ORIGINAL ARTICLE

Hypoxic mesenchymal stem cells (MSCS)-induced interleukin (IL)-10 alleviate systemic lupus erythematosus (SLE) inflammation through inhibiting interferon (IFN)-gamma production

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ABSTRACT

Aim To determine the effect of hypoxic mesenchymal stem cells (MSCs) on the interleukin (IL)-10 and interferon (IFN)-gamma in peripheral blood mononuclear cells (PBMCs) from systemic lupus erythematosus (SLE) patients.

Methods This study used a post-test control group design. Hypoxic MSCs were isolated and characterized according to their surface marker expression and differentiation capacities. PBMCs isolated from SLE patients were divided into three groups: control and two treatment groups. The treatment groups were treated by co-culturing MSCs to PBMCs with a ratio of 1:10 (T1) and 1:1 (T2) for 48 h incubation. Furthermore, IFN-gamma and IL-10 levels were determined by cytometric bead array (CBA) flow cytometry.

Results Hypoxic MSCs significantly decreased the IFN-gamma levels and increased the IL-10 levels in dose-dependent manner compared to the control group. The highest activity of hypoxic MSCs was noticed in T2 group.

Conclusion Hypoxic MSCs- induced IL-10 are important in the control of anti-inflammatory effect on SLE through inhibiting IFN-gamma.

Key words: hypoxic MSC, IFN-gamma, IL-10, inflammation, PBMC, SLE

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a highly complex and heterogeneous autoimmune disease characterized by a loss of the immune response tolerance to self-antigens that induce an excessive generation of autoantibodies against immune complex formation triggering systemic organ-specific inflammation (1). The host immune responses to these autoantibody-autoantigen complexes depositing into various tissues of the body including renal tissues induce a local inflammatory response up to severe tissue destructions such as lupus nephritis (LN) to renal failure (2). The persistent risk enhancement of these organ failures in SLE patients is a mortality trend, making SLE treatment by controlling the initial inflammation processes a challenge (3). On the other hand, interleukin IFN-gamma was involved in the LN development by inducing the T-cells to actively release the inflammatory mediators, thus decreasing the IFN-gamma level associated with LN's improvement in the SLE diseases (4,5). Hypoxic mesenchymal stem cells (MSCs) have a robust immunosuppressive capability to inflammatory cells (6), and therefore, the MSCs were assumed to be potentially controlling the onset of SLE by suppressing IFN-gamma.

MSCs represent a heterogeneous population of fibroblast-like multipotent cells derived from various sources, including umbilical cord (UC), bone marrow, dental pulp, and adipose tissues (7). These cells have the ability to differentiate into tissues of mesodermal lineage and are characterized by the high expression of several surface antigens, such as CD90, CD105, CD44, and CD73, and lack of expression of CD79 or CD19, CD14 or CD11b, CD45, CD34, and HLA-DR (8,9).

Nowadays, MSCs populations are being extensively investigated for their immunomodulatory properties. A previous study reported that hypoxic MSCs transplantation had a therapeutic effect on various autoimmune diseases including SLE (10,11). One reason for the use of MSCs in SLE studies is their ability to hamper excessive inflammation by enhancing the regulatory lymphocyte cells (Treg) modulation (12). The functional Treg can release several anti-inflammatory cytokines to inhibit the production of IFN-gamma (13,14).

As one of the pro-inflammatory cytokines released by antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs), IFN-gamma is responsible for mediating immune response (15). Several studies have reported that the overexpression of IFN-gamma in SLE diseases may trigger the differentiation of naive T helper into Th1 cells leading to the inflammation condition in renal (16). On the other hand, a previous study revealed that immunosuppressive properties of MSCs may control several autoimmune cells by enhancing the functionality of the Treg subset (17,18). The activation of functional Treg to reduce IFN-gamma level correlated with the improvement of inflammatory tissues (13). However, capability of human MSCs to decrease IFN-gamma production in peripheral blood mononuclear cells (PBMCs) from SLE patients in vitro remains unclear.

The aim of this study was to investigate the role of hypoxic MSCs in regulating the level of IL-10 and IFN-gamma derived from PBMCs of SLE patients *in vitro*.

MATERIALS AND METHODS

Materials and study design

This study was conducted in the Stem Cell and Cancer Research (SCCR) Laboratory, Semarang, in the period August – December 2019. In total, PBMCs were collected from SLE patients using an informed consent. Negative PBMCs control groups were treated with standard medium, and the treatment group was co-cultured with human UC-MSCs at doses of 1:10 and 1:1(MSCs:PBMCs).

This study was approved by and conducted in accordance with the review board of the Health Research Ethical Committee Medical Faculty of Universitas Sultan Agung (Unissula), Semarang (No. 698/TGL/KEP -RSUP HAM/2019).

Methods

MSCs isolation. MSCs were isolated from human umbilical cord with a specific informed consent. Briefly, the cords were rinsed using phosphate-buffered saline (PBS) (Gibco Invitrogen, NY, USA), and cord blood was removed. The washed cords were cut into smaller pieces, transferred into a T75 culture flask (Corning,

Tewksbury, MA, USA) containing low glucose Dulbecco's Modified Eagle Medium (Sigma-Aldrich, Louis St., MO); supplemented with 10% foetal bovine serum (Gibco Invitrogen, NY, USA), 1% penicillin-streptomycin (Gibco Invitrogen, NY, USA), and 0.25% amphotericin B (Gibco Invitrogen, NY, USA), and incubated under 37 °C and 5% CO. The cells derived from passage 5 were hired for the experiments (19).

MSCs differentiation capability assay. Adipogenic differentiation assay was analysed under Oil Red O staining assay. Briefly, adipogenesis was carried out in an adipogenic induction medium containing ADS medium supplemented with 0.5 mM isobutyl methylxanthine, 1μM dexamethasone, and 200 μM indomethacin. Adipogenic differentiation was confirmed by Oil-Red-O staining (20).

MSCs surface marker validation assay. Surface marker validation of MSCs was analysed using flow cytometry analysis based on manufacturer's instructions (the BD Bioscience, USA). Isolated cells were trypsinized and separated using 1900 rpm for 8 min centrifugation. Approximately 1×10^5 cells were resuspended in 100 μL staining buffer (BD Bioscience, San Jose, CA, USA). The cells were subsequently incubated using fluorescein isothiocyanate-, allophycocyanin (APC), peridinin-chlorophyll-protein (perCP)-CyTM5.5.1, and phycoerythrin (PE)-conjugated anti-human CD90, CD73, CD105, and Lin (CD45/CD34/CD11b/CD19/HLA-DR) antibodies (BD Bioscience, San Jose, CA, USA) for 30 min at room temperature. In addition, as the negative control an appropriate isotype-specific conjugated anti-IgG (BD Bioscience, San Jose, CA, USA) and unstained cells were used. The analysis was performed using a BD Accuri C6 Plus flow cytometer (BD Bioscience, San Jose, CA, USA). The capacity of the cell to differentiate into mature cells including osteogenic and adipogenic was validated using differentiation medium. Briefly, the cells were cultured at the density of 5×10^6 cells a 25 cm2 flask cultured with osteogenic and adipogenic differentiation medium following manufacturer protocols (21).

Hypoxic-MSCs isolation. MSCs were ready to precondition in hypoxic using hypoxic chamber (anaerobic environment; ThermoForma, Waltham, MA, USA) when it reached 70% confluency in flask containing complete medium, then they were washed twice with PBS and incubated in DMEM for 12 h. MSCs were cultured in mix gas mixture composing 5% CO_2, 10% H_2, and 85% N_2 then maintained at 37 °C. The oxygen level in the chamber was ~0.5% (22).

Determination of IFN-gamma and IL-10. After 48 h, the co-culture supernatants were analysed using the human IFN gamma and IL-10 single Flex (560154, BD Biosciences, San Jose, CA, USA) based on the manufacturer's instructions. After acquisition of sample data using BD Accuri C6 Plus Flow Cytometer (BD Biosciences, San Jose, CA, USA), the IFN gamma and IL-10 levels were determined using the FCAP Version 1.0 analysis software (BD Biosciences, San Jose, CA, USA).

Statistical Analysis

Data were presented as the mean±SE. Group comparisons were analysed using one way analysis of variance, followed by post hoc Fisher's LSD. The of $p<0.05$ was considered significant.

RESULT

Isolated cells from UC exhibited plastic adherent capability and fibroblast-like cell morphology, which are typical characteristics of MSCs (Figure 1A). Cells which were cultured in an osteogenic differentiation medium showed the capability to differentiate into osteogenic lineage through forming the bone matrix and oil droplets, according to the main criteria defined by the International Society for Cellular Therapy (23) (Figure 1B). The standard of osteogenic media were given for 21 days during the MSC-like osteogenic differentiation experiment. Alizarin red dye staining was utilized to identify calcium deposits as a red appearance (Figure 1C). Furthermore, flow cytometry analysis cells showed high expression of MSCs surface markers including CD90 (99.40%), CD105 (96.90%), CD73 (99.80%) and negative lineage of MSCs (0.30%) (Figure 1D).

IFN-gamma levels were measured after 48 hours of co-culture between hypoxic MSC and SLE-PBMC. The co-culture and PBMCs medium was collected for the CBA analysis. The CBA assay showed that hypoxic MSCs significantly decrease the IFN-gamma levels in the T1 (1408.33±119.68 pg/mL) and T2 groups (1091.67±101.75 pg/mL)

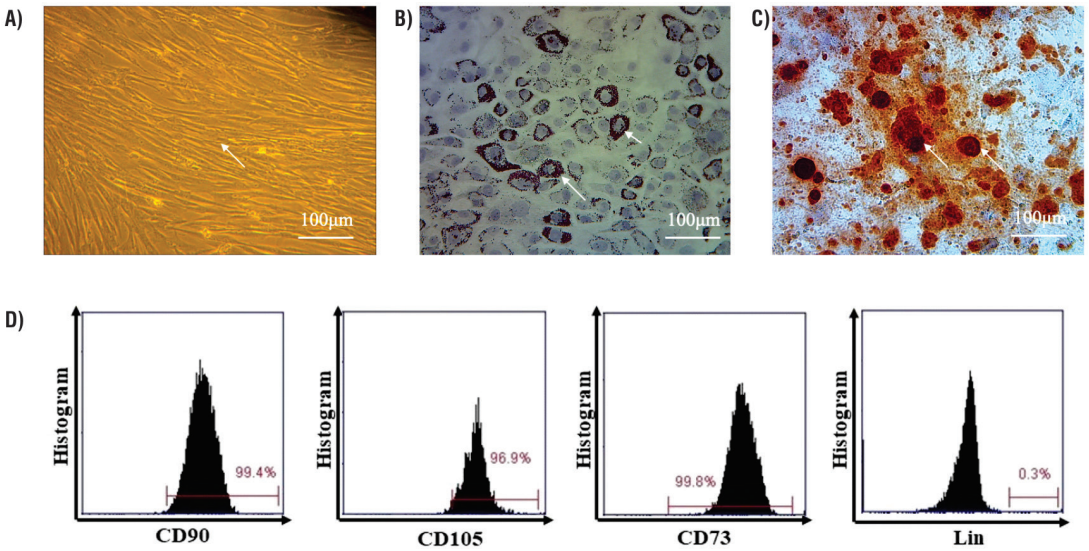

Figure 1. A) Mesenchymal stem cells (MSCs) characterization and differentiation (magnification 10x, scale bar 100 µm); B) MSCs differentiation - the white arrow denote a response to the lipid deposition under Oil-red O staining; C) a red bright colour marked by the white arrow in a response to the calcium deposition in osteocyte-differentiated MSCs via staining by alizarin red (magnification 40x, scale bar 100 µm); D) the surface marker analysis of the expression of CD90, CD105, CD73, and negative lineage

compared to the control group (4066±176.62 pg/mL) (Figure 2). On the other hand, we found that IL-10 increased significantly on the PBMC and MSC co-culture in the doses dependent manner, T1 (939±41.51 pg/mL) and T2 (1157±123.98 pg/mL) compared to the control group (429.33±53.16 pg/mL) (Figure 3).

DISCUSSION

In the present study, we assessed the anti-inflammatory effect of hypoxic MSCs on the co-culture PMBC SLE patient. The capacity of hypoxic MSCs to reduce the activation of cytokines most associated with proinflammation has provided new insights into SLE disease (11). MSCs con-

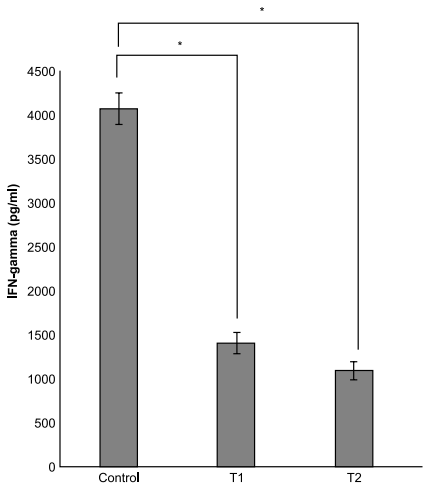

Figure 2. Mesenchymal stem cells (MSCs) suppress the IFN-gamma proinflammatory cytokines in the peripheral blood mononuclear cells (PBMCs) with systemic lupus erythematosus (SLE) condition. Data represented as mean±SD from 3 replicated experiment. The same results were observed for 48-h group (Control– SLE group without treatment; T1 group - treated with human MSCs:PBMC at dose of 1:10; T2 group - treated with human MSCs:PBMC at dose of 1:1).
*p<0.05

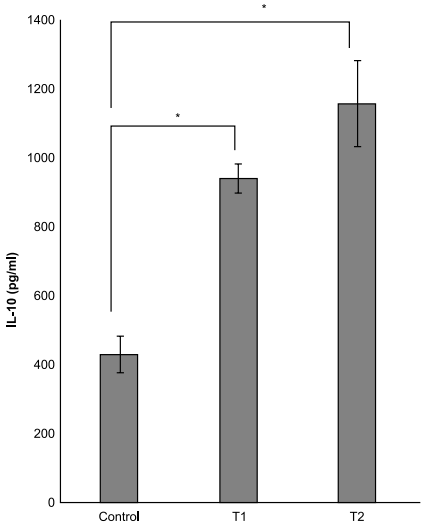

Figure 3. Mesenchymal stem cells (MSCs) increase the IL-10 anti-inflammatory cytokines in peripheral blood mononuclear cells (PBMCs) with systemic lupus erythematosus (SLE) condition. Data represented mean±SD from 3 replicated experiment. The same results were observed for 48-h group (Control - SLE group without treatment; T1 group - treated with human MSCs:PBMC at dose of 1:10; T2 group - treated with human MSCs:PBMC at dose of 1:1).
*p<0.05

trol inflammation in part by limiting IFN-gamma release by activated antigen presenting cells (APCs) such as macrophages and dendritic cells (DCs) to communicate with T-cell populations (24–26). Hypoxic MSCs were found to be able to supress the pro-inflammatory niche by drastically lowering IFN-gamma in dose-dependent manner. However, there was no difference in IFN-gamma among these doses. In addition, hypoxic MCSs also induced the IL-10 level in dose-dependent manner. These findings indicated that hypoxic MSCs can reduce the proinflammatory state of SLE patients even at low doses. MSCs increased TLR3 expression in the pro-inflammatory niche to stimulate the NF-kB and ERK pathways of type-1 MSCs, but these MSCs polarized into type-2 MSCs, which expressed TLR4 and released IL-10 (27,28). Therefore, our data, together with previous reports, suggest that the ability of MSCs to induce IL-10 release that causes MSCs to reduce inflammation in SLE conditions, through inhibition of IFN-gamma expression.

IL-10 is an immunosuppressive cytokine that has a role in maintaining a balanced immune response through T cell proliferation inhibitor and anti-inflammatory cytokine production (29). In this study, administration of hypoxic MSCs increased the serum levels of IL-10. In our co-culture condition, we found that the production of IFN-gamma was significantly decreased in line with the enhanced level of IL-10 compared with control cells. These observations suggest that SLE suppression by IL-10-hypoxic MSCs is associated with inhibition of IFN-gamma expression. The IL-10-induced changes in cytokine milieu, if they occur highly enough to overcome proinflammatory activity, would help hypoxic MSCs return to their native ability for immunosuppression (30). On the other hand, due to the overactivation of IFN-gamma-released Th1 and the depletion of Treg in SLE patients, IL-10 levels were dramatically decreased (3). The enormous production of IFN-gamma might be a part of positive feedback loop that activates APCs like macrophages and B cells, resulting in increased levels of many pro-inflammatory cytokines (15,31). By producing a high quantity of IL-10, MSCs were able to suppress this severe inflammatory response (30). Previous studies reported that IL-10 inhibits the production of Th1 and proinflammatory cytokines, including interferon IFN-gamma (9,32).

TLR-induced IFN gamma expression is negatively regulated by IL-10, according to a prior study (33,34). IL-10 can also repress both the IL-12a and IL-12b genes at the transcriptional level, which results in a decrease in IFN gamma levels (35). As a result, the high levels of IFN-gamma in SLE and lower levels of IL-10 result in a robust proliferation of Th1 and enormous inflammation. However, hypoxic MSCs could secrete IL-10 that regulated the expression of Treg cells leading to inhibiting anti-inflammatory cytokines. These data show that by limiting IFN-gamma expression by MSCs the inflammatory situation in human SLE may be controlled.

In conclusion, we have demonstrated that the co-culture administration of hypoxic MSCs on PMBC SLE patient generally inhibit expression of IFN-gamma through overexpressing IL-10 levels. These data provide a novel insight into the advantages of hypoxic MSCs as a cell therapy against SLE, which aims at suppression of the autoimmune response.

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TRANSPARENCY DECLARATION

Competing interests: None to declare.

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