IL-6 Production Stimulated by CD14\(^+\) Monocytes-paracrined IL-1\(\beta\) does not Contribute to the Immunosuppressive Activity of Human Umbilical Cord Mesenchymal Stem Cells

Ding Wang\(^1\), Yue Ru Ji\(^1\), Ke Chen\(^1\), Wei Ting Du\(^1\), Zhou Xin Yang\(^1\), Zhi-Bo Han\(^1,2\), Ying Chi\(^1\), Lu Liang\(^2\), Francis Bayard\(^3\) and Zhong Chao Han\(^1,2\)

\(^1\)The State Key Laboratory of Experimental Hematology, Institute of Hematology and Hospital of Blood Diseases, Chinese Academy of Medical Sciences and Peking Union of Medical College, Tianjin, \(^2\)National Engineering Research Center of Cell Products, AmCellGene Co. Ltd, TEDA, Tianjin, \(^3\)Institut de Médecine Moléculaire de Rangueil, Institut National de la Santé et de la Recherche Médicale, Unité 858, Université Toulouse III Paul Sabatier, Toulouse, *These authors contributed equally to this work

Key Words
Umbilical cord mesenchymal stem cells (UC-MSCs) • IL-6 • PGE2 • IL-1\(\beta\)

Abstract
Background/Aims: Human umbilical cord mesenchymal stem cells (hUC-MSCs) possess immunosuppressive activities but the mechanisms of such activities are not fully understood. Here, we investigated the role of IL-6, one of the characteristic factors of MSCs, in the immunoregulating effect of hUC-MSCs on CD4\(^+\) T lymphocytes. Methods: The condition media from human peripheral blood mononuclear cells (hPBMCs) or CD14\(^+/-\) cell were tested if stimulating IL-6 production by hUC-MSCs. The related signaling pathway of IL-6, and the immunosuppressive activity of IL-6 on CD4\(^+\) T lymphocytes were studied. Result: IL-6 production was dramatically increased by hUC-MSCs when co-culturing with resting or activated hPBMCs. CD14\(^+\) monocytes-paracrined IL-1\(\beta\) promoted the secretion of IL-6 by hUC-MSCs via JNK and NF-kB signaling pathway. Blocking of PGE2 synthesis did not affect the secretion of IL-6, anti-IL-6 antibody was not able to reverse hUC-MSCs-mediated inhibition on CD4\(^+\) T lymphocytes. IL-6 did not mediate the suppressive activity of IL-1\(\beta\)-hUC-MSCs- PGE2 on CD4\(^+\) T cell. Conclusion: CD14\(^+\) monocytes-paracrined IL-1\(\beta\) promotes IL-6 secretion by hUC-MSCs through activating JNK and NF-kB signaling pathway. However, increased IL-6 production does not contribute to immunosuppressive activity of IL-1\(\beta\)-hUC-MSCs-PGE2 on CD4\(^+\) T cells.

Introduction
Mesenchymal stem cells (MSCs) have been initially identified in the bone marrow (BM) as multipotent nonhematopoietic progenitor cells that differentiate into cells of the mesoderm lineage, including bone, fat and cartilage [1]. Although BM-MSCs were widely studied in the basic and clinical fields, human umbilical cord derived MSCs (hUC-MSCs) seem more competitive as
hUC-MSCs are not only poorly immunogenic but also have potent immunosuppressive activities. hUC-MSCs, like BM-MSCs, express human leukocyte antigen (HLA) major histocompatibility complex (MHC) class I, but not HLA MHC class II and co-stimulatory molecules such as CD40, CD80 and CD86 [7, 8]. MSCs can inhibit the activation and proliferation of T lymphocytes in a major histocompatibility complex (MHC)-independent manner [9] and also regulate the immune response indirectly through modulation of professional antigen presenting cells such as dendritic cells [10]. Some clinical trials have shown that MSCs contribute to the reduction of the incidence of graft-versus-host disease following hematopoietic stem cell transplantation [11], and to the improvement of multiple sclerosis and type 2 diabetes [12].

The mechanisms of the immunosuppression capability of MSCs are not fully understood, both cell-to-cell contact [13, 14] and soluble mediators are likely involved. A batch of soluble factors secreted by MSCs have been suggested to contribute to the immunosuppression including transforming growth factor-β1 (TGF-β1), hepatocyte growth factor (HGF) [15], indoleamine 2,3-dioxygenase (IDO) [16], nitric oxide (NO) [17], IL-6, vascular endothelial growth factor (VEGF) [18] and prostaglandin E2 (PGE2) [2]. Previously, we have demonstrated that PGE2 secreted by hUC-MSCs plays an important role in inhibiting the proliferation of human peripheral blood mononuclear cells (hPBMCs) in response to mitogenic or allogeneic stimulus but not TGF-β, IDO or NO [19].

IL-6 plays an important role in immunomodulation [20], angiogenesis [21], normal wound healing [22], and drug resistance of cancer [23]. And IL-6 has been demonstrated to be one of the characteristic factors of MSCs to contribute to the immunoregulation activities of MSCs and relates to the immunosuppression of PGE2 [18, 20]. However, the exact role of IL-6 in the immunoregulation of hUC-MSCs remains to be further illustrated. In this study, we have carefully investigated the mechanism of IL-6 production by hUC-MSCs and found that CD14+ monocytes secreted IL-1β play a key role in the induction of IL-6 production through activating JNK and NF-κB signaling pathway. Our previous study has shown that IL-1β promotes PGE2 production which mediates the immunosuppression of hUC-MSCs on CD4+ T cells, and IL-6 does not affect the secretion of IL-6 and IL-6 does not directly affect the suppressive activity of IL-1β-hUC-MSCs-PGE2 on CD4+ T cell. Our data indicate that IL-6 secreted by hUC-MSCs following IL-1β stimulation does not contribute to the immunosuppressive activity of hUC-MSCs on CD4+ T lymphocytes.

Materials and Methods

Isolation and culture of hUC-MSCs

hUC-MSCs 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. Briefly human umbilical cords were collected from normal, full-term infants delivered by cesarean section. The cord was cut into pieces of 2 mm diameter after flushing the residual blood off, and digested with type II collagenase (Sigma) at 37°C for 30 minutes. The digested fragments were treated with 0.125% trypsin (Gibco) for another 30 min with gentle agitation at 37°C. The digested mixture was then passed through 100 µm and 200 µm filters. The collected cells were resuspended in DMEM/F-12 (Gibco) containing 10% fetal bovine serum (FBS;HyClone), 10ng/mL epidermal growth factor (EGF; Sigma), 100U penicillin/streptomycin (Sigma), 2 mM L-glutamine (Gibco) and 0.1 mM nonessential amino acids. The cells were plated at a density of 1.0×10⁶cells/cm² in non-coated T-25 or T-75 flasks (Beckon Dickinson) and maintained in a humidified atmosphere at 37°C under 5% CO2 in air. The medium was changed in 24 hours, while adherent cells kept growing. Medium was changed twice a week, until 80% confluence was reached. Cells were collected by digesting with 0.125 % trypsin. The viability was examined by trypan blue exclusion and regularly found greater than 95%. Passages were carried out at 1x10⁶cells/cm² in complete DMEM/F-12 medium for cells expansion. Periodic control of cells markers by FACS as well as the differentiation ability were performed and showed stable up to, at least, the 10th passage. hUC-MSCs at P4 to P8 were used in this study.

Immunophenotype analysis of MSCs by flow cytometry

hUC-MSCs (n=5) were stained with PE conjugated antibodies specific for the following surface markers: CD3, CD13,CD14,CD19, CD29,CD31, CD44, CD49e, CD73, CD80, CD86,CD105, CD106, CD133,CD166, or with FITC-conjugated antibodies specific for the following surface markers: CD34, CD45, CD90, HLA-ABC and HLA-DR. Non-specific isotype matched antibodies served as controls. All the antibodies were purchased from BD Pharmingen, and the flow cytometry analysis was performed on a FACSCalibur using the CellQuest software (Becton Dickinson).

Multilineage differentiation

hUC-MSCs were plated in 24-well plate at a density of 3000 cells/well. Specific induction medium was added 24 hours.
after informed consent. CD4+ T cells and CD14+ monocytes (2200rpm×20min) from the venous blood of health volunteers were isolated by Ficoll-Paque (Axis-Shield) density gradient centrifugation (density 1.077 ± 0.002 at 2200rpm). Osteogenic differentiation was evaluated by Von Kossa assay three weeks afterward. For chondrogenic induction, the cells were cultured in DMEM-HG supplemented with 10% FBS, 100U penicillin/streptomycin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, pyridoxine hydrochloride (Gibco), 50 µg/ml L-ascorbic acid-2-phosphate (Sigma), 0.4 mM L-proline (Sigma), 10M dexamethasone, 1% ITS£ (Sigma) and 10 ng/ml recombinant human transforming growth factor-beta (TGF-beta; Peprotech). Chondrogenic differentiation was assessed by safranin O staining three weeks later.

Isolation of human peripheral blood mononuclear cells (hPBMCs) and CD14+ monocytes

hPBMCs were isolated by ficoll-Paque (Axis-Shield) density gradient centrifugation (density 1.077 ± 0.002 at 2200rpm×20min) from the venous blood of health volunteers after informed consent. CD4+ T cells and CD14+ monocytes were purified using relevant Magnetic MicroBead kits (Miltenyi Biotec). Briefly, hPBMCs were magnetically labeled with CD4 or CD14 microbeads (Miltenyi Biotec) respectively, and then loaded onto the column in a magnetic field according to the manufacturer’s instructions. The magnetically labeled cells were retained on the column and were eluted as the positively selected cell fraction. The purity of isolated cells was more than 90%.

Coculture of hUC-MSCs and the immune cells

Cells were maintained in culture in DMEM/F-12 medium or RPMI-1640 medium with 10% FBS, 100U penicillin/streptomycin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate. hUC-MSCs (20 Gy irradiated) were plated in 96-well flat-bottom plate 2-4 hours before the addition of hPBMCs or CD4+ T cells. For dose dependent experiment, various numbers of hUC-MSCs were incubated with hPBMCs (105/well) in the presence or absence of 10 ng/ml PGE2 ELISA kit. IFN-γ, IL-1β, and IL-6 ELISA kits were purchased from Jingmei Biotech Co, Ltd, PR China. PGE2 ELISA kit was purchased from Cayman Chemicals. All assays were carried out according to the protocols of the suppliers.

Preparation of condition medium

1x105 resting hPBMCs, 1x105 CD14+ monocyte, 1x105 CD14- cells or 5x105 hUC-MSCs were cultured in 24 well plate using 1ml PRMI 1640 medium with 10% FBS, 100U penicillin/streptomycin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate. After 18 hours, supernatants were collected and filtered by 0.4 µm filter as condition medium (CM), aliquoted and stored at -80°C.

Immunoblotting

Cell extracts were prepared using cell lysis buffer (Cell Signaling Technology), were centrifuged at 12,000 rpm×5 min after heating up at 95°C for 10 minutes. Proteins were then separated by SDS PAGE and transferred to PVDF membrane (0.2 µm, Amersham) using a wet tank transfer system (Bio-Rad). Membranes were blocked with 20 mM Tris, pH 7.4, 150 mM NaCl (TBS) containing 0.1% (v/v) Tween-20, 5% (v/v) calf serum for 2 hours at room temperature and incubated with either anti-iKB monoclonal antibody (2 µg/ml, Santa Cruz) or anti-β-actin monoclonal antibody (1 µg/ml, Santa Cruz) for 2 hours at room temperature. Membranes were washed three times (10 minutes each) with TBS, 0.1% (v/v) Tween-20, and the bounded antibodies were detected with goat anti-mouse HRP-conjugated secondary antibody (1:1000, Santa Cruz) and a chemiluminescence detection kit (Amersham) according to the manufacturer’s instructions. Protein bands were quantified by densitometric analysis using ImageJ software.

Statistical analysis

All experimental protocols were done with at least triplicate points and the results were analyzed using the GraphPad Prism software package (version 4, GraphPad Software). Data are presented as mean ± standard deviation (SD). Means between groups are compared with the unpaired student t test. Differences between groups are considered statistically significant when p< 0.05 (*) and highly significant when p< 0.01 (**).

Results

Characterization of hUC-MSCs

The immunophenotype of hUC-MSCs was analyzed by flow cytometry. hUC-MSCs were negative for CD3, CD14, CD19, CD31, CD34, CD45, CD80, CD86, CD133 and HLA-DR, but positive for CD13, CD29, CD44, CD49e, CD73, CD90, CD105, CD106, CD166 and HLA-DR.
ABC [19]. In addition, hUC-MSCs were able to differentiate into adipogenic, osteogenic and chondrogenic cells in lineage-specific induction conditions (Fig. 1).

**IL-6 is upregulated by hUC-MSCs upon coculture with hPBMCs**

The IL-6 level was very low in the culture supernatant of either hUC-MSCs or hPBMCs. The expression of IL-6 was significantly upregulated to 70 ng/ml and 100 ng/ml when hUC-MSCs were co-cultured with resting or activated hPBMCs at ratio 1:1. The IL-6 production was enhanced when the ratio of hUC-MSCs increased (Fig. 2A). Moreover, a dose and time-dependent upregulation of IL-6 was strongly evident in this coculture system, IL-6 expression was largely elevated after culturing for 24 hrs (Fig. 2B).

**CD14+ monocytes-paracrine IL-1β trigger IL-6 production in hUC-MSCs**

To determine the resource of IL-6, we cultured hUC-MSCs in cell-free condition medium (CM) of hPBMCs, which had been cultured for 18 hrs. The IL-6 production was largely enhanced after 24 hrs culture when 100ul of hPBMCs CM added. On the other hand, we cultured hPBMCs in cell-free condition medium (CM) of hUC-MSCs to see if hPBMCs could produce IL-6. However IL-6 level didn’t change even when 100 µl of hUC-MSCs CM added (Fig. 3A). We cultured hUC-MSCs in cell-free condition medium (CM) of CD14+ monocytes, CD14− cells or hPBMCs, respectively. As shown in Fig. 3B, IL-6 expression of hUC-MSCs increased in a volume-dependent manner only in the CM from CD14+ monocytes but not in the CM from CD14− cells. The level of IL-6 of hUC-MSCs cultured in CD14+ monocytes CM is similar to that in hPBMCs CM. These
results showed that hUC-MSCs were the main supplier of IL-6 in the coculture system, and the upregulation of IL-6 was in a volume-dependent manner. It also indicated that soluble factors in the CM of hPBMCs were the major driving force for IL-6 production by hUC-MSCs.

IL-1β was one of the characteristic factors of monocyte/macrophage [24, 25], and may induce the production of IL-6 [26, 27]. To confirm if IL-1β is one of the potential inducers of IL-6, we tested the concentrations of IL-1β in the hPBMCs, CD14+ monocytes CM, and CD14- monocytes CM. We found that concentrations of IL-1β in the hPBMCs and CD14+ monocytes CM were up to 10-fold higher than that in CD14- cells CM (Fig. 3C).

Fig. 3. CD14+ monocytes-paracrine IL-1β triggers IL-6 production in hUC-MSCs. (A) IL-6 expression in the supernatant was determined when hUC-MSCs were stimulated by hPBMCs CM or hPBMCs were stimulated by hUC-MSCs CM for 8 hours, the hPBMCs CM were collected after culturing 1*10^6 hPBMCs with 1ml PRMI 1640 medium with 10% FBS 100U penicillin/streptomycin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate (1640) and 5*10^5 hUC-MSCs with 1ml 1640 in 24 well plate for 18 hrs, respectively. (B) IL-6 expression in the supernatant was determined when hUC-MSCs were stimulated by the hPBMCs CM, CD14+ cells CM and CD14- cells CM for 8 hrs. CM were collected after culturing 1*10^6 hPBMCs, 1*10^6 CD14+ cells and 1*10^6 CD14- cells in 24 well plate using 1ml 1640 for 18 hrs. (C) The level of IL-1β was quantified in the CM of hPBMCs, CD14+ monocytes or CD14- cells. (D) IL-6 expression in the supernatant was determined when hUC-MSCs were stimulated by hPBMCs CM in addition of IL-1RA, and when Data represent the mean ± SD of experiments performed in three independent experiments (triplicate of each experiment), each performed with hUC-MSCs from different donors. * p < 0.05.

Fig. 4. PGE2 does not affect the production of IL-6. (A) the level of PGE2 were determined in the supernatant of coculture of hUC-MSCs with hPBMCs at different ratios for 3 days. (B) hUC-MSCs were stimulated by hPBMCs CM (100ul) in the presence of indomethacin (10µM) and NS-398 (50µM) for 8 hours, and IL-6 concentration in the supernatant was measured. Data represent the mean ± SD of experiments performed in three independent experiments (triplicate of each experiment), each performed with hUC-MSCs from different donors. * p < 0.05; ** p < 0.01.
hPBMCs CM. IL-6 production was significantly blocked by IL-1RA in a dose-dependent way (Fig. 3D). We also tested the mRNA expression of IL-6 in UC-MSCs incubated IL-1β by real time PCR, and we found that addition of IL-1β into the hUC-MSCs culture induced a notable increase (30 fold) in the IL-6 mRNA expression (data not shown). These data suggested that IL-1β may promote the production of IL-6 by hUC-MSCs. However, the IL-6 did not decrease to the basal level even when 500 ng/ml of IL-1RA added. IL-1β as one of the characteristic factors of monocyte/macrophage [24, 25] may induce the production of IL-6 [26, 27].

PGE2 does not affect IL-6 production

PGE2 has been suggested to be a candidate to promote the IL-6 expression by MSC [18]. The results shown in Fig. 4A indicate that the expression of PGE2 was significantly upregulated when coculturing of hUC-MSCs with resting hPBMCs in a dose dependent manner, and the tendency of IL-6 and PGE2 production were similar in coculture system. To identify the effect of PGE2 on IL-6 production, hUC-MSCs were cultured in hPBMCs CM containing indomethacin or NS-398 (PGE2 synthesis inhibitors) for 24 hrs and IL-6 level were determined. The results showed that blocking of PGE2 synthesis didn’t affect the secretion of IL-6 (Fig.4B). Collectively, the PGE2 and IL-6 secreted by hUC-MSCs were increased by allogeneic hPBMCs, but they were relatively independent.

JNK and NF-κB signaling pathways were activated in IL-1β-induced IL-6 production by hUC-MSCs.

To define the potential signaling pathway involved in IL-1β-induced IL-6 production, hUC-MSCs were pretreated with specific inhibitors of some signaling pathways. We selected JNK and NF-κB signaling pathways because of their known role in the regulation of IL-6 production. In Fig. 5, we show that both JNK and NF-κB were activated in IL-1β-induced IL-6 production by hUC-MSCs. (A) and (B) hUC-MSCs were stimulated with IL-1β (2ng/ml) for 8 hours after 1 hours pretreated with the specific inhibitors of different signaling proteins. Then the IL-6 level in each supernatant was detected by ELISA. Data represent the mean ± SD of experiments performed in three independent experiments (triplicate of each experiment), each performed with hUC-MSCs from different donors.

* p < 0.05. (C) hUC-MSCs were incubated without or with IL-1β for the indicated times. Total cellular proteins were extracted and subjected to immunoblot analysis using anti-IκB monoclonal antibody. Samples were also immunoblotted with anti-β-actin monoclonal antibodies in parallel as a loading control (triplicate of each experiment). (D) densitometric quantitation of IκB was measured for the relative units.
IL-6 did not mediate the suppressive activity of IL-1β-hUC-MSCs- PGE2 on CD4+ T cell. Irradiated hUC-MSCs (2*10^4/well) were cocultured with Dynabeads-stimulated CD4+ T cells (1*10^5/well) in a 96-well plate for 18 hrs in the presence (+) of indomethacin NS-398 (50 µM) or anti-IL-6 antibody (5 µg/ml) (A), or in the presence (+) of exogenous PGE2 (100 ng/ml) and/or IL-6 (20 ng/ml) (B). The level of IFN-γ was determined by ELISA. Percentage change of IFN-γ was obtained by direct comparison to the control of T cells alone with Dynabeads (=100%). Data represent the mean ± SD of experiments performed in three independent experiments (triplicate of each experiment), each performed with hUC-MSCs from different donors.* p < 0.05; **, p < 0.01.

IL-6 didn’t mediate the suppressive activity of IL-1β-hUC-MSCs- PGE2 on CD4+ T cell.

We have previously demonstrated that CD14+ monocytes promote hUC-MSCs-mediated immunosuppression probably via IL-1β-hUC-MSCs- PGE2 axis [28]. As IL-1β could induce IL-6 production by hUC-MSCs, we were wondering if IL-6 had effect on the immunosuppressive activity of hUC-MSCs. The cells were cocultured with anti-CD3/CD28 Dynabeads activated CD4+ T cells in the presence or absence of anti-IL-6 antibody or NS-398 (the PGE2 synthesis inhibitors), and IFN-γ level was determined. As we expected, IFN-γ secreted by activated CD4+ T cells was dramatically decreased, which indicated the immunosuppressive activity of hUC-MSCs. NS-398 almost completely abolished the inhibition of hUC-MSCs on IFN-γ secretion by activated CD4+ T cells and exogenous PGE2 partly mimicked the inhibitory effect of hUC-MSCs. These results demonstrated that PGE2 played an important role in the hUC-MSCs mediated immunosuppressive activity. However, the neutralizing anti-IL-6 antibody had little impact on the IFN-γ secretion by activated CD4+ T cells (Fig. 6A). Moreover, the exogenous IL-6 didn’t affect the IFN-γ expression as the difference was not significant (Fig. 6B, P>0.05), and the addition of exogenous IL-6 had no impact on the

| Table 1. Specific inhibitors for signaling pathways |
|-----------------------------------------------|
| Specific inhibitors | Signaling pathways |
|-------------------|------------------|
| PD98059           | extracellular signal-regulated kinase, ERK |
| SB20358           | p38 mitogen-activated protein kinase, p38 MAPK |
| SP600125          | c-jun-N-terminal kinase, JNK |
| H89               | protein kinase A, PKA |
| Wortmannin        | phosphatidylinositol 3-kinase, PI3K |
| IKK-NBD           | IKK-NBD |
| Go6983            | protein kinase C, PKC |
| Go6976            | protein kinase C, PKC |

IL-6 Production is not involved in the Immunosuppressive Activity of MSC

Cell Physiol Biochem 2012;29:551-560
function of exogenous PGE2 to hUC-MSCs (Fig. 6B, P>0.05). The results demonstrated that IL-6 didn’t mediate the suppressive activity of hUC-MSCs and had little effort on PGE2 for this suppression. This finding suggested that IL-6 was unlikely associated with immunosuppressive activity of IL-1β-hUC-MSCs- PGE2 on CD4+ T cells.

Discussion

CD14+ monocytes are capable of killing infected host cells via antibody and they are antigen presentation cells that lead to activation of T lymphocytes [29]. Monocytes play an important role in changing the level of characteristic factors and modulate the function of MSCs. It has been reported that the role of monocytes in the immunoinhibitory effects of BM-MSC was similar to that of human adipose-derived MSCs (hASCs) [30, 31]. Consistent with other studies, we have confirmed that CD14+ monocytes promote PGE2 secretion which may be responsible for hUC-MSCs-mediated immunosuppressive activity [28]. In this study, we found that not only PGE2 but also IL-6 were significantly upregulated once hUC-MSCs were cocultured with resting or activated MHC-unmatched hPBMCs, indicated that hUC-MSCs shared similar characteristic with BM-MSCs which also upregulated IL-6 in addition of allogeneic hPBMCs [2, 18]. We found that IL-6 was basically low expressed by hUC-MSCs but it dramatically increased in the coculture system. Furthermore, we discovered that CD14+ monocyte CM was sufficient for the induction of IL-6 by hUC-MSCs. IL-6 was another cytokine promoted by CD14+ monocytes, which motivated us to investigate the role of IL-6 in the immunosuppressive activity of hUC-MSC.

In this study, we demonstrated that IL-1β was a major inducer for IL-6 production by hUC-MSCs. IL-6 secretion significantly decreased when the specific inhibitors of JNK and NF-κB were added, and IkB was rapidly degraded in hUC-MSCs following IL-1β treatment, suggesting that IL-1β upregulated hUC-MSCs derived IL-6 expression through JNK and NF-κB signaling pathway. IL-1β has also been demonstrated to promote the secretion of IL-6 in astrocytes [32], intestinal epithelial cells [33], synoviocytes [34] and mast cells [35], and this effect was through p38 MAPK [34, 35] and NF-κB signaling pathway [32, 33, 35]. Interestingly we found that three MAPK (ERK, p38 MAPK, and JNK) signaling pathways but not NF-κB was involved in IL-1β-induced PGE2 production in hUC-MSCs (unpublished data). This indicated that IL-1β induced IL-6 and PGE2 production by hUC-MSCs through different signaling pathways. PGE2 has been illustrated to stimulate IL-6 secretion in BM-MSCs [18]. However, our previous study has shown that IL-6 was not effective on the induction of PGE2 by hUC-MSCs [28]. Here we confirmed that PGE2, a crucial soluble factor mediated the suppressive effect of hUC-MSCs, had no effect on the secretion of IL-6. Thus, we postulate that PGE2 and IL-6 secreted by hUC-MSCs do not interfere with each other and play a relatively independent role in the biological effectiveness of hUC-MSCs. We were interesting to note a recent observation that IL-6-activated PGE2 secretion mainly participates in immunosuppressive potential of MSCs in vivo [20]. Although this was different from our finding, the divergence may be explained by the possibility that the lock-out of IL-6 gene may lead to change of the microenvironment of mice, which reduces PGE2 secretion from MSC in the mice deficient IL-6 but not in normal mice. Meanwhile, Djouad F et al found that IL-6 was involved in the immunoregulatory mechanism mediated by MSC through a partial inhibition of DC differentiation, they studied IL-6 secretion by mice BM-MSCs inhibited DC differentiation which was responsible for a decrease in T-cell proliferation. While we emphasized IL-1β induced IL-6 secretion by hUC-MSCs and IL-6 was unlikely associated with immunosuppression activity of IL-1β-hUC-MSCs- PGE2 on CD4+ T cells, the role of IL-6 on DC differentiation need further study.

We have illustrated that hUC-MSCs exert their immunomodulatory effects via IL-1β-PGE2 axis [19]. The PGE2 synthesis inhibitors almost completely abolished the inhibition of hUC-MSCs on IFN-γ secreted by activated CD4+ T cells. Whereas here we showed that IL-6 had little effect on hUC-MSCs-induced inhibition on CD4+ cells. However, IL-6 may also mediate immunomodulatory on other immune cell. Hegde et al. indicated that IL-6 was important in the differentiation of monocytes to dendritic cells [36, 37], and MSCs modulate dendritic cell maturation, migration and function through IL-6 at different stages [18].

IL-6 is a key molecule for neutrophil to rescue from programmed cell death [38]. IL-6 also mediates the promotional effect of BM-MSCs and hUC-MSCs in megakaryocytopoiesis [39]. In addition, IL-6 is important in normal wound healing [22] and angiogenesis [21]. A few researchers have predicted that this proangiogenic effect of IL-6 may promote tumor growth [40]. It was also found that IL-6 mediated the promotional effect of BM-MSC on the proliferation of MCF-7 [41] and multiple
myeloma [37], the sphere formation and tumor initiation of colorectal tumor [42], the drug resistance, and overexpression of pluripotency markers of lung cancer [23]. As MSCs possess the ability to migrate to the tumor site with high selectivity following intravenous injection in experimental animals [43], further animal studies are required to evaluate the in vivo effect of hUC-MSCs on the potential risk of malignant transformation by the downregulation of antitumor immune and/or the direct impact of IL-6.

Several observations have indicated that allogeneic hPBMCs are required for the upregulation of IL-6 by BM-MSCs [2, 18]. In agreement with those reports, we found that IL-6 was basically low expressed by hUC-MSCs, and it increased dramatically when co-culturing with resting or activated MHC-unmatched hPBMCs. Furthermore we discovered that CD14+ monocyte CM was sufficient for the induction of IL-6 production in hUC-MSCs. IL-1β promoted the secretion of IL-6 by hUC-MSC, which was mediated by c-jun-N-terminal kinase (JNK) as well as NF-κB. Finally, our results indicate that IL-6 derived from the activated hUC-MSCs is not associated with the direct immunosuppressive effect of hUC-MSCs on CD4+ cells. And further studies on the role of IL-6 in the function of hUC-MSCs will be summarized in our next study.

Acknowledgements

We thank Youwei Wang, Bin Wang, Shulin Yan and Tian suo Zhao for excellent technical assistance. This work was supported by National Natural Science Foundation of China (30570357 and 30600238), 863 project of the Ministry of Science and Technology of China (2006AA02A110), Tianjin Municipal Science and Technology Commission, China (06YFSZSF01300 and 05YFJZJC01500).

References

1 Baksh D, Yao R, Tuan RS: Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem Cells 2007;25:1384-1392.
2 Aggarwal S, Pittenger MF: Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815-1822.
3 Friedman R, Betancur M, Boissel L, Tuncer H, Cetrulo C, Klingemann H: Umbilical cord mesenchymal stem cells: Adjuvants for human cell transplantation. Biol Blood Marrow Transplant 2007;13:1477-1486.
4 Lu LL, Liu YJ, Yang SG, Zhao QJ, Wang X, Gong W, Han ZB, Xu ZS, Lu YX, Liu D, Chen ZZ, Han ZC: Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoeis-supportive function and other potentials. Haematologica 2006;91:1017-1026.
5 Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, Fu YS, Lai MC, Chen CC: Mesenchymal stem cells in the wharton’s jelly of the human umbilical cord. Stem Cells 2004;22:1330-1337.
6 Zeddou M, Briquet A, Relic B, Josse C, Malaise MG, Gothot A, Lechanteur C, Beguin Y: The umbilical cord matrix is a better source of mesenchymal stem cells (msc) than the umbilical cord blood. Cell Biol Int 2010;34:693-701.
7 Le Blanc K, Ringden O: Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2005;11:321-334.
8 Weiss ML, Anderson C, Medecetty S, Seshareddy KB, Weiss RJ, VanderWerff I, Troyer D, McIntosh KR: Immune properties of human umbilical cord wharton’s jelly-derived cells. Stem Cells 2008;26:2865-2874.
9 Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, Mancardi G, Uccelli A: Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing t-cell anergy. Blood 2005;106:1755-1761.
10 Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N: Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 2005;105:4120-4126.
11 Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remmerber M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringden O: Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: A phase ii study. Lancet 2008;371:1579-1586.
12 Jiang R, Han Z, Zhuo G, Qu X, Li X, Wang X, Shao Y, Yang S, Han ZC: Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: A pilot study. Front Med 2011;5:94-100.
13 Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H, Galun E, Rachmilewitz J: Human mesenchymal stem cells alter antigen-presenting cell maturation and induce t-cell unresponsiveness. Blood 2005;105:2214-2219.

IL-6 Production is not involved in the Immunosuppressive Activity of MSC

Cell Physiol Biochem 2012;29:551-560
Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F: Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 2003;101:3722-3729.

Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM: Human bone marrow stromal cells suppress T lymphocyte proliferation induced by cellular or non-specific mitogenic stimuli. Blood 2002;99:3838-3843.

Meisel R, Zibert A, Laryea M, Gobei U, Daubener W, Dilloo D: Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 2004;103:4619-4621.

Sato K, Ozaki K, Oh I, Meguro A, Krampera M, Glennie S, Dyson J, Scott D: Interleukin 6 mediated recruitment of mesenchymal stem cells. Stem Cells Dev 2010;19:887-902.

Kostura MJ, Tocci MJ, Limjungo G, Chin J, Cameron P, Hillman AG, Chartrain NA, Schmidt JA: Identification of a monocyte specific pre-interleukin 1 beta convertase activity. Proc Natl Acad Sci USA 1989;86:5227-5231.

LeMay LG, Otterness IG, Vander AJ, Kluger MJ: In vivo evidence that the rise in plasma il 6 following injection of a fever-inducing dose of lps is mediated by il 1 beta. Cytokine 1990;2:199-204.

Sironi M, Breviario F, Proserpio P, Biondi A, Vecchi A, Van Damme J, Dejana E, Mantovani A: IL-1 stimulates IL-6 production in endothelial cells. J Immunol 1989;142:549-553.

Wang D, Chen K, Du WT, Han ZB, Ren H, Chi Y, Yang SG, Bayard F, Zhu D, Han ZC: CD14+ monocytes promote the immunosuppressive effect of human umbilical cord matrix stem cells. Exp Cell Res 2010;316:2414-2423.

Ziegler-Heitbrock L: The CD14+ CD16+ blood monocytes: Their role in infection and inflammation. J Leukoc Biol 2007;81:584-592.

Gonzalez-Rey E, Anderson P, Gonzalez MA, Rico L, Buscher D, Delgado M: Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 2009;58:929-939.

Groh ME, Maitra B, Szekely E, Koc ON: Human mesenchymal stem cells require monocyte-mediated activation to suppress allogeneic T cells. Exp Hematol 2005;33:928-934.

Sparacio SM, Zhang Y, Vilcek J, Benveniste EN: Cytokine regulation of interleukin-6 gene expression in astrocytes involves activation of an NF-kappa B-like nuclear protein. J Neuroimmunol 1992;39:231-242.

Fischer JE, Hasselgren PO: IL-6 production in human intestinal epithelial cells following stimulation with IL-1 beta is associated with activation of the transcription factor NF-kappa B. J Surg Res 1997;69:139-144.

Miyazawa K, Mori A, Miyata H, Akahane K: Nitric oxide plays a critical role in T-cell responses by indoleamine 2,3dioxygenase in Plasmodium berghei-infected mice. J Immunol 2005;174:488-496.