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Immunomodulatory effects of bone marrow-derived Mesenchymal stem cells in a BALB/c mouse model on induced Systemic lupus erythematosus (SLE)

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ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease that causes acute inflammation in most body tissues. The current study aims to determine levels of some cytokines and chemokines in BALB/c mice with SLE and treatment by using BALB/c Mesenchymal stem cells (BM-MSCs). Forty BALB/c male mice were divided into four groups equally. The first and second groups received activated lymphocyte-derived DNA (ALD DNA) for induction of SLE. The second group received BM-MSCs/IV after the appearance of SLE clinical signs. The third group received BM-MSCs only, while the fourth group (control group) received PBS. All the study groups examine levels of IL-10, IL-6, TGFβ1, VEGF, CCL-2, CCL-5/RANTES, IFNγ, and ICAM -1 by ELISA kits. The cytokines levels are determined in all the study groups. There was a significant increase in ANA and anti-dsDNA levels in the first group, while there was a decrease in the second group (treatment by BM-MSCs). There is no significant difference between the third and control groups in ANA and anti-dsDNA levels. The first group showed a significant increase in IL-6, CCL-5/RANTES, VEGF, ICAM, CCL-2, and IFNγ levels and a decrease in IL-10 and TGFβ1. The second group showed low levels of IL-6, CCL-5/RANTES, VEGF, ICAM, CCL-2/MCP-1, and IFNγ but a high level of IL-10 and TGFβ1 as compared with the control group. The third group has no significant differences from the control group in all the tested parameters. BM-MSCs have an essential therapeutic role in the functional regulation of cytokines and chemokines in mice with SLE.

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Introduction

Lupus is a long-term autoimmune disease; its causes are unknown. Humans and animals could be affected. Lupus occurs if the immune response is hyperactive and attacks the tissues (lungs, brain, heart, skin, joints, blood cells, and kidneys) and the antibodies (1). The conventional treatment of SLE is included immunosuppressive drugs, which inhibit the immune system activity, such as antimalarial drugs (Hydroxychloroquine), corticosteroids (prednisone), Immunosuppressant (azathioprine), and Biologics (belimumab) (2). These drugs have some disadvantages, particularly when used for a long time, such as stomach bleeding, kidney problems, damage to the eye retina, decreased weight, diabetes, high blood pressure, thinning bones, infection, cancer, hepatic tissue damage, and infertility. Furthermore, some cases do not respond to the therapy, and others show allergic clinical signs (3). If it becomes feasible to find an alternative, modern and safe way to treat SLE, especially in light of the spread of this disease worldwide, in humans and animals alike. Some studies have used alternative methods to treat this disease in newer ways, including using Mesenchymal stem cells. Mesenchymal stem cells are undifferentiated cells (4). When cultured in specific inducing media, it could express phenotypic markers and differentiate into several kinds of cells. Also, MSCs have immunomodulatory functions; by secreting some of the bio compounds based on cell-to-cell contacts, the secretion of cytokines is the main function of MSCs (5). The data about the relationship between the secreted cytokines and MSCs in lupus is minor and should focus on level changes of cytokines after treatment by MSC (6). The action mechanisms of MSCs are unknown, but they influence directly and indirectly on immune cells. SLE is autoimmune and shows a disorder in immunological response. Therefore, there is an urgent need for a new treatment method to induce immunosuppression without adverse side effects (7) (8). The present review focuses on the essential cytokines levels that changed after treatment by MSCs and their effect on autoimmune bodies in the mice serum.

Materials and Methods

The experimental animals

Forty BALB/c albino males mice at 6 weeks old with 20-30 grams were provided by (Jackson Laboratory, USA) and housed together under the same conditions. BALB/c mice are used in immunological experiments (9).

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The study design

forty BALB/c mice; divided into four groups, each group consisting of ten mice; the first group is administrated (ALD DNA) (50 µg/mouse/SC) in three doses at (0, 14, 28) days to induce SLE in BALB/c mice. The second group is administrated (ALD-DNA) (50 µg/mouse/SC), three doses at (0, 14, 28) days for inducing SLE; after onset, the clinical signs of lupus (after 28 days), also, ANA, and anti-dsDNA are examined for final diagnosis of SLE. Positive lupus in the second group is treated by BALB/c-MSCs (CellBiologics company, USA) (0.1×10^6) cells/for 10g/IV. The third group is administrated BALB/c-MSCs (0.1×106) cells/for 10g/IV. The fourth group (control group) administrated BPs only.

Induction of SLE

After receiving the shipped mice, some of the shipped mice out of the experimental animals (five mice) were used for the preparation of activated lymphocyte DNA (ALD DNA) for induction lupus in mice according to (10), as following steps:

-Preparation of splenocyte

removing the BALB/c mice spleens and kept in RPMI-1640 (Gibco, Ireland), then passing the cells several times on nylon mesh, then washing by RPMI-1640 then, Add fetal bovine serum (Gibco, Ireland) (10%) with Mglutamine 2m (Sigma, USA), penicillin G and streptomycin (100) mg/ml each one to prevent the contamination. Then, splenocytes were diluted to (2×10^6) cells/ml with concanavalin A (5 ug/ml) for 48 hours (ConA can stimulate mouse T-cells for giving rise to many folds (11) (12).

-DNA extraction: gDNA extracted activated splenocytes were treated with proteinase K (Sigma, USA) and S1 nuclease (TaKaRa, Japan) and then purified by kit (Genetech, China). The nanodrop (Thermo Fisher Scientific, USA) is used at (260) nm for determining DNA concentrations.

-The immunization: the mice were classified into four groups. Each one consists of ten mice (G1, G2, G3, G4). 1st and 2nd groups were only immunized with activated ALD DNA (0.2) ml/ SC with PBS and (CFA) (Sigma, USA), at three doses of ALD DNA, the period between one and another was two weeks, at (0, 14, 28) day. Before the third dose, the animals showed clinical signs of SLE syndrome (nose bleeding).

ANA and anti-dsDNA Examination

ELISA kit antinuclear antibodies (ANA) (MyBioSource, USA) and ELISA kit anti-double strand DNA (Anti-dsDNA) (MyBioSource, USA) is used for the detection of antibodies levels in animals in all the study groups, before and after treatment by BM-MSCs and PBS, according to company directions.

The BALB/c BM-MSCs preparation

BALB/c- BMMSCs are provided by (Cell biologics Company, USA) from the bone marrow of pathogen-free. BALB/c-MSCs could be used in immunosuppressive trials. The cells are thawing water bath (37°C) for (60) seconds until the disappearance of the ice bit. The provided cells were put in a centrifuge tube with a culture Medium (8-10) ml for five minutes at 120 rpm. Remove the supernatant layer then put the cell in a culture Medium (6) ml. Add suspended cells into a flask, place the T25 flask in a humidified CO_2 (5%), then incubate at (37°C). The media are changed daily (for four days) to remove non-adherent cells and spread the nutrient elements.

Administration of BALB/c- BM- MSCs

The 2nd and 3rd group received BALB/c- BM-MSCs at dose (0.1×10^6) Cells (IV) through the tail vein to decrease and prevent the trapping in the lung.

The used ELISA kits

The tested cytokines in the study are IL-10, IL-6, CCL-2, TGFβ1, IFNγ, CCL-5, ICAM, and VEGF using ELISA kits to the company directions as shown in table (1).

Statistical Analysis

The results were analyzed by a one-way ANOVA and two-way ANOVA tests using SPSS, V27; the USA). LSD test was used to determine the significant difference between the groups at the significant level ($P\geq0.05$) (13).

Results

ANA and Anti dsDNA levels

Our findings showed that the Anti-dsDNA levels before treatment were (16.98±0.83) ng/ml, (17.29±0.77) ng/ml, (3.10±3.10) ng/ml, and (3.06±5.01) ng/ml. In contrast, Anti-dsDNA levels after treatment were (16.8±0.90) ng/ml, (2.45±0.52) ng/ml, (3.00±0.30) ng/ml, and (2.98±0.28) ng/ml in the study groups respectively, wherever does not show significant differences except for the 2nd group which showed significant differences between before and after treatment by BM-MSCs at ($P<0.05$).

Table 1. The used ELISA kits in the study.

The ELISA kit	The company	Origen
Interleukin-10 (IL-10)	Abcam™	UK
Interleukin-6 (IL-6)	Invitrogen™	USA
Transforming growth factor β (TGFβ1)	Invitrogen™	USA
chemokine ligand Monocyte chemotactic protein-1 (CCL-2/MCP-1)	Invitrogen™	USA
C-C motif ligand 5 (CCL-5/RANTES)	Abcam™	UK
Vascular endothelial growth factor (VEGF)	Quantikine™	USA
Intercellular adhesion molecule -1 (ICAM -1)	Invitrogen™	USA
Interferon gamma (IFNγ)	abcam™	USA
Mouse Anti-nuclear antibody (ANA)	MyBioSource™	USA
Anti -double-stranded DNA (anti -dsDNA)	MyBioSource™	USA

Table 2. level of ANA and Anti dsDNA in serum before and after treatment by BM-MSCs.

	Anti dsDNA ng/ml		ANA ng/ml	
	Before treatment by BM-MSCs	After treatment by BM-MSCs	Before treatment by BM-MSCs	After treatment by BM-MSCs
G1	16.98 ±0.83^{Aa}	16.8±0.90^{Aa}	18.15±1.45^{Aa}	18.07±1.09^{Aa}
G2	17.29 ±0.77^{Aa}	2.45 ±0.52^{Bb}	17.55±0.83^{Aa}	2.71±0.61^{Bb}
G3	3.10±3.10^{Ba}	3.00±0.30^{Bb}	3.70±4.1^{Ba}	3.22±0.64^{Bb}
G4	3.06±5.01^{Ba}	2.98±0.28^{Bb}	3.63±4.87^{Ba}	3.30±0.75^{Bb}
	0.584		0.861	

Our findings showed that ANA levels before treatment were (18.15±1.45) ng/ml, (17.55±0.83) ng/ml, (3.70±4.1) ng/ml, and (3.63±4.87) ng/ml in the first, second, third, fourth group. The ANA levels after treatment were (18.07±1.09) ng/ml, (2.71±0.61) ng/ml, (3.22±0.64) ng/ml, and (3.3±0.75) ng/ml in the first, second, third, and fourth group respectively. It does not show significant differences except for the 2nd group, which showed significant differences between before and after treatment by BM-MSCs at ($P<0.05$), as a table (2).

The cytokine levels

According to figure (1), the first group showed decrease significant differences in IL-10 levels (7.18±0.47) ng/ml as compared with the second group (29.1±1.1) ng/ml, the third group (32.09±2.89), and the fourth group (32.14±3.54) ng/ml. In contrast, the fourth group showed a higher level of IL-10 as compared with the study groups.

According to figure (2), the first group showed an increase significant (360.89±4.48) ng/ml of IL-6 levels as compared with the second group (201.14±5.55) ng/ml, the third group (190.89±3.7c) ng/ml, and the fourth group (191.37±3.26).

According to figure (3), levels of TGF-β1 were (2354.2±341.7 ng/ml, (8222.54±570.3) ng/ml, (8225.6±390.1) ng/ml, and (8250.8±384.8) ng/ml in the first, second, third, and fourth group respectively. The first group showed a decrease significant in TGF-β1 levels compared with the second, third, and fourth groups. However, there are no significant differences between the second, third, and fourth groups in the TGF-β1 level.

According to figure (4), the first group showed an increase in significant differences (162.98±11.48) ng/ml of CCL-2/MCP-1 levels as compared with the second group (52.09±6.15) ng/ml, the third group (48.1±10.2) ng/ml, and the fourth group (49.4±9.75) ng/ml. There are no significant differences between the second, third, and fourth groups.

The 1st group showed an increase in significant differences in CCL-5/RANTES level (41.74±4.9) ng/ml as compared with the 2nd group (7.37±1.45) ng/ml, the 3rd (7.68±1.47) ng/ml, and the 4th group (7.61±1.51) ng/ml. There are no significant differences among the 2nd, 3rd, and 4th groups, as shown in figure (5).

According to figure (6), the 1st group showed increasing significant differences (41.84±2.89) ng/ml of VEGF-A levels as compared with the 2nd group (11.03±3.30) ng/ml, the 3rd group (9.63±2.97) ng/ml, the 4th group (9.72±3.46) ng/ml. At the same time, there are no significant diffe-

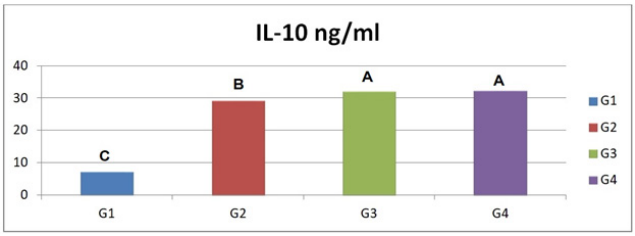

Figure 1. Concentration of IL-10 in study groups.

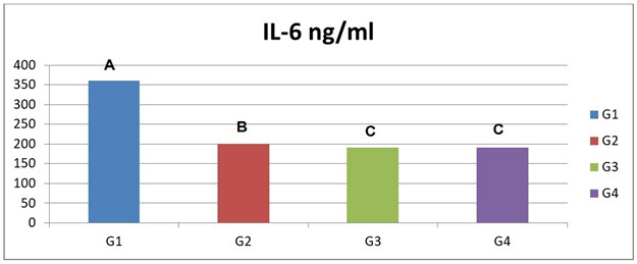

Figure 2. Concentration of IL-6 in study groups.

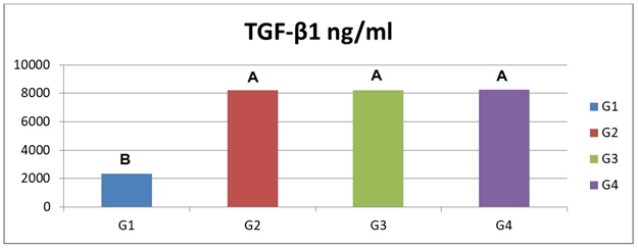

Figure 3. Concentration of TGF-β1 in study groups.

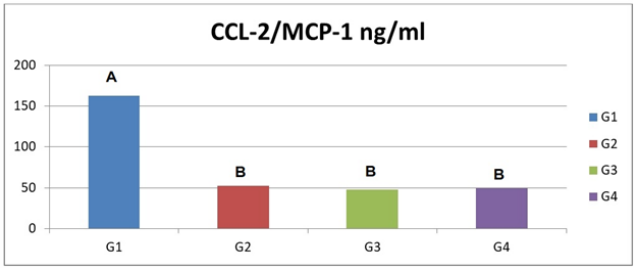

Figure 4. Concentration of CCL-2/MCP-1 in study groups.

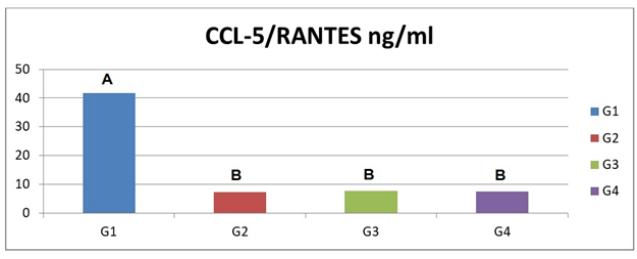

Figure 5. Concentration of CCL-5 in study groups.

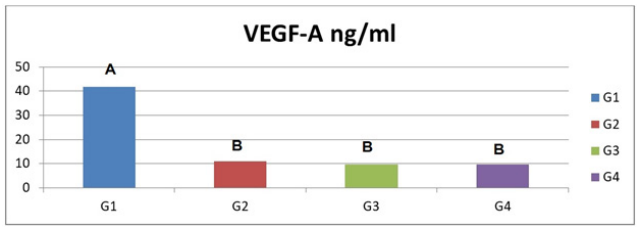

Figure 6. Concentration of VEGF-A in study groups.

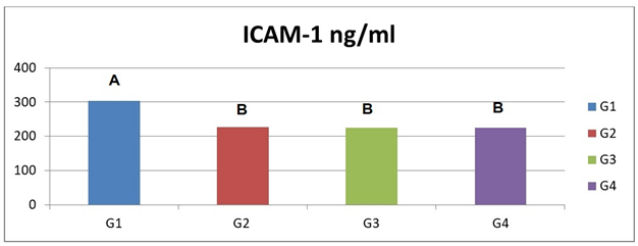

Figure 7. Concentration of ICAM-1 in study groups.

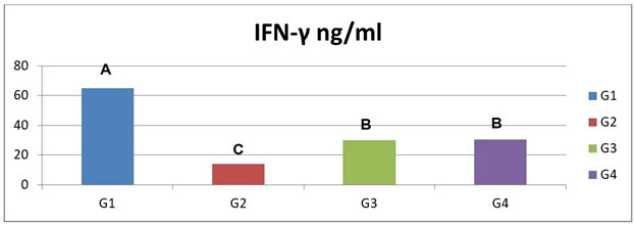

Figure 8. Concentration of IFN-γ in study groups.

rences among all the study groups.

According to figure (7), the 1st group showed increased significant differences (302.9±28.31) ng/ml of ICAM-1 level as compared with the 2nd group (226.8±11.69) ng/ml, the 3rd group (225.3±6.26) ng/ml, and the 4th group (225.1±8.33) ng/ml, while there are no significant differences among the 2nd, the 3rd, the 4th group.

According to figure (8), the 1st group showed increased significant differences (64.85±4.10) ng/ml in IFN-γ levels as compared with the 2nd (14.12±2.10) ng/ml, the 3rd group (30.21±2.33) ng/ml, and the 4th group (30.37±2.85) ng/ml, while the 3rd and the 4th group were showed increased significant differences as compared with the 2nd group.

Discussion

Lupus is an immune and inflammatory disorder in most body tissues due to disorders in the levels and function of the cytokines (14). High expression of th17 cells and down-regulation of Treg cells occur in patients with lupus due to high levels of autoantibodies, which leads to complications and death (15). The autoantibodies are causing a dramatic series of clinical findings of SLE (16).

MSCs are used in treating autoimmune diseases because they regulate immunological responses (17). Moreover, MSCs have immunomudualry effects (18). Lupus is included immune disorder and the absence of immune regulation (19).

Our findings showed that anti-dsDNA levels decreased after administration of BM-MSCs in the second group and showed significant negative differences compared with other groups after treatment. The second group showed significant positive differences in ANA levels before the BM-MSCs administration as compared to after treatment by MSCs. The second group showed normal values of ANA after the administration of BM-MSCs close to the values in the control group.

The anti-dsDNA and ANA are antibodies produced by the lymphocytes against self-DNA due to poor identification of the exact antigenic components (20). Anti-dsDNA and ANA antibodies are increased in lupus, according to many reports (21), as found and agree with our results.

Our findings showed that the IL-10 level decreased in the first group compared with the control group. IL-10 level was a comeback to the normal level in the second (after treatment), with slightly significant differences between both groups.

IL-10 has anti-inflammatory effects, produced at low levels in lupus cases compared with healthy individuals (22), suppressed by many immunobiological factors secreted in lupus involvement (23). The treated group by MSC (the second group) showed an increase in IL-10 cytokine levels as compared with the first group due to MSC effects that are unknown yet (24). Some reports indicated that MSC could produce and help to secret the exosomes, the cytokines, and the chemokines that directly regulate and control the immune response; furthermore, MSC can be balanced between th17 cells and Treg cells (25).

Our results showed that TGF-β1 levels were decreased in the first group (induced lupus) compared to the control group, but they come back to their normal level after treatment by MSC (the second group).

TGF-β1 is a cytokine that has a vital play in the pathogenesis of lupus diseases. The patients with lupus showed a significantly lower level of TGF-β1 compared to the control group. The lowest levels of TGFβ1 attributed to low activity and low numbers of Treg cells in lupus cases. A low level of TGFβ1 is associated with lupus nephritis in humans and animals. As lupus nephritis shows a lower level of TGFβ1, it will indicate more kidney tissue damage (26).

The reports demonstrated that TGF-β1 has anti-inflammatory effects and showed low concentration in lupus due to less activity of Treg cells that secreted it, which reveals agreement with our findings (27).

IL-6, CCL-2/MCP-1, CCL-5/RANTES, VEGF-A, ICAM-1, and IFN-γ levels showed a significant increase in the first group (lupus group) compared with other groups based on our study. All mentioned cytokines are pro-inflammatory cytokines produced by th17 cells. Undoubtedly, SLE is an acute autoimmune condition that includes high pro-inflammatory cytokines and autoantibodies (28).

SLE is an autoimmune disease that includes cytokine imbalances, triggers inflammation, immune cell dysfunction, and cytokine storm. The primer cytokine of the SLE pathogenesis is interferon-alpha, which is stimuli by immune compounds that increase some inflammatory proteins (29) (30).

SLE is a disease that includes dysregulation of the cytokine and chemokines levels with its receptors expressed against the target antigens and organs. Interferons play the main role in SLE and can activate rest cytokines. Interferons are keys to controlling and treating lupus, such as TNF-α used for rheumatoid arthritis (31). Absent coordination between the cytokine is important to development of the active lupus. IL-17 is produced by th17 cells, which act as pro-inflammatory cytokines that play a role in lupus

development and loss of tolerance (32).

IL-6, CCL-2/MCP-1, CCL-5/RANTES, VEGF-A, ICAM-1, and IFN-γ levels decreased in the 2nd group (treated group by MSC) compared with the first group, and there are no significant differences between the third group and fourth group based on our findings.

Nowadays, MSC is considered therapy for SLE to improve the clinical signs of the SLE by inhibiting the activity of Th1, Th17, and B cells and increasing the activity of Th2 and Treg cells. Treatment by MSC has benefits for enhancing the immunosuppression in lupus, although MSC therapy is reported ineffective in some studies, and that attributed to some patients with lupus do not respond to the therapy due to strange sources of MSC or patient-related factors or patients haven't able to respond to cellular immunological stimulation by treatment by MSC (33).

MSC plays a role in regulating SLE immunity (34). MSCs have immunosuppressive effects on the hyperactivities of immunological reactions. Administration of MSCs IV will ameliorate the clinical signs of lupus. Allogeneic MSCs suppress autoimmunity in mice and humans (35). Treatment by MSC for animals with SLE decreased autoimmune bodies such as ds-DNA and ANA (36). MSCs used to treat lupus in drug-resistant cases, such as cortisone and corticosteroid drugs used normally to treat lupus (37). The meta-analysis studies assessing MSCs showed that MSCs are a new treatment for SLE without side effects in humans and lab animals (38).

MSC secrete immunomodulatory factors that restore immune balance and produce good results in treating SLE. MSCs do not express major histocompatibility complex II (MHC II). Therefore, it is less rejected after MSC transplantation. MSCs have no immunomodulatory effects only, but they have great ability in regenerative medicine due to their ability to differentiate into several types of cells (39).

The third group does not have significant differences compared with the control group. We establish the third group to confirm that MSCs do not cause a cross-immunological reaction such as histological and immunological mismatching. It supports that MSC only has an essential role in lupus cases and has no role in healthy animals.

In the end, we can conclude that BM-MSCs can regulate and maintenance of cytokines and chemokines in mice with lupus. BM-MSCs could use for the treatment of lupus in mice.

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there is no fund

Competing interests
The authors have declared that no competing interests exist.

Authors' contribution
Ghassan: experiments design, data analysis, study validation, writing. Tarek and Khalil: supervision, provision of study materials.

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