Original Paper

Mesenchymal Stem Cells Support Proliferation and Terminal Differentiation of B Cells

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Key Words

Mesenchymal stem cells • B cells • PGE2 • IL-6

Abstract

Background: Mesenchymal stem cells (MSC) play important roles in modulating the activities of T lymphocytes, dendritic cells and natural killer cells. These immunoregulatory properties of MSC suggest their therapeutic potential in autoimmune diseases. However, the effects of MSC on B cells are still poorly understood. The present study was designed to investigate the interaction between MSC and B cells both *in vitro* and *in vivo*, and to determine the possible mechanism of action. **Design and Method:** The effect of human umbilical cord mesenchymal stem cells (UC-MSC) on proliferation and differentiation of B-cells were characterized *in vitro*, and we also tested the immunoregulatory properties of mouse bone marrow MSC (BM-MSC) on T cell dependent and independent antibody production *in vivo* in mice. **Results:** Treatment with human UC-MSC resulted in an increase of proliferation, differentiation of B cells into plasma cells and production of antibodies *in vitro*. Mouse BM-MSC significantly enhanced T cell dependent and independent antibodies production *in vivo* in mice. PGE2 partially mediated the immunosuppressive activity of human UC-MSC but IL-6 did not regulate this activity. **Conclusion:** MSC promote proliferation and differentiation of B cells *in vitro* and *in vivo* partially through PGE2 but not IL-6.

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Introduction

Mesenchymal stromal cells (MSC), initially described by Friedenstein in 1970 [1], can be obtained from a variety of tissues, including bone marrow (BM-MSC), adipose (AD-MSC) [2], umbilical cord blood (UCB-MSC) [3], placenta [4], umbilical cord (UC-MSC) [5], amniotic fluid (AF-MSC) [6] and many other tissues [7]. MSC affect both the phenotype and the function of a number of cells that belong to the innate and adaptive immune system including T and B lymphocytes, dendritic cells, and natural killer cells [8]. In addition, their immunomodulatory effects have been tested in a variety of animal models related to alloreactive immunity (GVHD) [9], autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) [10], collagen-induced arthritis (CIA) [11], systemic lupus erythematosus (SLE) [12], and systemic sclerosis (SSc) [13]. Actually, human UC-MSC are being explored as a promising candidate for many potential clinical applications due to their easy isolation and expansion in large quantities [5], and to their immunosuppressive effect on T cells [14, 15]. We have previously demonstrated that UC-MSC exert their immunosuppression via IL-1 β -PGE2 axis [14, 16]. However, the clinical application of MSC was focused on TH1 type autoimmune diseases such as collagen induced arthritis [11, 17], experimental autoimmune encephalomyelitis [10] and type 1 diabetes mouse models [18]. Th1 cells are able to promote cell-mediated immunity and Th2 response is often associated with the humoral response. MSC ameliorated the clinical and histopathological severity of autoimmune diseases through down-regulation TH1 responses [19] and activation of CD4+CD25+FoxP3+ Tregs [20]. However, allogeneic MSC did not benefit New Zealand black (NZB) X New Zealand white (NZW) F1 (NZB/W) mice and appeared to exacerbate systemic lupus erythematosus (SLE) which belonged to TH2-type SLE [21]. MSC supported *in vitro* proliferation and differentiation to immunoglobulin-secreting cells (ISCs) of B cells isolated from SLE patients [22].

B cells play an important role in autoimmune diseases both as immunoglobulin-secreting cells and regulatory cells to produce cytokines, which influence T cells, macrophages, natural killer cells in the pathogenesis of autoimmunity disease [23]. B-cell development occurs in the bone marrow and is strictly dependent on close interaction between B-cell progenitors and stromal cells that produce cytokines capable of supporting B-cell survival and proliferation. However, there is no further information on the immunomodulation effects of MSCs on antibodies production *in vivo*.

In this study, we investigated the immunomodulation effect of human UC-MSCs on the *in vitro* proliferation and differentiation of B cells into immunoglobulin-secreting cells and the possible mechanism, and we also tested mice BM-MSC on T cell dependent and independent humoral immunity in mice *in vivo*, and we found that human UC-MSC promote B cell proliferation and differentiation *in vitro* partially through PGE2 but not IL-6, mice BM-MSC increased the T cell dependent and independent IgM and IgG production *in vivo*. Our results suggest that MSC promote humoral immunity both *in vitro* and *in vivo* partially through PGE2 but not IL-6. This study raises important questions on MSC as a therapeutic tool in some autoimmune diseases especially associated with auto-antibodies.

Materials and Methods

Human UC-MSC and murine bone marrow MSC preparation

Human UC-MSC were isolated from umbilical cords obtained from local maternity hospitals with donors' informed consent. Human tissue collection for research was approved by the institutional review board of the Chinese Academy of Medical Science and Peking Union Medical College. UC-MSC isolation and *ex vivo* expansion were done according to the procedures described previously [5]. UC-MSC were negative for CD3, CD14, CD19, CD34, CD45, CD31, CXCR4, Stro-1, HLA-DR, CD80, and CD86 expression but positive for CD13, CD44, CD73, CD90, CD105, CD106, CD166, CD29, CD49e, and HLA-ABC expression (data not shown). In addition, UC-MSC were able to differentiate into adipogenic, osteogenic and chondrogenic cells in lineage-specific induction conditions as previously described [14]. UC-MSC at passage 4-6 were preplated

and subjected for 30 Gy irradiation and then allowed to adhere 2 hours at 37° C before co-culture with B cells.

Murine MSC were isolated from bone marrow BALB/c mice. The whole BM cells were flushed out of the mouse femur and tibia, and cultured in 25 cm² tissue culture flasks (5x10⁵ cells/cm²) using L-DMEM (Gibco) containing 10% FBS, 5ng/ml recombinant murine FGF-basic (Peprotech), and 20ng/ml mouse EGF(Peprotech) at 37°C in air plus 5% CO₂ and 5% O₂. After 3 day culture, the nonadherent cells were decanted by changing the medium. The adherent cells were trypsinized and replated at 1:3 ratio when cultures reached 70% to 80% confluence. After two passages, the cells were harvested and stained with fluorescein-conjugated monoclonal antibody against CD14, CD19,CD29, CD45, CD80, and HLA-DR, CD90 (biolegend) and the flow cytometry analysis was performed on a FACSCalibur using the CellQuest software (Becton Dickinson).The capacity of BM-MSc that differentiate along adipogenic and osteogenic lineages was also evaluated following the supplier's instruction by Adipogenesis Differentiation Kit and Osteogenesis Differentiation Kit (Gibco). BM-MSc were negative for CD14, CD19, CD45, CD80 HLA-DR expression but positive for CD90, CD29 expression (data not shown).

Flow Cytometry Analysis

Phycoerythrin (PE)-CD19,-CD138, were purchased from Becton Dickinson(BD). 1*10⁵ B cells were harvested, washed once with and resuspended in PBS. Cells were treated at RT for 30 min with the isotype control mAbs or with the following specific anti-human antibodies, and flow cytometry analysis was performed on a FACSCalibur using the CellQuest software (Becton Dickinson).

B Cells Isolation and Culture

Peripheral blood samples were generated from healthy donors after informed consent was obtained. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque (Axis-Shield) density gradient centrifugation. CD19⁺ B cells were isolated using CD19 microbeads (Miltenyi Biotec) according to the manufacturer's instructions. Briefly, the PBMC were magnetically labeled with CD19 microbeads, and then loaded onto the column in a magnetic field. The magnetically labeled cells were retained on the column and were eluted as the positively selected cell fraction. The purity of B cells was more than 95%. The purified B cells were cultured in PRMI 1640 medium containing 10% FBS (Hyclone), 100U penicillin/streptomycin (Gibco) in the absence or presence of the follow stimuli: 2.5µg/ml CpG 2006 (Sangon Biotech), 2.5ug/ml AffiniPure F(ab')₂ Fragment Goat Anti-Human IgA + IgG + IgM (H+L) (Jackson Immunoresearch Laboratories), 1ug/ml soluble CD40L (Peprotech) and 100U/ml IL-2 (Peprotech).

Proliferation Assay

Peripheral blood CD19⁺ B cells (10⁴ per well) were seeded in flat-bottom 96 well plates, and treated with or without irradiated UC-MSc (10⁴ per well) in PRMI 1640 medium containing 10% FBS, 100U penicillin/streptomycin for 4 days in the absence or presence of stimuli (2.5µg/ml CpG 2006, 2.5ug/ml AffiniPure F(ab')₂ Fragment Goat Anti-Human IgA + IgG + IgM (H+L), 1ug/ml soluble CD40L and 100U/ml (IL-2). The proliferation of purified B cells was measured using the cell proliferation BrdU ELISA kit (Roche Diagnostics) according to manufacturer's instructions. BrdU was added for the last 18h of incubation. After fixation, the mouse monoclonal anti-BrdU-POD antibody was added. The immune complexes were revealed by the subsequent substrate reaction and add 1M H₂SO₄ to stop the solution, then quantified by measuring the absorbance at 450 nm with a reference wavelength at 575 nm in a microliter plate reader.

Enzyme-Linked Immunosorbent Assay

Peripheral blood B cells (10⁴ per well) were seeded in flat-bottom 96 well plates, and cocultured with indicated doses of UC-MSc (10⁴ per well) for 4 or 7 days. Cell-free supernatants were collected and kept frozen at 80 °C until assayed for cytokines concentration by enzyme-linked immunosorbent assays (ELISA). The PGE₂ and IgA, IgG, IgM expressions were measured in the 7-day culturing cell-free supernatants, and IL-6 level were determined in the 4-day culturing cell-free supernatants by ELISA (Westang Biotech Co, Ltd., PR China, and Cayman Chemicals) following the supplier's instruction.

Fig. 1. UC-MSC promote Proliferation of activated purified peripheral blood B-cells. 10^4 purified peripheral blood B-cells were treated with or without 10^4 irradiated UC-MSC per well in 96-well plates for 90 h, in the absent or in the present of the following stimuli: CpG 2006, rCD40L, anti-immunoglobulin antibodies and IL-2. (A) without UC-MSC in the absent of stimuli; (B) without UC-MSC in the present of stimuli; (C) with UC-MSC in the absent of stimuli; (D) with UC-MSC in the present of stimuli; Magnification: 40 $\times$. (E) Proliferation data were evaluated by BrdU ELISA Kit. Each data represent the mean $\pm$ SEM of three independent experiments, each performed in triplicate. *** $P < 0.001$.

Immunization and ELISA assay for anti-TNP Abs

Immunization and ELISA were performed as described by Franzoso et al. [24]. Briefly, female Eight-week-old male BALB/c mice were provided by the Institute of Experimental Animals, Chinese Academy of Medical Sciences, and immunized by a IP injection of 100 μ g 2,4,6-Trinitrophenyl Keyhole Limpet Hemocyanin (TNP-KLH), 50 μ g 2,4,6-Trinitrophenyl lipopolysaccharide (TNP-LPS), and 25 μ g 2,4,6-Trinitrophenyl Ficoll (TNP-Ficoll) (all from Biosearch Technologies). Immediately after the antigen treatment, the mice were randomly divided into groups and injected intravenously with 1×10^6 BM-MSC in 100 μ l PBS per animal or with 100 μ l PBS alone. Sera were collected on day 7 and day 14 postimmunization. Serial dilutions of serum in PBS-Tween were placed in a TNP-BSA (Biosearch Technologies)-coated 96-well plate (ebioscience) and refrigerated overnight. Following three washes with PBS-Tween, the plates were incubated for 1 h at RT with HRP-conjugated goat anti-mouse isotype-specific Abs (Southern Biotechnology Associates), and then incubated at RT for 30 minutes with the HRP substrate ABTS (ebioscience) after another three washes. The reaction was stopped with 1M H_2SO_4 , and the absorbance was measured at 450 nm.

Statistical Analysis

Data are presented as mean $\pm$ SEM. When applicable, a Student's unpaired t-test and one-way ANOVA were used to determine significance, * $p < 0.05$, was considered to be statistically significant. Statistical analyses were performed using the GraphPad Prism software.

Results

UC-MSC promote proliferation of activated B-cells

UC-MSC could be isolated and expanded easily in large quantities *in vitro* and resemble BM-MSC in many respects. We examined the effects of human UC-MSC on purified human peripheral blood B cells *in vitro*. The purified peripheral blood B-cells were treated with or

Fig. 2. UC-MSCs promote terminal differentiation and immunoglobulin production of Peripheral blood B-cells both *in vitro* and *in vivo*. (A-B) Purified peripheral blood B cells were cultured for 7 days without or with irradiated UC-MSC at 1:1 ratios in the presence of CpG, rCD40L, anti-immunoglobulin antibodies, IL-2. After culture, B cells were stained with PE-CD138 mAb and analyzed by flow cytometry. Results obtained from 7 different experiments. *** $P < 0.001$. (C) Immunoglobulin concentrations in 7-day supernatants of purified peripheral blood B cells with or without UC-MSC. Each data represent the mean $\pm$ SEM of 5-9 independent experiments. (*** $P = 0.0002$ for IgM $N = 9$; ** $P = 0.0044$ for IgA, $N = 5$; *** $P < 0.0001$ for IgG, $N = 6$).

without irradiated UC-MSC at ratio 1:1 for 90h in the absence or presence of the stimuli (CpG 2006, rCD40L, anti-immunoglobulin antibodies and IL-2). As shown in Fig. 1, B cells do not proliferate without stimulation no matter cultured alone (Fig. 1A) or cocultured with UC-MSC (Fig. 1B). B cells were activated by the stimuli and grow as cluster (Fig. 1C) while the increased number of B cells was observed and the cells were well spread when cocultured with UC-MSC in the presence of stimuli (Fig. 1D). Later proliferating cells number was calculated by Brdu ELISA (Fig. 1E), the OD in cocultured wells was not changed compared with B cells in the absence of the stimuli, while activated B cells had a significant increase in proliferation when cocultured with UC-MSC compared with activated B cells alone (1.7592 ± 0.09473 versus 1.237 ± 0.05293 , $P < 0.0001$). Thus we demonstrate that UC-MSC drastically increase activated B cells proliferation *in vitro*.

MSC promote terminal differentiation and immunoglobulin production of B-cells in vitro

The effect of human UC-MSC on purified peripheral blood B cell differentiation was subsequently addressed by examining the expression of CD138, a special marker at the B cell terminal stage. The CD138⁺ B-cells is only $2.18\% \pm 0.416$ when activated B cells were cultured alone for 7 days, and it went up to $15.08\% \pm 1.305\%$ ($P < 0.001$) when cocultured with irradiated UC-MSC at ratio 1:1 in the presence of CpG, rCD40L, anti-immunoglobulin antibodies and IL-2 (Fig. 2A and 2B). The increase of CD138 proved that UC-MSC promote B cell terminal differentiation.

In order to assess the immunomodulation of UC-MSC on immunoglobulin secretion, the immunoglobulin production in the 7-day culturing supernatants of purified peripheral

Fig. 3. Soluble mediators mediate the immunomodulation of UC-MSCs on purified peripheral blood B cells. 5×10^4 irradiated UC-MSCs were plated onto the upper chamber of a 24-well Transwell plate 2 h before the addition of 5×10^4 /well purified peripheral blood B cells in the presence of CpG, rCD40L, anti-immunoglobulin antibodies and IL-2. The cells were cultured for 4 days. The growth of B cells was observed by 40 $\times$ magnification. (A) Transwell (B) Purified activated peripheral blood B cells alone, and (C) addition of BrdU in the last 18 h to the culture and testing of the BrdU incorporation by BrdU ELISA. Data represent the mean $\pm$ SEM of three independent experiments, each performed with UC-MSCs from different donors, *** $P < 0.0001$.

blood B cells without or with UC-MSCs at a 1:1 ratio was assessed by ELISA. As shown in Fig. 2C, UC-MSCs increased three kinds of immunoglobulin production (IgM: 1.8 fold, IgA: 1.6 fold, IgG: 2.2 fold). Thus, UC-MSCs promote the terminal differentiation and immunoglobulin production of B cells *in vitro*.

Immunomodulation of UC-MSCs on purified peripheral blood B cells depends on soluble factors

Previous studies have shown that MSCs exert immunosuppressive effects either through direct cell-cell contact or by soluble factors [8, 11, 25-29]. To determine this, we performed co-culture experiments using the transwell system in which purified peripheral blood B cells and UC-MSCs were physically separated by a membrane permeable for soluble factors. Even in the absence of direct cell-cell contact, UC-MSCs dramatically supported the growth of B cells observed in 40 $\times$ magnification (Fig. 3A and 3B). In addition, we also tested B cell proliferation by BrdU ELISA. BrdU incorporation dramatically increased in the transwell group than in the B cells group, and there was no significant difference between cell-cell contact groups and transwell groups (Fig. 3C $p < 0.05$), indicating that the immunomodulation of UC-MSCs is largely mediated by soluble factors.

PGE2 but not IL-6 is an important soluble factor for UC-MSCs-mediated immunomodulation

A number of soluble factors including TGF- β , IL-10, NO, PGE2, HLA-G have been proposed as possible mediators of MSC-mediated immune regulation [27-29]. PGE2 influences B cell Ig class-switch recombination, modulates B-cell differentiation [30-33]. Our previous data have demonstrated that PGE2-mediated mechanism by which UC-MSCs exert their immunomodulatory effects on T cells [14, 34]. We assessed PGE2 and IL-6 in 4-day culturing cell-free supernatants, and we found PGE2 and IL-6 concentrations were both higher (PGE2: 34.49 ± 5.792 ng/ml, IL-6: 33.68 ± 3.121 ng/ml) in coculture system than UC-MSCs alone (PGE2: 17.84 ± 3.809 ng/ml $P = 0.0339$, IL-6: 19.70 ± 2.17 ng/ml $P = 0.0072$) (Fig. 4A). Coculture experiments were performed in the presence or absence of specific inhibitors of PGE2 (indomethacin, 10 mM) and neutralizing antibodies of IL-6. Indomethacin inhibited the percentage change in proliferation of B cells in coculture system from 34.4% to 22.1% (Fig. 4B), and indomethacin also inhibited percentage change in immunoglobulin production

Fig. 4. PGE2 but not IL-6 is a soluble factor responsible for UC-MSC-mediated immunomodulation. 10^4 /well irradiated UC-MSC were plated in 96-well plate 2h before the addition of CpG, rCD40L, anti-immunoglobulin antibodies, IL-2 -stimulated purified peripheral blood B cells. (A) 4-day culturing cell-free supernatants of UC-MSC alone and coculture system were collected to test IL-6 and PGE2 by ELISA. Data represent the mean $\pm$ SEM of three independent experiments, each performed with UC-MSC from different donors * $P < 0.05$, ** $P < 0.01$. (B) Irradiated UC-MSC were co-cultured with purified peripheral blood B cells (at ratio of 1:1) activated by CpG, rCD40L, anti-immunoglobulin antibodies, IL-2 in the presence or absence of PGE2 inhibitors, indomethacin and neutralizing antibodies of IL-6, B cells proliferation was detected by Brdu ELISA at day 4. Three independent experiments were shown * $P < 0.05$) and (C) 7-day cell-free supernatants were collected to test IgM, IgA and IgG by ELISA. Data represent in three independent experiments * $P < 0.05$. (D) activated B cells were cultured with/without irradiated UC-MSC (at ratio of , B cells proliferation was detected by Brdu ELISA at day 4. Three independent experiments were shown ** $P < 0.01$, *** $P < 0.0001$).

(IgM: 87.33% vs 34.9195% $P < 0.001$, IgA: 67.65% vs 22.28% $P < 0.05$, IgG: 131.5% vs 35.85% $P < 0.05$) (Fig. 4C). However, neutralizing antibodies of IL-6 only slightly reduce IgM and IgG production in coculture system, and there was no statistically significant difference (Fig. 4C). Moreover, neutralizing antibodies of IL-6 did not affect the proliferation and IgM production in coculture system. We also added exogenous PGE2 (10ng/ml) and indomethacin (10 mM) in the culture systems, and found that exogenous PGE2 could increase the B cells proliferation in coculture system while PGE2 secretion could be inhibited by indomethacin (Fig. 4D, $P < 0.0001$). Exogenous PGE2 did not affect the proliferation of B cells (Fig. 4D, $P > 0.05$). Therefore MSCs derived PGE2 might cooperate with other soluble cytokines to regulate B-cell proliferation and differentiation.

BM-MSC promote immunoglobulin production of B-cells in vivo

1×10^6 murine BM-MSC were injected IP to mice immunized with TNP-KLH (T cell dependent antigen, TD-Ag), TNP-LPS (T cell independent antigen-I, TI-IAG), and TNP-Ficoll (T cell independent antigen-II TI-IIAG), and TNP-specific Abs were measured in serum at day 7 and day 14 post cell injection. The titers of TNP-specific IgM increased in TNP-LPS immunized mice 7 days after BM-MSC injection (Fig. 5A, $P < 0.05$), however, BM-MSC didn't

Fig. 5. Murine BM-MSCs promote immunoglobulin production *in vivo*. (A) 100 μ g TNP-KLH, 50 μ g TNP-LPS and 25 μ g TNP-Ficoll were injected i.p. into separate groups of Bach/C female mice with or without 106 BM-MSC per mouse. 7-day serum samples were collected for TNP-specific IgM. (5 samples from two independent experiments; * $p < 0.05$). (B) 14-day serum samples were collected for TNP-specific IgG. (5 samples from two independent experiments; * $p < 0.05$). TNP-KLH: T cell dependent antigen TD-Ag; TNP-Ficoll: T cell independent antigen-II, TI-IIAg; TNP-LPS: T cell independent antigen-I, TI-Iag.

affect the TNP-specific IgM expression in TNP-KLH or TNP-Ficoll immunized mice ($P > 0.05$). the TNP-specific IgG-titer was elevated in immunized mice after 14 days treating with BM-MSC (Fig. 5B, IgG of TNP-LPS enhanced from 1.436 ± 0.01978 to 1.275 ± 0.06232 $p = 0.0414$; IgG of TNP-KLH from 0.8253 ± 0.01788 to 0.9434 ± 0.04361 $p = 0.0155$; IgG of TNP-Ficoll from 1.135 ± 0.02166 to 1.051 ± 0.01264 $p = 0.0031$). These results clearly show that BM-MSC increased the immunoglobulin production *in vivo*.

Discussion

In this study, we demonstrated that MSC were able to support B cell proliferation and differentiation both *in vitro* and *in vivo*, and that the immunomodulation effect of MSC on B cells mainly depended on soluble factor PGE2 but not IL-6 secreted by UC-MSC. We further demonstrated that BM-MSC could promote production of antibodies in B cells *in vivo*.

UC-MSC could be isolated and expanded easily in large quantities *in vitro* and resemble bone marrow MSC (BM-MSC) in immunosuppression on T cell [16]. We examined the effects of UC-MSC on purified human peripheral blood B cells *in vitro* and found UC-MSC promoted the terminal differentiation of B cells to plasma cells and immunoglobulin production. MSC exert their immunomodulatory effects via cell-cell contact or soluble factors such as TGF- β , IL-10, NO, HLA-G and PGE2. We have also detected the expression of soluble factors (PGE2, IL-6, IL-10, HLA-G, TGF- β) secreted by UC-MSC when UC-MSC were cocultured with activated B cells by real time PCR. We found that UC-MSC expressed IL-6, PGE2, HLA-G, and TGF-beta but not IL-10, and only IL-6 and PGE2 had statistically significant difference (data not shown). The results kept consistent with our previous data, which found only PGE2 and IL-6 increased when UC-MSC were cocultured with peripheral blood mononuclear cells. It has been known that PGE2 secreted by MSC affects immune cells such as T cells [14, 16, 34], NK [35] and DC [36]. PGE2 as an important regulator of the immune response shifts the balance towards a T helper type 2 response, and promotes memory cell formation [33, 37]. In addition, PGE2 affected B cell Ig class-switch recombination and modulated B-cell differentiation [30-33]. We found that the level of PGE2 was 2-fold higher in UC-MSC and B cells coculture system than UC-MSC alone, and that PGE2 inhibitor indomethacin partially but not completely (Fig. 4B and 4C) abolished the immunomodulation on the proliferation and immunoglobulin production of B cells. We found that exogenous PGE2 could increase the B cells proliferation in the MSC and B cells coculture systems (Fig. 4D) while exogenous PGE2 did not play any role in B cells cultured alone. Some studies had demonstrated PGE2 strongly enhanced isotype

differentiation to IgE and IgG in the presence of IL-4 [32], So we think that the exogenous PGE2 alone can not substitute the effects of MSCs on B cells, but give rise to a critical role of MSCs. These results indicated that PGE2 only partially mediated the immunomodulation effect of MSCs on B cells. PGE2 might cooperate with other MSC derived soluble cytokines to regulate B-cell proliferation and terminal differentiation. IL-6 was another soluble factor mediated the immunomodulation of MSC especially on DCs [38]. We also found that IL-1 β promoted the secretion of IL-6 by UC-MSC via JNK and NF- κ B signaling pathway. However IL-6 is not directly associated with the immunoinhibitory activity UC-MSC on CD4⁺ T cell [39]. IL-6 was originally isolated and cloned as a B-cell differentiation factor that induced terminal B-cell differentiation and supported the production of immunoglobulin G (IgG) [40, 41]. We observed that although the level of IL-6 was higher in coculture system, the neutralizing antibodies to IL-6 did not affect the immunomodulation on B cells. In conclusion, we presumed that IL-6 does not participate in the immunoinhibitory activity of UC-MSC on B cells.

We then tested if MSC modulate immunoglobulin production *in vivo*. We firstly injected human UC-MSC into BALB/c mice, and we found that mice spleen was dramatically increased, so we presumed allogeneic MSC possessed immunogenicity. In order to test the effect of MSC on B cells, we used female bone marrow MSC of BALB/c mice to observe the effect on immunoglobulin production with two type different antigens. Mice BM-MSC supported both the antibodies production of both TD-Ag (TNP-KLH) and TI-Ag (TI-I Ag: TNP-LPS, TI-II Ag: TNP-Ficoll) *in vivo*. BM-MSC promoted IgM antibodies production of TNP-LPS (shown in Fig. 5A) on day 7, and TNP-LPS is a kind of TI-I antigens which can activate the innate immunity at the early time. BM-MSC also increased the IgG antibodies production of both T dependent and independent antigens (shown in Fig. 5B). We demonstrated that BM-MSC supported immunoglobulin production in normal mice *in vivo* for the first time, and it suggest an important interaction between MSC and humoral immunity.

These data are in line with a previous observation made by Traggiai et al. [22], who reported an increase in proliferation and differentiation into plasma cells of transitional and naïve B cells isolated from peripheral blood both healthy human and systemic lupus erythematosus patient mediated by MSC *in vitro*. Youd and colleagues also demonstrated that co-culturing MSC with plasma cells purified from NZB/W mice led to an increase in immunoglobulin G antibody production, and suggested that MSC therapy may not be beneficial in Th2-type and B cell-driven diseases [21]. And our results kept consistent with the studies which demonstrated MSC [21] and PGE2 [33] shifting the balance towards a T helper type 2 response and humoral immunity. However, our present data were in contrast with a previous report describing MSC-mediated inhibition of proliferation and differentiation into immunoglobulin secretion cells of human peripheral B cells by Anna Corcione [42] and other teams [43-46]. And Nan Che also found human UC-MSC suppressed mice spleen B-cell proliferation and differentiation. The discrepancies might reflect differences in B cells source and purification procedures, culture conditions, and timing of analysis. We emphasize that different culture conditions containing B cell active factors and B cell secretion factors may polarize MSC to have different immunomodulation activities on B cells. For example, IFN- γ promoted the immunosuppressive capacity of MSCs [47] and overruled the growth promoting effect of MSC on primary purified follicular lymphoma B cells [48]. We compared the culture conditions between Anna Corcione [42] and Traggiai E [22] and found that IL-4 could partly reverse the immunomodulation of UC-MSC on B cell (data not shown) while IL-4 stimulated B activity and class switching. The microenvironment plays a determinative role in the immunomodulation of MSCs. Further study will be needed to determine which inflammatory factors trigger the different immunomodulation of MSCs. In addition, we presume that the modulation of MSC on B cell is why MSC play a double-edged sword in some autoimmunity diseases.

In conclusion, we have shown that UC-MSC promoted the proliferation and terminal differentiation of B cells both *in vitro* and *in vivo*, and the promoting effect partially depends on PGE2 but not IL-6. Our study casts important questions on UC-MSC as a therapeutic tool

in autoimmune diseases especially TH2 type autoimmune diseases in which B-cell activation is crucially implicated in the pathogenesis of the disease. However, further studies are still necessary to compare the immunomodulation of MSC on B cells activities in TH1-type autoimmune diseases and TH2-type autoimmune diseases.

Disclosure Statement

The authors declare no competing financial interests.

Acknowledgements

We thank Ke Chen, Ding Wang and for excellent technical assistance. This study was supported by the 863 project (Grant no. 2011AA020118) and the 973 program of China 2011 CB964800 (Grant no. 2011CB964802) from the Ministry Science & Technology of China.

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