MSC-EVs: A Promising Modulator for Immune Response in Systemic Lupus Erythematosus

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Abstract

At present, human umbilical cord mesenchymal stem cells(hUCMSCs) have been used in the clinical treatment of systemic lupus erythematosus(SLE), However, the detailed mechanism of MSCs remains unclear. In this study, we investigated whether hUCMSCs derived extracellular vesicles (hUCMSC-EVs) could regulate abnormal immune responses of T cells or B cells in SLE. We isolated splenic mononuclear cells from MRL/lpr mice, a classical animal model of SLE. PBS(Phosphate-buffered saline), 2×10^5 hUCMSCs, 25 μ g/ml hUCMSC-EVs, 50 μ g/ml hUCMSC-EVs were co-cultured with 2×10^6 activated splenic mononuclear cells for 3 days in vitro, respectively. The proportions of CD4⁺T cell subsets and the concentrations of cytokines were detected. Both hUCMSCs and hUCMSC-EVs inhibited CD4⁺T cells, increased the production of T helper(Th)17 cells, promoted the production of interleukin-17 and transforming growth factor beta 1(TGF- β 1) ($P < 0.05$), although they had no significant effects on Th1, Th2, T follicular helper (Tfh), regulatory T (Treg) cells and IL-10 ($P > 0.05$); only hUCMSCs inhibited CD19⁺ B cells, promoted the production of interferon-gamma (IFN- γ) and IL-4 ($P < 0.05$). hUCMSCs exert immunoregulatory effects on SLE at least partially through hUCMSC-EVs in vitro, hUCMSC-EVs play novel and potential regulator roles in SLE.

Introduction

In recent years, animal experiments and clinical evidence have demonstrated that stem cell transplantation could treat refractory systemic lupus erythematosus (SLE) and improve its prognosis. Therefore, stem cell transplantation has become an important treatment method for SLE and has been widely used in clinical practice[1, 2]. At present, adult stem cells for treating refractory SLE are mainly derived from hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs)[3–5]. Allogeneic HSCs transplantation could result in a high incidence of graft-versus-host disease(GVHD) and high transplant-related mortality. While autologous HSCs transplantation does not fundamentally correct the abnormal immune system of SLE, leading to a high recurrence rate after transplantation[4, 6]. These problems have been troubling the clinical application of HSCs transplantation in SLE, and have become the technical bottleneck of stem cell transplantation. Fortunately, Studies indicated that MSCs have brought us a new opportunity in recent years.

Due to its features of low cellular immunogenicity and strong immunomodulatory function, MSCs have brought great hope for the treatment of autoimmune diseases, especially SLE. There are studies suggested that MSCs transplantation can reduce the production of inflammatory factors and autoantibodies, and promote the repair of lupus nephritis (LN) [7]. However, the detailed mechanisms underlying the treatment of LN by MSCs transplantation remain unclear. Current studies have also shown that MSCs may replace damaged renal parenchymal cells by differentiating into functional cells, which is called the differentiation mechanism. However, some scholars believe that MSCs cannot regenerate renal parenchymal organs by differentiating into plenty of renal parenchymal cells [8]. In addition, other researchers believe that MSCs play an immune effect to improve kidney damage by secreting anti-

inflammatory biological factors (such as IL-10, PGE2, IL-1R, TGF- β 1), which is the secretion mechanism [9]. For example, scholars only use the supernatant obtained from MSCs to treat diseases, and the survival of renal tubular cells was also independently promoted [10]. These findings suggest that MSCs may reshape immune function through the secretion mechanism to treat LN. However, the detailed secretion mechanism of MSCs remains unclear.

As a hot topic of life science research in recent years, extracellular vesicles (EVs) are membrane vesicles with a bilayer lipid membrane secreted by a paracellular mechanism. According to the diameter of membrane vesicles, EVs can be divided into exosomes, microvesicles, and apoptotic bodies; currently the former two are the most widely reported. EVs contain active components derived from parent cells such as proteins, lipids, nucleic acids (eg. miRNA, etc.), and organelles. These components are transferred to recipient cells through EVs to mediate the communication between parent cells and recipient cells, and thus participating in physiological and pathological processes such as immune response, cell phenotype regulation, and angiogenesis [11]. Recent studies have shown that mesenchymal stem cells derived extracellular vesicles (MSC-EVs) play an important role in autoimmune diseases. In addition, the transfer of signal molecules to immune cells through MSC-EVs mediated by MSCs has become a novel mechanism for the immunotherapeutic effect of MSCs [7]. Researches have indicated that MSCs-EVs have therapeutic effects in a variety of disease, such as GVHD[12], type 1 diabetes [13] and inflammatory arthritis [14]. In 2019, the finding that MSC-EVs inhibit inflammatory responses by regulating immune cells in inflammatory tissues [15]. Unfortunately, so far, the effects of MSC-EVs in SLE remain unclear in related researches. Therefore, in this study, we investigated whether hUCMSC derived extracellular vesicles (hUCMSC-EVs) could regulate the immune response of immune cells in SLE.

Results

1. Characterization of hUCMSC-EVs

In this study, hUCMSC-EVs were isolated by differential ultracentrifugation. Nanoparticle tracking analysis (NTA) was used to detect the particle size distribution of the purified EVs. The particle size fluctuated between 70-400nm and peaked at about 150 nm (Fig. 1A). hUCMSC-EVs were showed a bilayer-membrane structure of about 150nm in diameter under the transmission electron microscope (Fig. 1B). WB results showed that the hUCMSC-EVs expressed the positive marker CD9, TSG101, but not the negative marker Calnexin (Fig. 1C, Figure S8).

2. Regulation of hUCMSC-EVs on T and B cells in MRL/lpr mice splenic mononuclear cells

2.1 hUCMSC-EVs inhibited CD4⁺T cells in MRL/lpr mice splenic mononuclear cells in vitro

The results showed that the proportion of CD4⁺T cells in hUCMSCs group or 50µg/ml hUCMSC-EVs group was lower than that in PBS control group,the difference was statistically significant($P < 0.05$, Fig. 2A,Figure S1). The proportion of CD4⁺T cells in hUCMSCs group was lower than that in 50µg/ml hUCMSC-EVs group, while there was no significant difference ($P > 0.05$, Table S1).It indicates that the effects of hUCMSC-EVs in the inhibition of CD4⁺T cells were comparable with those of hUCMSCs .

2.2hUCMSC-EVs promoted Th17 cells differentiation in MRL/lpr mice splenic mononuclear cells in vitro

The results showed that the proportion of Th17 cells in hUCMSCs group or 50µg/ml hUCMSC-EVs group was higher than that in PBS control group,the difference was statistically significant($P < 0.05$, Fig. 2B,Figure S2). The effects of hUCMSC-EVs in the differentiation of Th17 cells were comparable with those of hUCMSCs($P > 0.05$, Table S2).

2.3 hUCMSC-EVs had no significant effects on other CD4⁺ T cell subsets in MRL/lpr mice splenic mononuclear cells in vitro

The results showed that there was no significant difference in the proportion of T helper(Th)1,Th2, T follicular helper (Tfh) and regulatory T (Treg) cells among all groups (Fig. 2(C,D,E,F), Figure S(3,4,5,6), Table S(3,4,5,6)).

2.4 hUCMSC-EVs had no effect on CD19⁺ B cells in MRL/lpr mice splenic mononuclear cells in vitro

The results showed that the proportion of CD19⁺ B cells in hUCMSCs group was significantly lower than that in PBS control group in MRL/lpr mice ($P < 0.05$). However, the proportion of CD19⁺ B cells were not affected by hUCMSC-EVs(Fig. 3, Figure S7, Table S7)

3.the Effects Of Hucmsc-evs On Cytokines In Co-culture Supernatant

3.1 hUCMSC-EVs had no effect on the cytokine of IFN-γ in supernatant in vitro

The concentration of IFN-γ in the co-culture supernatant of hUCMSCs group was significantly higher than that of PBS control group ($P < 0.05$). No significant difference was observed in the concentration of IFN-γ in 25µg/ml hUCMSC-EVs group or 50µg/ml hUCMSC-EVs group compared with that in PBS control group ($P > 0.05$) (Fig. 4A, Table S8).

3.2 hUCMSC-EVs had no effect on the cytokine of IL-4 in supernatant in vitro

The concentration of IL-4 in the co-culture supernatant of hUCMSCs group was significantly higher than that of PBS control group ($P < 0.05$). No significant difference was observed in the concentration of IL-4 in 25µg/ml hUCMSC-EVs group or 50µg/ml hUCMSC-EVs group compared with that in PBS control group ($P > 0.05$) (Fig. 4B, Table S9).

3.3 hUCMSC-EVs increased the cytokine concentration of IL-17 in the supernatant in vitro

The concentration of IL-17 in the co-culture supernatant of hUCMSCs group or hUCMSC-EVs group was significantly ($P < 0.05$) higher than PBS control group. The effects of hUCMSC-EVs promoting IL-17 production were weaker than hUCMSCs ($P < 0.05$). (Fig. 4C, Table S10). It indicates that hUCMSCs at least partially regulated the expression of IL-17 through hUCMSC-EVs.

3.4 hUCMSC-EVs increased the cytokine concentration of TGF- β 1 in the supernatant in vitro

The concentration of TGF- β 1 in the co-culture supernatant of hUCMSCs group or hUCMSC-EVs group was significantly higher than that of PBS control group ($P < 0.05$). The effects of hUCMSC-EVs promoting TGF- β 1 production were weaker than hUCMSCs ($P < 0.05$). It indicates that hUCMSCs at least partially regulated the expression of cytokine TGF- β 1 through hUCMSC-EVs. (Fig. 4D, Table S11).

3.5 hUCMSC-EVs had no effect on the cytokine of IL-10 in supernatant in vitro

The results showed that there was no significant difference in the concentration of IL-10 among all groups. (Fig. 4E, Table S12).

Discussion

Multiple paracrine factors of MSCs induce peripheral immune tolerance and regulate immune response, but the specific mechanism has not been fully elucidated. EVs are one of the important paracrine materials, and current researches suggest that MSC-EVs as a promising candidate for a new cell-free therapy of a wide range of immune diseases. To date, among a series of published researches on the animal models or clinical trials of SLE, there is no experimental study reporting the immunoregulatory effects of MSC-EVs on T or B cells. In this study, we reported that both hUCMSCs and hUCMSC-EVs inhibited CD4⁺T cells and increase the proportion of Th17 cells in splenic mononuclear cells; hUCMSCs inhibited B cells; both hUCMSCs and hUCMSC-EVs had no significant effects on Th1, Th2, Tfh and Treg cells. Meanwhile, both hUCMSCs and hUCMSC-EVs promote the expression of IL-17 and TGF- β 1; hUCMSCs promote the production of IFN- γ and IL-4; hUCMSCs and hUCMSC-EVs had no significant regulatory effect on cytokine IL-10. We report that hUCMSC-EVs at least partly mimic the immunomodulatory effects of MSC in SLE, such as inhibiting CD4⁺T cells, promoting Th17 cells, and increasing the production of IL-17 and TGF- β 1. Therefore, hUCMSC-EVs play novel and potential immunoregulation roles in SLE.

SLE is a chronic systemic inflammatory autoimmune disease characterized by the breakdown of autoimmune tolerance. Previous studies have shown that abnormal CD4⁺T cell apoptosis or proliferation may play a key role in the initiation and promotion of autoreactive humoral immunity. The gene mutation of lymphoproliferation (Lpr) in MRL/lpr mice resulted in a lack of functional *Fas* receptor in vivo [17, 18], which mainly promoted T cell expansion [17] and hindered T cell apoptosis [18]. On the one hand, renal infiltration of CD4⁺T cells promoted the development of Lupus nephritis in MRL/lpr mice [19]. On the

other hand, CD4⁺T cell-deficient MRL/lpr mice produced significantly less autoantibody and prolonged survival[20]. Furthermore, anti-CD4 monoclonal antibody therapy for SLE significantly reduced the incidence of vasculitis, and glomerulonephritis, and significantly lowered the levels of antinuclear antibody, total IgG, and anti-dsDNA [20]. Studies showed that MSCs delayed disease progression by promoting CD4⁺T cell apoptosis or inhibiting CD4⁺T cell proliferation in MRL/lpr mice[1, 21]. And in this study hUCMSCs or hUCMSC-EVs inhibited CD4⁺T cells in MRL/lpr mice. Therefore, hUCMSCs may slow the progression of lupus disease by inhibiting CD4⁺T cells through paracrine extracellular vesicles.

IFN- γ and IL-4 are two typical cytokines representing the Th1 and Th2 subsets, respectively. Th2 cells are mainly involved in phenotypic changes from primary T cells to effector T cells and humoral immune activation [22, 23]. Clinically, SLE is diagnosed by detecting a variety of pathogenic autoreactive antibodies against nucleoproteins and nucleic acids, hence Th2 cells may play an important role in the pathogenesis of SLE. Previous studies have differed on the therapeutic effect of MSCs on lupus disease, For example, MSCs promote Th1 cell differentiation [1, 2, 24] and Th2 cell differentiation [25] or inhibit immune response of Th1 and Th2 in lupus models or patients [26]. The results in this study indicated that hUCMSCs or hUCMSC-EVs have no effects on Th1 and Th2 cells in MRL/lpr mice in vitro, while hUCMSCs increased the production of cytokines IFN- γ and IL-4 in the supernatant. This phenomenon explained by the following reasons:(1)the cytokine concentration in the supernatant measured by ELISA was actually the total amount of cytokines produced by mononuclear cells. Cell types determined by flow cytometry for intracellular cytokine detection were actually to confirm whether mononuclear cells differentiate into Th1 or Th2 cells at the single-cell level. (2)The cytokine network of SLE is extremely complex, which is related to the disease state, the specific tissues involved, and the genetic constitution. These diseases present complex symptoms, suggesting that the cytokine network in SLE may also be very complicated. These factors may contribute to the differences in results of various laboratories.

There were studies indicating that MSCs promoted the production of Th17 cells and Treg cells in peripheral blood mononuclear cells, as well as production of IL-17 and TGF- β in SLE patients[27]. In non-SLE diseases, MSCs also promoted the differentiation of activated CD4⁺T cells into Th17 cells and the production of IL-17. Our study indicated that hUCMSCs or hUCMSC-EVs promoted the differentiation of CD4⁺T cells into Th17 cells and increased the levels of IL-17 and TGF- β 1.

Follicular helper T cells(Tfh) as another subset of CD4⁺T cells, also play an important role in B cell differentiation, maturation and antibody secretion [28]. These results showed that hUCMSCs inhibited Tfh cells, while hUCMSC-EVs only had a tendency to inhibit Tfh cells. It has been documented that MSCs inhibit the differentiation of CD4⁺T cells into Tfh cells through cell-to-cell contact [29–31]. Whether hUCMSCs can inhibit Tfh cell differentiation through paracrine extracellular vesicles needs to be further verified by expanding the sample size or increasing the intervention amount of EVs.

IL-10 is a multifunctional cytokine that plays an important role in regulating the growth and differentiation of B cells and the production of autoantibodies [32]. Most IL-10 came from monocytes and B cells, with a small amount from T cells [33, 34]. In 1993, it was first reported that PBMCs in newly

diagnosed SLE patients produced more IL-10 than healthy control group [35], and serum IL-10 was significantly associated with lupus disease activity and anti-ds-DNA titers [36]. Anti-IL-10 monoclonal antibody therapy can reduce urinary protein and autoreactive IgG levels and inhibit in vitro cellular immune responses of peripheral blood mononuclear cells in SLE patients [37]. IL-10 can also inhibit the production of active TGF- β [38]. Hence IL-10 may be the hub connecting Treg cells, Tfh cells and B cells. Previous studies have also shown that MSCs alleviate lupus-like diseases by reducing the number of CD19⁺B cells [39]. The secretions of MSCs reduce serum IL-10 levels in lupus mice [40]. This study demonstrated that hUCMSCs inhibited B cells; hUCMSC-EVs had no effects on B cells, and hUCMSCs or hUCMSC-EVs had no significant regulatory effects on IL-10. It is possible that hUCMSCs regulate B cells through other mechanisms, or the small sample size or intervention amount of EVs in this study resulted in no statistical significance among groups.

In summary, hUCMSCs exert immunoregulatory effects on SLE at least partially through hUCMSC-EVs in vitro, and hUCMSC-EVs play a novel and potential regulatory role in SLE. However, we lack in vivo experiments to further verify the regulatory effects of hUCMSC-EVs on immune cells in MRL/lpr mice and the therapeutic effect on the condition of mice.

Conclusion

This study investigated the immunoregulatory effects of hUCMSC-EVs on CD4⁺T cell subsets and CD19⁺B cells in splenic mononuclear cells of MRL/lpr mice. This study shows that hUCMSCs inhibit the total CD4⁺T cells and regulate the proportion of Th17 cells, and promote the expression of soluble cytokines IL-17 or TGF- β 1 at least partially through hUCMSC-EVs in vitro. hUCMSC-EVs play a novel and potential immunoregulation role in SLE. This further elucidates another novel mechanism by which MSCs play an immunoregulatory role in SLE. This study may provide a theoretical basis for the application of MSC-EVs in the treatment of autoimmune diseases.

Methods

Experimental animal

Female MRL/lpr mice (stock number 000485 and induced from Jackson laboratory, USA) were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. and raised under the specific-pathogen-free facilities in the Laboratory Animal Center of the Second Xiangya Hospital, Central South University until they were used. All experimental applications were carried out in accordance with ARRIVE guidelines. The MRL/lpr mice used in this experiment is a classic animal model of SLE. The onset of autoimmune diseases in MRL/lpr mice was monitored by measuring proteinuria (100 mg/dl) [1, 16], and MRL/lpr mice have symptoms such as lymphoproliferation and skin damage, which are in line with the description in Jackson Laboratory. Please see the Jackson laboratory website for details: <https://www.jax.org/jax-mice-and-services>. Female MRL/lpr mice were euthanized at age of 20 weeks and mononuclear cells were isolated from the spleen for in vitro experiments. Splenic mononuclear cells from the same mouse were

separated into four groups(2×10^6 cell/group) as phosphate-buffered saline (PBS) control group, MSC group, 25 μ g/ml hUCMSC-EVs group, 50 μ g/ml hUCMSC-EVs group. The Ethical approval was obtained from the Institutional Animal Care and Use Committee of the Second Xiangya Hospital, Central South University (Approval No. 2020344) for the study, animal experimentation guidelines were exactly followed.

hUCMSC-EVs extraction and identification

The hUCMSCs were subcultured and expanded by the primary hUCMSCs isolated from healthy human umbilical cord wharton's jelly. The 3-8 passages of hUCMSCs were cultured in serum-free complete medium (Clin-Biotechnology, China). When the cells grew to 80%-90% fusion, the surface of the cells was cleaned with PBS solution, repeated twice, and then basic medium (Clin-Biotechnology, China) was added for 3 days of starvation culture. Next, the supernatant was collected and hUCMSC-EVs in supernatant was isolated by ultracentrifugation, specific steps were as follows: Centrifugation at 300g for 15min to remove residual cells; centrifugation was performed at 2,000g for 15min to remove large cell fragments; small fragments and apoptotic bodies were removed by centrifugation at 10,000g for 30min. After that, the centrifuged supernatant was filtered with a needle filter of 0.22 μ m to remove smaller fragments and larger vesicles. Ultra-15 10-KDA MWCO test tubes (Merck Millipore, Ireland) were centrifugated at 4,000g for 30min to concentrate the supernatant after the above treatment and the concentrated supernatant was collected. Finally, centrifugation was performed at 120,000g for 2 hours (SW 41 Ti 41,000 RPM, Beckman Optima XPN-100, USA). The prepared EVs were ultimately resuspended in appropriate amount of PBS. All centrifugation processes were carried out under 4°C. The total protein concentration of EVs was measured by BCA protein quantitation method (Thermo Scientific, USA).

The purified EVs were diluted 10,000 times, and Nanoparticle Tracking Analysis (NTA) (ZetaView, Germany) was used to measure the particle size distribution of EVs. 10 μ l EVs were dropped onto the ultrathin carbon film copper mesh, then EVs were treated with glutaraldehyde, uranyl oxalate, and methylcellulose-UA in turn, and after drying, the morphology of EVs was observed by the transmission electron microscope (Hitachi, Japan).

To detect EVs specific markers and negative markers, the samples were incubated with the antibodies of anti-CD9, anti-TSG101, and anti-Calnexin (Abcam, UK), and then incubated with enzyme-labeled anti-rabbit IgG antibody (Sigma, USA), and finally a western blot developer machine was used.

Mononuclear cells were co-cultured with hUCMSCs or hUCMSC-EVs

Spleen mononuclear cells were isolated by density gradient centrifugation with mouse ficoll according to the manufacturer's instructions. Briefly, female MRL/lpr mice for 20 weeks were euthanized and the spleen was removed aseptically. Spleen grinding liquid were layered over ficoll-paque PREMIUM 1.084g/ml (GE, catalog No. 17544602-1, USA) and centrifuged at 400g for 30-40min at 18-20°C. The mononuclear cells were collected at the interface, then the cells were mixed with 3 times the volume of PBS to wash twice by centrifugation at 60-100g for 10min at 18-20°C. Then the cells were resuspended

in RPMI1640(Gibco, USA) supplemented with 10% fetal bovine serum(Gibco, USA). The final concentration of mononuclear cells was adjusted to 2×10^6 /ml. hUCMSCs were pretreated with 10 μ g/ml mitomycin C(Sigma, USA) and inoculated at a density of 2×10^5 /well into a 12-well plate (Corning, USA) and added with RPMI1640 medium (Gibco, USA) containing 10% fetal bovine serum, 100U/ml penicillin as well as 100 mg/ml streptomycin overnight. On the second day, 1ml suspension of 2×10^6 /ml mouse splenic mononuclear cells was added into the well with hUCMSCs, and the ratio of hUCMSCs and splenic mononuclear cells =1:10. In the experimental well of hUCMSC-EVs immune response to T cells, to activate T cells under Th0 condition, 1.0 μ g/ml anti-CD3e and anti-CD28 were added to the co-culture mentioned above. In order to activate B cells, 6 μ g/ml ODN 1826 B cell activators were added to the above co-culture system. Then the final volume of each well was adjusted to 1.2ml with PBS and hUCMSCs were co-cultured with mononuclear cells for 3 days. In the hUCMSC-EVs group, fresh hUCMSC-EVs preparations of 25 μ g/ml or 50 μ g/ml were added to study the effect of hUCMSC-EVs in the same procedure and time. As 25 μ g/ml hUCMSC-EVs had no significant regulation effect on T cells, only 50 μ g/ml hUCMSC-EVs were used to study the regulation effect of hUCMSC-EVs on B cells. The control group was treated with PBS.

CD4⁺T cell subsets and CD19⁺B cells were detected by a flow cytometer

Suspended mononuclear cells were recovered from the culture medium, washed with PBS, and the proportions of CD4⁺T cell subsets and CD19⁺B cells were detected by a flow cytometer (Cytex, USA). Cells were stained with anti-mouse antibodies that are fluorescent-conjugated for CD4 (BD Pharmingen, USA), IFN- γ (BD Pharmingen, USA), IL-4 (BD Pharmingen, USA), IL-17a (BD Pharmingen, USA), CD25 (BD Pharmingen, USA), Foxp3 (BD Pharmingen, USA), CD185 (BD Pharmingen, USA), CD19 (BD Pharmingen, USA). CD4⁺IFN- γ ⁺ T cells , CD4⁺IL-4⁺T cells ,CD4⁺IL-17⁺T cells, CD4⁺CD25⁺Foxp3⁺T cells and CD4⁺CD185⁺T cells were thought as Th1, Th2, Th17, Treg and Tfh cells, respectively. For intracellular staining, the cells were treated with cytokine stimulation blockers (BD Pharmingen, USA). The Flowjo10.5.3 program was used to analyze the data.

Cytokines were measured by ELISA

Cytokine levels in the co-culture supernatant were measured by commercial ELISA kits. Mice were tested for IFN- γ (USCN, catalog No. SEA049Mu, China), IL-4 (elabscience, catalog No. E-EL-M0043c, China), IL-17a (elabscience, catalog No. E-EL-M0043c, China), TGF- β 1 (USCN, catalog No. SEA124Mu, China), and IL-10 (USCN, catalog No. SEA056Mu, China)in accordance with the manufacturer's instructions.

Statistical analysis

We used the One-way analysis of variance for statistical analysis for parametric data and the Kruskal-Wallis test for non-parametric data. We performed statistical analyses and maps with GraphPad Prism 5 software and considered a *P* value less than 0.05 as significant. Data are shown as means $\pm$ standard error of mean (means $\pm$ SEM).

List Of Abbreviations

hUCMSCs: human umbilical cord mesenchymal stem cells

hUCMSC-EVs: human umbilical cord mesenchymal stem cells derived extracellular vesicles

PBS: phosphate-buffered saline

IFN- γ : Interferon- γ

IL: Interleukin

TGF- β 1:transforming growth factor- β 1

Th: T Helper

Tfh: T Follicular helper

Treg: Regulatory T

SLE: Systemic lupus erythematosus

HSCs: Hematopoietic stem cells

GVHD: Graft-versus-host disease

LN: Lupus nephritis

NTA: Nanoparticle Tracking Analysis

Declarations

Ethics approval and consent to participate

The studies involving animals were reviewed and approved by the institutional ethics board of the Second Xiangya Hospital of Central South University (No. 2020344).

Consent for publication

Not applicable.

Competing interests

The authors declare that the they have no competing interests.

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Authors' contributions

MX and CW: study conception and design and manuscript writing. CL, MH, MX, SL and ZS: development of methodology and statistical analysis. JT, FW, JM and WW: data collection. All authors manuscript formatting and editing.

Data availability statement

The original contributions presented in the study are included in the article/ supplementary material. Further inquiries can be directed to the corresponding author.

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Figures

Figure 1

hUCMSC-EVs characterization. (A) NTA particles size distribution. (B) Transmission electron microscopy. (C) Western blot.

Figure 2

In vitro immunomodulatory effect of hUCMSCs and hUCMSC-EVs on MRL/lpr mice splenic T cells. Flow cytometric analysis of the proportion of CD4⁺T cell (2A), CD4⁺T cell subsets(Th1(2B),Th2(2C),Th17(2D) , Tfh (2E), Treg (2F)) in splenic mononuclear cells .Data were expressed as the means±SEM ,n=9 for each group ,* $P \leq 0.05$ vs PBS control group.

Figure 3

In vitro immunomodulatory effect of hUCMSCs and hUCMSC-EVs on MRL/lpr mice splenic B cells. Flow cytometric analysis of the proportion of CD19⁺B cell in splenic mononuclear cells. Data were expressed as the means±SEM, n=11 for each group, * $P \leq 0.05$ vs PBS control group.

Figure 4

In vitro immunomodulatory effect of hUCMSCs and hUCMSC-EVs on cytokines. (A,B,CD,E) Culture media supernate levels of IFN- γ , IL-4, IL-17, TGF- β 1 and IL-10 in each group. Data were expressed as the means $\pm$ SEM, $n=10$ for each group, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.0001$ vs PBS control group. # $P \leq 0.05$, ### $P \leq 0.0001$, hUCMSCs vs 50 μ g/ml hUCMSCs -EVs.

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