Mesenchymal Stem Cells Suppress Dendritic Cells and Modulate Proinflammatory Milieu Through Interleukin-10 Expression in Peripheral Blood Mononuclear Cells of Human Systemic Lupus Erythematosus

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ABSTRACT

Background: Immune-mediated inflammatory injury among systemic lupus erythematosus (SLE) individuals may be involved by dendritic cells (DCs) abnormality though the underlying mechanism remains incompletely understood. Objective: This study aimed to elaborate MSCs’ potential in suppressing abnormal DCs cell function on peripheral blood mononuclear cells (PBMCs) among SLE patients. Methods: MSCs were isolated from human umbilical cord blood. On the other side, human PBMCs were isolated from 20 active SLE patients and 5 healthy controls. The PBMCs of SLE patients were divided into 5 groups: sham (Sh) and control (C) groups were treated with standard medium, and the treatment groups (T1, T2 and T3) was co-cultured with UUC-MSC at doses of 1:1, 1:25, and 1:50 (MSCs:PBMCs). The expression of CD11c in DCs was analyzed using flow cytometry, while the level of TNF-α, IFN-γ-IL-6 and IL-10 was analyzed using cytometric bead array (CBA). Results: The MSCs significantly downregulates CD11c of dendritic cells in all treatment groups. MSCs also significantly suppress the level of TNF-α, IFN-γ-IL-6 and IL-10 in all treatment groups. Conclusion: Therefore, MSCs could suppress DCs through regulating the proinflammatory milieu in PBMCs of SLE patients.

Keywords: MSC, SLE, Dendritic Cells, Autoimmune disease.

1. BACKGROUND

Systemic lupus erythematosus (SLE) is a systemic autoimmune inflammatory disorder characterized by high levels of circulating autoantibodies against nuclear antigens and immune complex deposition, thus triggering multi-systemic inflammation (1, 2). Considering its complicated interaction with auto-immunities, there are no effective curative treatments for SLE, though the global rheumatology association had pharmacologically recommended antimalarial, conventional immunosuppressive agents, nonsteroidal anti-inflammatory drugs (NSAIDs), and disease-modifying antirheumatic drugs (DMARDs) to control the disease activity though possessing potentially serious side effects in some occurrence (3-5). Due to its important role in SLE pathology, various immune cells and inflammatory mediators have been recognized as harmful aspects of SLE, especially the dysfunctional dendritic cells (6, 7). An abnormal function of the Dendritic cells (DCs) might crucially promote dysregulated T and B cell functions, inducing the production of pro-inflammatory cytokines e.g., interferon-γ (IFN-γ), IL-6, and tumor necrosis factor-alpha (TNF-α) from type-1 helper T cells (8).

To date, the emergence of mesenchymal stem cells (MSCs) in the scientific forums had been considered a novel-yet-potentially impactful treatment due to its unique immune properties. It has been extensively investigated and characterized to identify an alternative stem cell source, which is expected to hold similar potential for immune-modulating, immunosuppressive, and regenerative capacities (9). Theoretically, MSCs are adult stem cells that can be obtained from different sources e.g., bone marrow, adipose tissue, and umbilical cord tissue.
(Wharton’s Jelly) or amniotic fluid (the fluid surrounding a fetus) (10). Umbilical cord-derived mesenchymal stem cells (UC-MSCs) are a source of MSCs which have multi-directional differentiation properties, and are highly self-renewal and thus easier to collect and preserve (11, 12). UC-MSCs also express CD90, CD73, and CD105 but lack CD45, CD34, CD14 (or CD11b), CD79A (or CD19), and HLA-DR surface molecules (13), partially explaining its differentiating ability into osteoblasts, adipocytes, and chondrocytes under standard in vitro conditions (14).

The beneficial effects of MSCs in autoimmune disorders are not completely relying only on direct cell-to-cell interaction, but also utilizing its paracrine ability to upregulate anti-inflammatory cytokine. While the pluripotential properties initially place MSCs in the crucial stage of an alleged era of regenerative medicine, their unique immunoregulatory property framed them as an attractive option for treating autoimmune diseases, especially SLE. Furthermore, the cytokine secretion profile of peripheral blood DCs or PBMC (peripheral blood mononuclear cells) is also might be influenced by MSCs transplantation, hence shifting the immune reaction from a “pro-inflammatory immune” state to anti-inflammatory cytokines. The DCs function is also expected to be regulated even further through inhibition of co-stimulatory molecular expression, which in turn may suppress their capacity to initiate naïve T cells and immune responses. Nevertheless, MSC’s role in regulating DCs through expressing IL-10 and modulating proinflammatory cytokines in SLE remains unclear (15-17).

2. OBJECTIVE

This study aims to explore the therapeutic properties of MSCs in regulating the DC population and inflammatory milieu in SLE PBMCs through expressing IL-10.

3. METHODS

Research design

This study was conducted at the Stem Cell and Cancer Research (SCCR) Laboratory, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, from September-October 2019. We included 20 individuals from Kariadi General Hospital, Semarang, Indonesia with confirmed SLE according to the 1997 international guideline by American College of Rheumatology (ACR), along with 5 healthy individuals as a comparator group. Each written consent had been obtained prior to each patient’s registration at the hospital administration specifically to collect the peripheral blood sample and umbilical cord tissue. This study was also ethically approved by the Ethical Committee of Faculty of Medicine, Universitas Sumatera Utara, Medan, under the issued letter of No. 698/TGL/KEPK FK USU-RSUP HAM/2019. We divide the participants into 5 different groups e.g., sham (Sh) and control (C) groups which treated without any hUC-MSCs yet different in procedural approaches; and the treatment groups (T1, T2, and T3) which were administered with hUC-MSC at different doses of 1:1, 1:25, and 1:50.

MSCs Isolation, Characterization, and Differentiation Assay

MSCs were isolated from Wharton jelly obtained from labored healthy mothers under aseptic condition after detailed consent. The cords were rinsed, cut and seeded into a flask (Corning, Tewksbury, MA, USA) containing low-glucose Dulbecco’s modified Eagle’s medium (Sigma-Aldrich, Louis St, MO), 10% fetal bovine serum (FBS) (Gibco™ Invitrogen, NY, USA) 1% penicillin (100 U/mL)/streptomycin (100 µg/mL) (Gibco), and 0.25% amphotericin B (Gibco); at 37°C in a humidified 5% of CO₂. The medium was replaced every 3 days. The cells were passaged after reached 80% confluence and then transferred into new flask. The cells from passage 5 were used for the following experiments.

MSC-like surface markers were assessed by flow cytometry (FC) according concordant to manufacturer’s instructions. Fluorescein isothiocyanate (FITC)-, 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) were used to stain the cells for 30 minutes at room temperature in the dark. Cells were resuspended in buffer (BD Bioscience). Post-acquisition analysis was conducted using BD Accuri C6 Plus software (BD Bioscience).

To determine the differentiation potential of MSC-like, the cells were induced into osteogenic and adipogenic differentiation using standard induction media. MSC-like were cultured in a 24-well plate using standard medium and incubated at 37°C, 5% CO₂, and ≥ 95% humidity. After 95% confluency, the standard medium was replaced with an osteogenic differentiation medium containing Human MesenCult™ Osteogenic Basal Medium (Stem Cell Technologies, Singapore), supplemented with 20% Human MesenCult™ Osteogenic Differentiation SX Supplement (Stem Cell Technologies), 1% L-glutamine, 1% penicillin/streptomycin (100 U/mL; respectively) and 0,25% amphotericin B. On the other side, for adipogenic differentiation, the cells were cultured using adipogenic differentiation medium that contained Human MesenCult™ Adipogenic Basal Medium (Stem Cell Technologies), Human MesenCult™ Adipogenic Differentiation Supplement (Stem Cell Technologies), 1% L-Glutamine, 1% penicillin/streptomycin (100 U/mL; respectively) and 0,25% amphotericin B. The differentiation medium was renewed every 3 days. After bone matrix and adipose cells formation occurred, the differentiation potential was assessed by alizarin red and oil red o for osteogenic and adipogenic differentiation staining, respectively.

Isolation of PBMCs and MSCs co-culture

Human peripheral blood mononuclear cells (PBMCs) were isolated from 20 active SLE patients and 5 healthy controls using Ficoll-Paque (Sigma-Aldrich) density gradient centrifugation with specific informed consent. The separated buffy coat was collected, washed, and pelleted by centrifugation at 1900 rpm for 8 minutes. Then, PBMCs were cultured in 2 ml of advanced RPMI 1640 culture medium (Gibco); supplemented with 10% FBS, 100 U/mL penicillin and streptomycin, and 2 mM glutamine; and incubated at 37°C in a humidified atmosphere with 5% CO2. After 24 hours of incubation, for the treatment groups, PBMCs were co-cultured with MSCs using a Corning Costar 0.4 µm Trans well plate containing RPMI supplemented with 10% FBS, 1% penicillin-streptomycin, and 0.25% amphotericin B at an MSC to PBMC ratio of 1:1, 1:25, and 1:50 (T1, T2, and T3, respecti
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Flow cytometry analysis

According to the manufacturer’s instructions, Dendritic cell markers in the PBMCs population were assessed by flow cytometry analysis after 72 hours of incubation of MSCs. The PBMCs were pelleted by centrifugation, resuspended in 100 µl buffer and incubated using perCP-, FITC- and PE-conjugated anti-human HLA-DR, Lin and CD11c (BD Bioscience). These cells were incubated for 30 minutes at room temperature in the dark. The post-acquisition analysis was conducted using the BD Accuri C6 Plus software.

The level of TNF-α, IFN-γ, IL-6 and IL-10 were also analyzed in co-culture supernatants using custom cytometric bead array kit (CBA; BD Biosciences) following the manufacturer’s instructions. Co-culture supernatants were incubated using the CBA for 30 min and combined with the phycoerythrin (PE)-conjugated antibodies mixed cocktail. The acquisition of TNF-α, IFN-γ, IL-6 and IL-10 were measured via PE fluorescence quantification in reference to a standard curve using BD Accuri C6 Plus Flow Cytometer. The proprietary FCAP analysis software (BD Biosciences) was used to calculate the particular concentration.

Data analysis

Statistical significance was calculated using SPSS 16.0 (IBM Corp., Armonk, NY, USA), and the data were presented as mean ± SD. cytokine levels were analyzed by paired ANOVA and then followed by post hoc Fisher’s LSD. All statistical methods have been stated in the figures. A P value of < 0.05 was considered statistically significant.

4. RESULTS

Characteristics and differentiation capability of MSCs

MSCs isolated from the umbilical cord were analyzed based on their adherent capability under standard culture conditions, antigen-specific surface markers, and differentiation capability after 5 passages. The cell morphology of MSCs at the fourth passage exhibited typical monolayers of spindle-shaped fibroblast-like cells, with adhering capability to the plastic flask (Figure 1a). The ability of MSCs to differentiate into osteogenic and adipose cells was analyzed by cultivating the cells in a differentiation medium for 21 and 30 days, respectively. After incubation, the calcium and adipose deposition were visualized as red color after alizarin red and oil red o solution administration, respectively (Figure 1b and 1c). On the other hand, to characterize MSCs surface antigens, we performed flow cytometry analysis as indicated by the International Society for Cellular Therapy (ISCT). We found a high level of CD90 (99.7±0.75%), CD105 (95.1±1.35%), CD73 (99.4±0.85%) and lacked the expression of CD45/CD34/CD11b/CD19/HLA-DR represented as Lin (0.2±0.06%; Figure 1d).

MSCs downregulated CD11c+ DCs population in the co-cultured PBMCs

Samples were cultured in the presence/absence of MSCs and then analyzed by flow cytometry gating strategy. DCs cells were gated by Lin, HLA-DR, and CD11c. Based on this gate representative flow cytometry dot plots and histograms are shown in Figure 2a. Based on the flow cytometric analysis, expression of CD11c of dendritic cells was remarkably decreased in all MSC co-culture with significance (P <0.05) in all treatment groups. Indeed, the group with the lowest CD11c expression was clearly detected at a dose of MSCs 1:1 with 0.22±0.19%. On the contrary, the untreated group did not lead to CD11c down-regulation.

MSCs downregulated IL-6, TNF-α and IFNγ levels in the co-cultured PBMCs

To investigate the capacity of MSCs in suppressing proinflammatory cytokines, the level of IL-6, TNF-α, and IFNγ were analyzed by flow cytometry after 72 hours incubation of MSCs and PBMCs. The CBA analysis showed that there was a significant decrease in IL-6 levels in
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To investigate the capacity of MSCs in upregulating anti-inflammatory cytokines, the level of IL-10 measurement was performed after 72 hours incubation of MSCs and PBMCs co-culture using CBA analysis. The CBA showed that there was a significant enhancement of IL-10 levels in the T2 and T3 groups, compared to the control group (p<0.05). The group with the highest level of IL-10 was clearly detected at a dose of MSCs 1:1 with 2473.18±619.29 pg/mL.

**5. DISCUSSION**

For the last decade, MSCs are known as a promising therapeutic agent for complex diseases involving immune system dysfunction e.g., autoimmunity, or SLE to be specified. These cells represent regulatory properties on several immune cells, though their potentiation to regulate DCs in SLE has been insufficiently investigated. In this study we prevailed that MSCs possess an immunoregulatory effect on DCs, unveiling their potential to control SLE’s disease activity (17). Previous studies also have suggested that MSCs can inhibit several pro-inflammatory cytokines, such as IFN-γ, IL-1, and IL-6; as well as increase the anti-inflammatory molecules, including IL-10 secretion (9, 15). Furthermore, MSCs can down-modu-
late the DCs maturation markers and inhibit the secretion of TNF-α, leading to higher tolerance in an auto-immunological state, therefore favoring a better outcome (18). These studies guided us to further explore the interaction between MSCs and DCs to provide future clinical therapeutic approaches to SLE patients.

We also explored the possibility of MSCs affecting the DCs population by evaluating the PBMCs in SLE patients, in which co-cultured with MSCs in low (1:50), moderate (1:25), and high (1:1) populations of MSCs. In the presence of MSCs, we analyzed the significant decrease of CD11c+ DCs after 72 hours of incubation of MSCs in all treatment groups, indicating the hampered proliferation of DCs. Our observation is in line with a previous study that reported the decrease of BDCA-1, a specific marker for myeloid DCs type 1 after co-cultured with MSCs (19). Other studies also reported the hampered generation of several myeloid DCs subtypes, including CD4+ DCs, CD14+ monocytes, and CD11c+ Ecad+ Langerhans DCs after the co-culture between MSCs and dendritic commitment of UC-HSCs (20).

Aside from DCs maturation’s suppression, MSCs also revealed a potency to hamper DCs activity and increase regulatory T cells subset (18,20-23). Our previous studies reported the enhanced population of CD4+CD25+FoxP3+ Treg cells in co-cultures between MSCs and PBMCs of SLE patients (23,24). Similar results showed the induction of FoxP3+ T cells in co-cultures between BMSCs and CD4+ T cells (25). Regarding these previous studies, we suggest that the reduction of DCs proliferation efficiency after MSCs co-culture could predispose the increase of Treg cell generation whilst modulating the inflammatory microenvironment at the same time. Our suggestion was supported by a previous study that reported the MSCs’ potency to enhance the CD4+FoxP3+ T cells generation among the decreased DCs efficiency to stimulate CD4+ and CD8+ T cells (19). This suggestion was also confirmed by our study reported the enhanced level of IL-10 post-co-culture between MSCs and PBMCs of SLE patients.

Laidlaw et al. and Murai et al. revealed that CD4+CD25+FoxP3+ Treg cells are one of the IL-10 sources that play a major role in modulating inflammatory responses (26, 27). Other studies also reported that Treg cells can release IL-10 through IL-10R binding in a feed-forward loop (28). Specifically, this binding mechanism involves activation of Janus tyrosine kinases-1 (JAK1) and tyrosine kinase-2 (TYK2) which phosphorylate the IL-10R1, which eventually leads to the generation of signal transducer and activator of transcription-3 (STAT3) docking sites. Concisely, STAT3 will translocate into the nucleus and induces the expression of the IL-10 gene (29). The vigorous production of IL-10 could result in the inhibition of the pro-inflammatory cytokines release e.g., TNF-α, IFN-γ, and IL-6. The dimerization of STAT3 also promotes the transcription of the suppressor of cytokine signaling-3 (SOSC3). The latter molecule can inhibit the nuclear factor kappa B (NF-kB) nuclear translocation and extracellular signal-regulated protein kinase (ERK)1/2, the pathways known to induce the expression of pro-inflammatory genes, including TNF-α, IFN-γ, and IL-6 (30). These mechanisms are in line with our observations that reported the decreased level of TNF-α, IFN-γ, and IL-6 post-MSCs co-culture on PBMCs of SLE patients.

We believe our study might potentially act as a supporting investigation to induct MSCs utilization in SLE though some limitations may apply i.e., we did not analyze the population of CD4+CD25+FoxP3+ Treg cells. We also did not analyze the intracellular proteins and mRNA expression of cytokines released by either MSCs or PBMCs of SLE patients-derived DCs, so we could not confirm the exact molecules expressed whether from MSCs or DCs. Moreover, the expression of several transcription molecules on PBMCs that have a role in inflammatory regulation, such as STAT3, SOSC3, and NF-kB is also unclear.

6. CONCLUSION

In summary, our present work showed that MSCs could inhibit the DCs proliferation. This is confirmed by the decrease of surface markers related to DCs, such as CD11c. Furthermore, MSCs displayed an ability to upregulate the level of IL-10. This was accompanied by the suppression of the pro-inflammatory cytokine secretion, including IL-6, IFN-γ, and, TNFa. Our observation reaffirms the MSCs’ immunoregulatory potential, specifically in SLE patients. MSCs could play a role in homeostasis through these properties, helping to restore equilibrium to an environment disturbed by SLE. Our data shows that MSCs maintain the homeostatic role in experimental conditions and therefore could be explored as potential tools for clinical immune-related therapy in SLE.

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• Availability of data and materials: The data generated in the present study may be requested from the corresponding author.

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• Declaration of competing interest: None declared.


