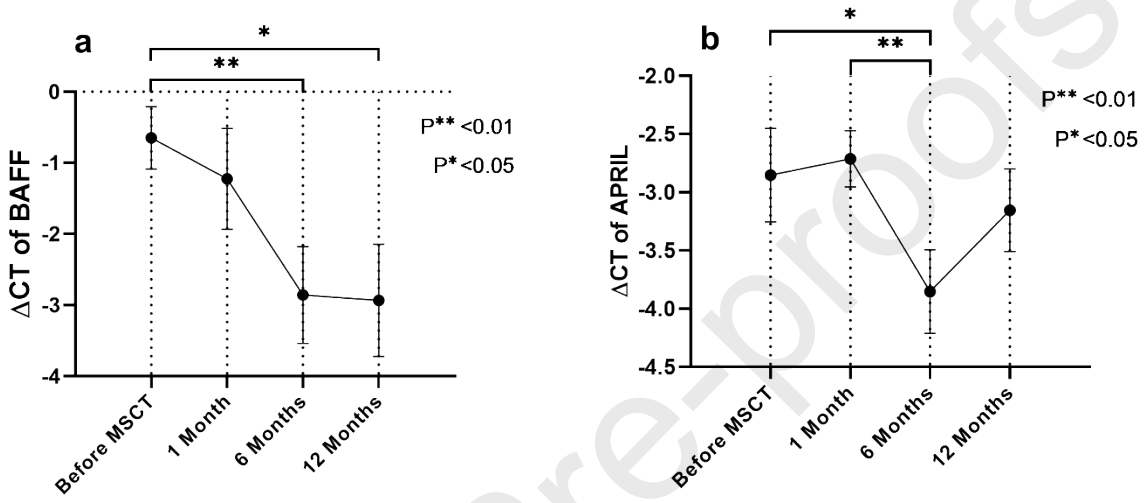


Figure 10: The gene expression of BAFF (a) and APRIL (b) on PBMCs of patients with refractory RA before and after the mesenchymal stem cell transplantation. ΔCt value is directly related to the target gene expression at the mRNA level. Data are presented as mean ± SEM.

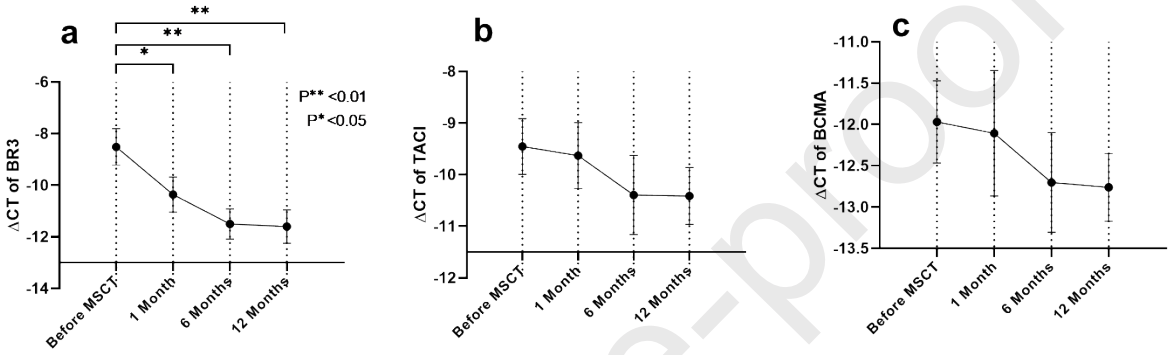


Figure 11: The gene expression of BR3 (a), TACI (b) and BCMA (c) in the PBMCs of patients with refractory RA before and after the mesenchymal stem cell transplantation. ΔCt value is directly related to the target gene expression at the mRNA level. Data are presented as mean ± SEM.

Gene Name	Primers Sequence	Amplicon

Table 1:

		size (bp)
Human APRIL	Fwd: 5'-CATGGGTCAGGTGGTGTCTC-3' Rev: 5'-AAATGGAAGACACCTGCGCT-3'	126
Human BAFF	Fwd: 5'-ACAGAAAGGGAGCAGTCACG-3' Rev: 5'-GACAGAGGGGCTTTCCTTCC-3'	105
Human BR3	Fwd: 5'-CCGGGAATCTCTGATGCCAC-3' Rev: 5'-CTATTGTTGCTCAGGGCCGG-3'	153
Human TACI	Fwd: 5'-CAAGTCTTCCCAGGATCACGC-3' Rev: 5'-CCACTGTCTGGGATGTGTGG-3'	216
Human BCMA	Fwd: 5'-GGCAGTGCTCCCAAAATGAA-3' Rev: 5'-CACTGAATTGGTCACACTTGCA-3'	131
Human GAPDH	Fwd: 5'-CACTAGGCGCTCACTGTTCTC-3' Rev:5'-CCAATACGACCAAATCCGTTGAC-3'	101

Forward and reverse primer sequences for target genes and the housekeeping gene.

Table2: Clinical and laboratory indicators before and after the mesenchymal stem cell transplantation. Data are presented as mean ± SEM.

Clinical and laboratory indicators	Before MSCT	1 month	6 months	12 months
VAS score	7.92 ± 0.54	6.67 ± 0.56	5.76 ± 0.67	5.61 ± 0.70
DAS28-ESR	5.56 ± 0.40	5.04 ± 0.44	5.06 ± 0.34	4.72 ± 0.50
CRP (mg/l)	14.12 ± 5.09	9.63 ± 3.64	8.53 ± 2.03	9.71 ± 3.64
ESR (mm)	23.75 ± 7.73	14.58 ± 4.62	14.58 ± 3.69	15.41 ± 3.74

Table 3: The level of RF and Anti-CCP before and after the mesenchymal stem cell transplantation. Data are presented as mean ± SEM.

serologic characteristics	Before MSCT	1 month	6 months	12 months
Rheumatoid Factor (RF) IU/ml	93.32 ± 18.70	82.96 ± 17.10	56.48 ± 16.72	70.16 ± 16.14
Anti-CCP IU/ml	203.46 ± 32.78	222.24 ± 33.09	204.97 ± 33.40	175.85 ± 29.40

Table 4: BAFF and APRIL concentration before and after the mesenchymal stem cell transplantation. Data are presented as mean ± SEM.

Plasma levels of	Before MSCT	1 month	6 months	12 months

cytokines				
BAFF (ng/ml)	5.88 ± 0.52	3.84 ± 0.33	4.06 ± 0.36	6.80 ± 0.62
APRIL (ng/ml)	37.34 ± 1.92	29.52 ± 2.33	37.40 ± 2.81	36.48 ± 2.34

Table 5: Expression of CD19+B cells in PBMCs population and BR3, TACI, BCMA on CD19+ B cells before and after the mesenchymal stem cell transplantation. Data are presented as mean ± SEM.

Flow cytometer markers	Before MSCT	1 month	6 months	12 months
Percentage of CD19+B cells in PBMCs	1.42 ± 0.18	1.31 ± 0.23	1.99 ± 0.49	1.17 ± 0.29
Percentage of BR3+CD19+ B cells	0.27 ± 0.07	0.10 ± 0.04	0.10 ± 0.03	0.025 ± 0.004
GMFI of BR3 on B cells	7.68 ± 1.01	4.50 ± 0.56	5.08 ± 0.71	4.51 ± 0.34
Percentage of TACI+CD19+ B cells	5.64 ± 1.29	4.04 ± 0.83	3.69 ± 0.81	2.55 ± 0.40
GMFI of TACI on B cells	7.06 ± 0.59	5.37 ± 0.40	5.54 ± 0.34	3.84 ± 0.13
Percentage of BCMA+CD19+ B cells	13.27 ± 1.55	7.58 ± 1.07	6.80 ± 0.62	4.40 ± 0.37
GMFI of BCMA on B cells	23.75 ± 7.73	14.58 ± 4.62	18.66 ± 3.69	15.41 ± 3.74

Table 6: SYBR green real-time PCR (ΔCt values) to quantify the expression of BAFF, APRIL, BR3, TACI and BCMA in PBMCs of patients with refractory RA before and after the mesenchymal stem cell transplantation. ΔCt value is directly related to the target gene expression at the mRNA level. Date are presented as mean ± SEM.

Gene expression	Before MSCT	1 month	6 months	12 months
BAFF	-0.64 ± 0.44	-1.22 ± 0.71	-2.85 ± 0.68	-2.93 ± 0.79
APRIL	-2.85 ± 0.40	-2.71 ± 0.24	-3.85 ± 0.35	-3.15 ± 0.35
BR3	-8.51 ± 0.71	-10.36 ± 0.68	-11.50 ± 0.58	-11.60 ± 0.65
TACI	-9.45 ± 0.53	-9.63 ± 0.64	-10.39 ± 0.76	-10.41 ± 0.55
BCMA	-11.96 ± 0.49	-12.10 ± 0.75	-12.70 ± 0.60	-12.76 ± 0.40

Reference

Akiyama, K., Chen, C., Wang, D., Xu, X., Qu, C., Yamaza, T., Cai, T., Chen, W., Sun, L. and Shi, S., 2012. Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. Cell stem cell 10, 544-555.

Ancuta, C., Pomirleanu, C., Mihailov, C., Ancuta, E. and Opris, D., 2017. BAFF System in Rheumatoid Arthritis: from Pathobiology to Therapeutic Targets, New Developments in the Pathogenesis of Rheumatoid Arthritis. IntechOpen.

Asari, S., Itakura, S., Ferreri, K., Liu, C.-P., Kuroda, Y., Kandeel, F. and Mullen, Y., 2009. Mesenchymal stem cells suppress B-cell terminal differentiation. Experimental hematology 37, 604-615.

Bosello, S., Youinou, P., Daridon, C., Tolusso, B., Bendaoud, B., Pietrapertosa, D., Morelli, A. and Ferraccioli, G., 2008. Concentrations of BAFF correlate with autoantibody levels, clinical disease activity, and response to treatment in early rheumatoid arthritis. The Journal of rheumatology 35, 1256-1264.

Brandau, S., Jakob, M., Bruderek, K., Bootz, F., Giebel, B., Radtke, S., Mauel, K., Jäger, M., Flohé, S.B. and Lang, S., 2014. Mesenchymal stem cells augment the anti-bacterial activity of neutrophil granulocytes. PLoS One 9, e106903.

Cislo-Pakuluk, A. and Marycz, K., 2017. A promising tool in retina regeneration: current perspectives and challenges when using mesenchymal progenitor stem cells in veterinary and human ophthalmological applications. Stem Cell Reviews and Reports 13, 598-602.

Corcione, A., Benvenuto, F., Ferretti, E., Giunti, D., Cappiello, V., Cazzanti, F., Risso, M., Gualandi, F., Mancardi, G.L. and Pistoia, V., 2006. Human mesenchymal stem cells modulate B-cell functions. Blood 107, 367-372.

Craxton, A., Magaletti, D., Ryan, E.J. and Clark, E.A., 2003. Macrophage-and dendritic cell—dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF. Blood 101, 4464-4471.

De Bari, C., 2015. Are mesenchymal stem cells in rheumatoid arthritis the good or bad guys? Arthritis research & therapy 17, 113.

DelaRosa, O., Dalemans, W. and Lombardo, E., 2012. Mesenchymal stem cells as therapeutic agents of inflammatory and autoimmune diseases. Current opinion in biotechnology 23, 978-983.

Dörner, T., Jacobi, A.M. and Lipsky, P.E., 2009. B cells in autoimmunity. Arthritis research & therapy 11, 247.

Elsaeed, A.M., Mokhtar, E.R., Abdelsalam, E.M., EL-Saied, M.E.-f. and Nageb, H.M., 2017. Relationship of Serum BAFF Levels and BAFF Receptor (BAFF-R) Expression on B Lymphocytes in Early and Late Rheumatoid Arthritis Egyptian Patients. Clinical Medicine and Diagnostics 7, 67-74.

Eseonu, O.I. and De Bari, C., 2014. Homing of mesenchymal stem cells: mechanistic or stochastic? Implications for targeted delivery in arthritis. Rheumatology 54, 210-218.

Fan, L., Hu, C., Chen, J., Cen, P., Wang, J. and Li, L., 2016. Interaction between mesenchymal stem cells and B-cells. International journal of molecular sciences 17, 650.

Fekete, A., Soos, L., Szekanecz, Z., Szabo, Z., Szodoray, P., Barath, S. and Lakos, G., 2007. Disturbances in B-and T-cell homeostasis in rheumatoid arthritis: suggested relationships with antigen-driven immune responses. Journal of autoimmunity 29, 154-163.

Ferri, F.F., 2009. Ferri's Clinical Advisor 2010 E-Book: 5 Books in 1, Elsevier Health Sciences.

Franquesa, M., Mensah, F., Huizinga, R., Strini, T., Boon, L., Lombardo, E., DelaRosa, O., Laman, J., Grinyo, J. and Weimar, W., 2015. Human adipose tissue-derived mesenchymal stem cells abrogate plasmablast formation and induce regulatory B cells independently of T helper cells. Stem cells 33, 880-891.

Gaugler, B., Laheurte, C., Bertolini, E., Pugin, A., Wendling, D., Saas, P. and Toussirot, E., 2013. Peripheral Blood B cell Subsets and BAFF/APRIL Levels and their Receptors are Disturbed in Rheumatoid Arthritis but not in Ankylosing Spondylitis. J Clin Cell Immunol 4, 2.

Ghoryani, M., Shariati-Sarabi, Z., Tavakkol-Afshari, J., Ghasemi, A., Poursamimi, J. and Mohammadi, M., 2019. Amelioration of clinical symptoms of patients with refractory rheumatoid arthritis following treatment with autologous bone marrow-derived mesenchymal stem cells: A successful clinical trial in Iran. Biomed Pharmacother 109, 1834-1840.

Kendrick, N., 2012. A gene's mRNA level measured by microarrays or RT-PCR does not necessarily predict its protein level: R 2 (or Rs) for plots of mRNA versus protein is< 0.4. Kendrick Labs Inc.

Kim, N. and Cho, S.-G., 2013. Clinical applications of mesenchymal stem cells. The Korean journal of internal medicine 28, 387.

Klinker, M.W. and Lundy, S.K., 2012. Multiple mechanisms of immune suppression by B lymphocytes. Molecular medicine 18, 123-137.

Koarada, S. and Tada, Y., 2011. RP105-negative B cells in systemic lupus erythematosus. Clinical and Developmental Immunology 2012.

Koarada, S. and Tada, Y., 2012. RP105-negative B cells in systemic lupus erythematosus. Clin Dev Immunol 2012, 259186.

Kornicka, K., Marycz, K., Marędziak, M., Tomaszewski, K.A. and Nicpoń, J., 2017. The effects of the DNA methyltranfserases inhibitor 5-Azacitidine on ageing, oxidative stress and DNA methylation of adipose derived stem cells. Journal of cellular and molecular medicine 21, 387-401.

Lam, Q.L.K., Ko, O.K.H., Zheng, B.-J. and Lu, L., 2008. Local BAFF gene silencing suppresses Th17-cell generation and ameliorates autoimmune arthritis. Proceedings of the National Academy of Sciences 105, 14993-14998.

Lapidot, T. and Kollet, O., 2010. The brain-bone-blood triad: traffic lights for stem-cell homing and mobilization. ASH Education Program Book 2010, 1-6.

Li, H., Gu, Z.-K., Li, X.-S., Hou, C.-M., Tang, P.-H. and Mao, N., 2007. Functional and phenotypic alteration of intrasplenic lymphocytes affected by mesenchymal stem cells in a murine allosplenocyte transfusion model. Cell transplantation 16, 85-95.

Ma, X., Che, N., Gu, Z., Huang, J., Wang, D., Liang, J., Hou, Y., Gilkeson, G., Lu, L. and Sun, L., 2013. Allogenic mesenchymal stem cell transplantation ameliorates nephritis in lupus mice via inhibition of B-cell activation. Cell transplantation 22, 2279-2290.

Marycz, K., Mierzejewska, K., Śmieszek, A., Suszynska, E., Malicka, I., Kucia, M. and Ratajczak, M.Z., 2016. Endurance exercise mobilizes developmentally early stem cells into peripheral blood and increases their number in bone marrow: implications for tissue regeneration. Stem Cells International 2016.

Massberg, S., Schaerli, P., Knezevic-Maramica, I., Köllnberger, M., Tubo, N., Moseman, E.A., Huff, I.V., Junt, T., Wagers, A.J. and Mazo, I.B., 2007. Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. Cell 131, 994-1008.

Menard, C., Pacelli, L., Bassi, G., Dulong, J., Bifari, F., Bezier, I., Zanoncello, J., Ricciardi, M., Latour, M. and Bourin, P., 2013. Clinical-grade mesenchymal stromal cells produced under various good manufacturing practice processes differ in their immunomodulatory properties: standardization of immune quality controls. Stem cells and development 22, 1789-1801.

Moura, R.A., Canhão, H., Polido-Pereira, J., Rodrigues, A.M., Navalho, M., Mourão, A.F., Resende, C., Campanilho-Marques, R., Dias, J.M. and da Silva, J.A.P., 2013. BAFF and TACI gene expression are increased in patients with untreated very early rheumatoid arthritis. The journal of Rheumatology 40, 1293-1302.

Nell, V., Machold, K.P., Stamm, T.A., Eberl, G., Heinzl, H., Uffmann, M., Smolen, J.S. and Steiner, G., 2005. Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis. Annals of the rheumatic diseases 64, 1731-1736.

Pérez-Simon, J.A., Lopez-Villar, O., Andreu, E.J., Rifon, J., Muntion, S., Campelo, M.D., Sánchez-Guijo, F.M., Martinez, C., Valcarcel, D. and del Cañizo, C., 2011. Mesenchymal stem cells expanded in vitro with human serum for the treatment of acute and chronic graft-versus-host disease: results of a phase I/II clinical trial. Haematologica 96, 1072-1076.

Ratajczak, M.Z., Marycz, K., Poniewierska-Baran, A., Fiedorowicz, K., Zbucka-Kretowska, M. and Moniuszko, M., 2014. Very small embryonic-like stem cells as a novel developmental concept and the hierarchy of the stem cell compartment. Advances in Medical Sciences 59, 273-280.

Rochas, C., Hillion, S., Saraux, A., Mageed, R.A., Youinou, P., Jamin, C. and Devauchelle, V., 2009. Transmembrane BAFF from rheumatoid synoviocytes requires interleukin-6 to induce the expression of recombination-activating gene in B lymphocytes. Arthritis Rheum 60, 1261-71.

Rosado, M.M., Bernardo, M.E., Scarsella, M., Conforti, A., Giorda, E., Biagini, S., Cascioli, S., Rossi, F., Guzzo, I. and Vivarelli, M., 2014. Inhibition of B-cell proliferation and antibody production by mesenchymal stromal cells is mediated by T cells. Stem cells and development 24, 93-103.

Roschke, V., Sosnovtseva, S., Ward, C.D., Hong, J.S., Smith, R., Albert, V., Stohl, W., Baker, K.P., Ullrich, S. and Nardelli, B., 2002. BLyS and APRIL form biologically active heterotrimers that are expressed in patients with systemic immune-based rheumatic diseases. The Journal of Immunology 169, 4314-4321.

Roth, W., Wagenknecht, B., Klumpp, A., Naumann, U., Hahne, M., Tschopp, J. and Weller, M., 2001. APRIL, a new member of the tumor necrosis factor family, modulates death ligand-induced apoptosis. Cell death and differentiation 8, 403.

Samuels, J., Ng, Y., Paget, D. and Meffire, E., 2004. Impaired early B cell tolerance in patients with rheumatoid arthritis, ARTHRITIS AND RHEUMATISM. WILEY-LISS DIV JOHN WILEY & SONS INC, 111 RIVER ST, HOBOKEN, NJ 07030 USA, pp. 4081-4082.

Seyler, T.M., Park, Y.W., Takemura, S., Bram, R.J., Kurtin, P.J., Goronzy, J.J. and Weyand, C.M., 2005. BLyS and APRIL in rheumatoid arthritis. The Journal of clinical investigation 115, 3083-3092.

Shabgah, A.G., Shariati-Sarabi, Z., Tavakkol-Afshari, J. and Mohammadi, M., 2019. The role of BAFF and APRIL in rheumatoid arthritis. Journal of cellular physiology.

Smith, K.M., Pottage, L., Thomas, E.R., Leishman, A.J., Doig, T.N., Xu, D., Liew, F.Y. and Garside, P., 2000. Th1 and Th2 CD4+ T cells provide help for B cell clonal expansion and antibody synthesis in a similar manner in vivo. The Journal of Immunology 165, 3136-3144.

Sohni, A. and Verfaillie, C.M., 2013. Mesenchymal stem cells migration homing and tracking. Stem cells international 2013.

Thorn, M., Lewis, R.H., Mumbey-Wafula, A., Kantrowitz, S. and Spatz, L.A., 2010. BAFF overexpression promotes anti-dsDNA B-cell maturation and antibody secretion. Cellular immunology 261, 9-22.

Vincent, F.B., Saulep-Easton, D., Figgett, W.A., Fairfax, K.A. and Mackay, F., 2013. The BAFF/APRIL system: emerging functions beyond B cell biology and autoimmunity. Cytokine & growth factor reviews 24, 203-215.

Wang, S., Qu, X. and Zhao, R.C., 2012. Clinical applications of mesenchymal stem cells. Journal of hematology & oncology 5, 19.

Wei, F., Chang, Y. and Wei, W., 2015. The role of BAFF in the progression of rheumatoid arthritis. Cytokine 76, 537-544.

Woo, Y.-J., Yoon, B.-Y., Jhun, J.-Y., Oh, H.-J., Min, S., Cho, M.-L., Park, S.-H., Kim, H.-Y. and Min, J.-K., 2011. Regulation of B cell activating factor (BAFF) receptor expression by NF-κB signaling in rheumatoid arthritis B cells. Experimental & molecular medicine 43, 350.

Yan, H., Wu, M., Yuan, Y., Wang, Z.Z., Jiang, H. and Chen, T., 2014. Priming of Toll-like receptor 4 pathway in mesenchymal stem cells increases expression of B cell activating factor. Biochem Biophys Res Commun 448, 212-7.

Yan, X., Cen, Y. and Wang, Q., 2016. Mesenchymal stem cells alleviate experimental rheumatoid arthritis through microRNA-regulated IκB expression. Scientific reports 6, 28915.

Yap, H.-Y., Tee, S., Wong, M., Chow, S.-K., Peh, S.-C. and Teow, S.-Y., 2018. Pathogenic role of immune cells in rheumatoid arthritis: implications in clinical treatment and biomarker development. Cells 7, 161.

Zhao, J., Guo, J., Wang, L., Zhou, W. and Zhang, Z., 2014. The role of a proliferation-inducing ligand (APRIL) in the pathogenesis of rheumatoid arthritis. Scandinavian journal of rheumatology 43, 462-469.

Rheumatoid Arthritis	RA
Refractory Rheumatoid Arthritis	RRA
established Rheumatoid Arthritis	ERA
Mesenchymal Stem Cell Transplantation	MSCT
Mesenchymal Stem Cell	MSC
anti-cyclic citrullinated peptide	Anti-CCP
Rheumatoid Factor	RF
B-Cell-Activating Factor	BAFF
A proliferation-inducing ligand	APRIL
BLyS receptor 3	BR3
Transmembrane activator and CAML interactor	TACI
B cell maturation antigen	BCMA
Peripheral blood mononuclear cell	PBMC
recombination activating gene 1	RAG-1
TNF Receptor Superfamily Member 13B	TNFRSF13B
TNF Receptor Superfamily Member 17	TNFRSF17
TNF Receptor Superfamily Member 13C	TNFRSF13C
Bone marrow	BM
hematopoietic stem/progenitor cells	HSPCs
endothelial progenitor cells	EPCs
peripheral blood	PB
Multiple Sclerosis	MS
Diabetes Mellitus	DM
Systemic Lupus Erythematosus	SLE
Graft-versus-host disease	GvHD
Autoimmune Hepatitis	AIH
Peripheral Blood Lymphocytes	PBLs

marginal zones	MZs
Blimp1	B lymphocyte-induced maturation protein-1

Author contributions:

Arezoo Gowhari Shabgah: designed and performed the experiments, Writing- Original draft preparation, performed the analytic calculations.

Zhaleh Shariati-Sarabi: (Rheumatologist) Introduced refractory rheumatoid arthritis patients to participate in the project.

Jalil Tavakkol-Afshari: Helped manage the project.

Ali Ghasemi: Bone marrow aspiration.

Mohsen Ghoryani: Helped to carry out the experiment.

Mojgan Mohammadi: Conceptualization, Methodology, Contributed to the final version of the manuscript, Supervised the project.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Plasma concentration of BAFF and APRIL significantly decreased following MSCT in Patients with Refractory Rheumatoid Arthritis.
- MSCT inhibited B cells by reducing the expression of BAFF and APRIL genes.
- APRIL, BAFF, and BAFF receptors play a major role in the pathogenesis of RA, and MSCT seems to be a good choice to inhibit these immunological markers.