Clinical Immunology (2010) 135, 448–458

available at www.sciencedirect.com

Clinical Immunology

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Clinical Immunology Society

Human umbilical cord mesenchymal stem cells hUC-MSCs exert immunosuppressive activities through a PGE_2-dependent mechanism

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Received 6 November 2009; accepted with revision 27 January 2010
Available online 5 March 2010

KEYWORDS
Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs);
Prostaglandin E_2 (PGE_2);
Immunosuppression

Abstract Human umbilical-cord-derived mesenchymal stem cells (hUC-MSCs) constitute an attractive alternative to bone-marrow-derived MSCs for potential clinical applications because of easy preparation and lower risk of viral contamination. In this study, both proliferation of human peripheral blood mononuclear cells (hPBMCs) and their IFN-γ production in response to mitogenic or allogeneic stimulus were effectively inhibited by hUC-MSCs. Co-culture experiments in transwell systems indicated that the suppression was largely mediated by soluble factor(s). Blocking experiments identified prostaglandin E_2 (PGE_2) as the major factor, because inhibition of PGE_2 synthesis almost completely mitigated the immunosuppressive effects, whereas neutralization of TGF-β, IDO, and NO activities had little effects. Moreover, the inflammatory cytokines, IFN-γ and IL-1β, produced by hPBMCs upon activation notably upregulated the expression of cyclooxygenase-2 (COX-2) and the production of PGE_2 by hUC-MSCs. In conclusion, our data have demonstrated for the first time the PGE_2-mediated mechanism by which hUC-MSCs exert their immunomodulatory effects.
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doi:10.1016/j.clim.2010.01.015

Introduction

Human umbilical-cord-derived mesenchymal stem cells (hUC-MSCs) are being explored as a promising candidate for many potential clinical applications because they can be isolated and expanded easily in large quantities in vitro [1]. hUC-MSCs resemble bone marrow MSCs (BM-MSCs) in many respects, including plastic adherence, surface marker expression, self-renew ability, and potential to differentiate into osteocyte, chondrocyte, adipocyte, cardiomyocyte, skeletal myocyte, endothelial cell, and dopaminergic neuron [2–4]. Recent studies have provided encouraging results regarding the utility of hUC-MSCs in tissue repair and regeneration in several disease models, such as rescuing visual functions in a rodent model of retinal disease [5], ameliorating apomorphine-induced rotations in hemiparkinsonian rats [6], accelerating neurological functional recovery of rats after stroke [7], and treating rat liver fibrosis[8]. There is also evidence showing that hUC-MSCs can be transfected with either DNA or mRNA, suggesting that hUC-MSCs may serve as a useful cellular vector for gene therapy [9].

One of the characteristic features of hUC-MSCs is its apparently low immunogenicity. Like BM-MSCs, hUC-MSCs express low levels of human leukocyte antigen (HLA) major histocompatibility complex (MHC) class I, but they do not express HLA MHC class II and co-stimulatory molecules (CD40, CD80, and CD86) [1,10]. It was demonstrated that a single injection of porcine UC-MSCs did not elicit a detectable adaptive immune response in recipient pigs [11], and no immune rejection was observed when porcine UC-MSCs were transplanted into rat brain [12,13]. In addition, hUC-MSCs appear to directly suppress T cell activation in an antigen-independent fashion. Weiss et al. showed that hUC-MSCs were able to suppress the proliferation of mitogen-stimulated rat splenocytes (xenograft model) or hPBMCs (allogeneic transplant model) in vitro [10]. There are a number of other studies that have investigated the mechanism(s) of immunosuppression by MSCs derived from BM, but there are still discrepancies in the literature. For example, Plumas et al. reported that MSC inhibited activated T cell proliferation by inducing apoptosis [14]. However, this report was contradicted by Benvenuto et al., who found that MSCs may in effect protect T cells from apoptosis [15]. In addition, both cell–cell contact-dependent and -independent mechanisms have been proposed [16,17], and possible soluble factors that may be involved in the suppression include transforming growth factor-β1 (TGF-β1), hepatocyte growth factor (HGF) [18], indoleamine 2,3-dioxygenase (IDO) [19], nitric oxide (NO) [20], and prostaglandin E_2 (PGE_2) [21]. One prominent candidate for hPBMCs suppression is PGE_2, a catabolin of arachidonic acid that possesses potent immunomodulatory property. The synthesis of PGE_2 is regulated by cyclooxygenase (COX), of which there are two isoforms: one constitutive form (COX-1) and one inducible form (COX-2), which can be induced by some inflammatory cytokines, such as IL-1β, IL-6, IFN-γ, and TNF-α [22–25].

In this study, we examined the immunosuppressive effects of hUC-MSCs on the activation of hPBMCs in response to allogeneic or mitogenic stimulation, and we further investigated the underlying mechanisms that may have important implications for the optimized clinical applications of hUC-MSCs. Our data reveal that the immunosuppression of hUC-MSCs is largely cell contact-independent with PGE2 being the principal soluble mediator.

Materials and methods

Reagents

Indomethacin (Sigma) and NS-398 (Cayman Chemicals) were used at 10, and 50 μM, respectively. PGE_2 was purchased from Sigma and used at 1 ng/ml to 200 ng/ml. IFN-γ (Peprotech) was used at 30 ng/ml, and IL-6 (Peprotech) was used at 20 ng/ml. TNF-α (Peprotech) was used at 10 ng/ml. IL-1β was purchased from eBioscience and used at 10 ng/ml. PHA and SEB were purchased from Sigma.

Generation of human UC-MSCs and BM-MSCs

MSCs were isolated from umbilical cords or bone marrow aspirates 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. The details of isolation and ex vivo expansion of MSCs were essentially as described previously [1] and provided in the supplement.

Immunophenotype analysis of MSCs by flow cytometry

hUC-MSCs ($n=5$) and hBM-MSCs ($n=5$) were stained with PE-conjugated antibodies specific for the following surface markers: CD3, CD14, CD19, CD31, CXCR4, CD80, CD86, CD13, CD44, CD73, CD105, CD106, CD166, CD29, CD49e, SSEA-4, and OCT3/4, or with FITC-conjugated antibodies specific for the following surface markers: CD34, CD45, CD90, Stro-1, Nestin, Flk-, HLA-ABC, and HLA-DR. Non-specific isotype-matched antibodies served as controls. All the antibodies were purchased from BD Pharmingen, and flow cytometry analysis was performed on a FACSCalibur using the CellQuest software (Becton Dickinson).

hPBMCs and MSCs co-culture experiments

hPBMCs were isolated by Ficoll-Paque (Axis-Shield) density gradient centrifugation (density, 1.077 ± 0.002) from the venous blood of health volunteer donors. All cultures were carried out in complete DF-12 medium (Gibco) containing 10% fetal calf serum (FCS) (HyClone), 2 mM glutamine, 100 U/ml penicillin and streptomycin, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate. hUC-MSCs (20 Gy irradiated) were plated and allowed to adhere for 2 h at 37 °C. Co-culture experiments were performed in 96-well flat-bottom plate (responder hPBMCs were plated at 10^5 per well) for BrDU ELISA colorimetric assay or in 24-well flat-bottom plate (responder hPBMCs were plated at 10^6 per well) for CFSE labeling and BrDU incorporation assays. For mixed lymphocyte reaction (MLR), an equal number of responder and irradiated (20 Gy) stimulating hPBMCs were co-cultured in the presence or absence of hUC-MSCs for 7 days. In some

experiments, exogenous PGE_2 or PGE_2 synthesis inhibitors (indomethacin or NS-398) were added to the culture system.

Transwell experiments

Irradiated hUC-MSCs (20 Gy, 5×10^5 per well) were plated into the inner transwell chamber with 0.3-μm pore size membrane of a 6-well plate (Corning Costar) for 2 h before hPBMCs (3×10^6 per well) stimulated by PHA (10 μg/ml) were added into the lower compartment. BrDU was added for the last 18 h of the 4-day culturing. hPBMCs were harvested and evaluated by flow cytometry for BrDU incorporation and IFN-γ concentrations were determined using the IFN-γ ELISA kit.

Cell proliferation assays

Cell proliferation was assessed by three different methods. BrDU incorporation and BrDU ELISA colorimetric assays were performed using the Kits purchased from BD Biosciences and Roche. BrDU was added for the last 18 h of the total 4- or 7-day culturing. Then cells were harvested and prepared for flow cytometry or detected by colorimetric assay directly in the culture plate according to the manufacturer's instructions. In some experiments, hPBMCs proliferation was detected by Carboxy Fluorescein Diacetate Succinimidyl Ester (CFSE) labeling (Invitrogen). Briefly, hPBMCs were labeled with 10 μM CFSE in pre-warmed phosphate-buffered saline (PBS) containing 0.1% BSA at a final concentration of 10^6 cells/ml. After incubation at 37 °C for 10 min, cells were washed with fresh medium containing 10% FCS. After co-culture, hPBMCs were washed by cold PBS and analyzed by flow cytometer.

Determination of cytokine concentrations by ELISA

Cell-free supernatants were collected and kept frozen at −80 °C until assayed for cytokine concentrations by enzyme-linked immunosorbent assays (ELISA). ELISA assay kits for IL-1β, IL-6, IFN-γ, and TNF-α were used following the supplier's instruction (Jingmei Biotech Co, Ltd., PR China). PGE_2 ELISA kits was purchased from Cayman Chemicals and used according to the protocol of the manufacturer.

Quantitative analysis of COX-2 mRNA expression

Total RNA was extracted by using Trizol (Invitrogen), and cDNA synthesis was done using the MLV RT kit (Invitrogen) for 50 min at 37 °C in the presence of oligo-dT primer. Real-time reverse transcriptional quantitative polymerase chain reaction (RT-PCR) analyses for COX-2 and HPRT were performed by SYBR Green Master Mix (Applied Biosystems) on an Applied Biosystems 7500 Real-Time PCR System. PCR cycling conditions were 10 min at 95 °C, 15 s at 95 °C, and 1 min at 60 °C, followed by the final single peak-melting curve program. Each sample was done in triplicate, and mean values were used for quantification. The ratio of COX-2 and HPRT was calculated according to the formula:

$$\text{ratio} = 2^{-ddCt}(\textit{ddCT} = \textit{mean Ct gene} - \textit{mean Ct housekeeping}).$$

Human COX-2 primers were as follows: 5′-ACTCTGGCTAGA-CAGCGTAA-3′ (forward) and: 5′-ACCGTAGATGCTCAGGGAC-3′ (forward). Human HPRT primers were 5′-TGACACTGG-CAAAACAATGCA-3′ (forward) and 5′-GGTCCTTTTCACCAG-CAAGCT-3′ (forward).

Statistical analysis

The data were analyzed for statistic significance using the GraphPad Prism software. Data are presented as mean ± 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.

Results

Characterization of hUC-MSCs

Expression of specific cell surface and intracellular markers by hUC-MSCs and BM-MSCs were examined by flow cytometry. Both hUC-MSCs and hBM-MSCs 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 (Supplement Table 1). However, Nestin, CD54, SSEA-4, and OCT-4 were expressed at significantly higher levels on hUC-MSCs than on hBM-MSCs (Fig. 1). The surface maker expression on hUC-MSCs was not altered by passaging or by cryopreservation at least before passage 10 (data not shown). In addition, hUC-MSCs, which exhibited a fibroblast-like morphology (Supplement Fig. 1A), were able to differentiate toward osteogenic, adipogenic, and chondrogenic lineages (Supplement Fig. 1B, C, D) in lineage-specific induction condition, indicating that hUC-MSCs possess multilineage differentiation potential.

hUC-MSCs possess potent immunosuppressive effects on activation of hPBMCs

To examine the immunogenicity of hUC-MSCs, hUC-MSCs were co-cultured with MHC-mismatched allogeneic hPBMCs in vitro. Neither un-irradiated nor irradiated hUC-MSCs elicited a proliferative response by allogeneic hPBMCs (data not shown). Consistent with the findings from previous studies on human BM-MSCs [17], these data indicated that hUC-MSCs were also hypoimmunogenic. Next, MLR experiments were performed to determine the suppressive effects of hUC-MSCs on allogeneic T cell activation. The data showed that hUC-MSCs could suppress the activation of hPBMCs in a dose-dependent manner when assessed by either cell proliferation or IFN-γ production. The suppression was potent, as the inhibitory effects were already significant when the hPBMCs:hUC-MSCs ratio was 20:1 (proliferation) or 100:1 (IFN-γ secretion) (Fig. 2A). The suppressive effects of hUC-MSCs on hPBMCs stimulated with the mitogen PHA were also examined, and similar results were again obtained (Fig. 2B). Furthermore, the immunosuppressive properties appeared to be a consistent and stable feature of hUC-MSCs, because similar extent of inhibition was observed with hUC-MSCs prepared from eight different donors and with different numbers of cell passaging (P4-P9) (Fig. 2C).

PGE_2 is the major soluble mediator of the immunosuppression by hUC-MSCs

There are discrepancies in the literature with regard to the importance of direct cell–cell contact [16,17] and soluble factors [18,19] for the immunomodulatory activities of MSCs. To investigate this, cell co-culture experiments were conducted in the transwell systems to physically separate hUC-MSCs from hPBMCs with cell proliferation and IFN-γ secretion as read-outs. The results shown in Fig. 3A demonstrated that hUC-MSCs were still able to block hPBMCs proliferation and IFN-γ secretion in response to PHA stimulation even in the absence of direct cell contact. Although there appeared to be slight reduction in the degree of inhibition compare with that seen with direct cell–cell contact, the difference was not statistically significant ($p>0.05$) (Fig. 3A), indicating that the inhibitory effects of hUC-MSCs were largely mediated by soluble mediators.

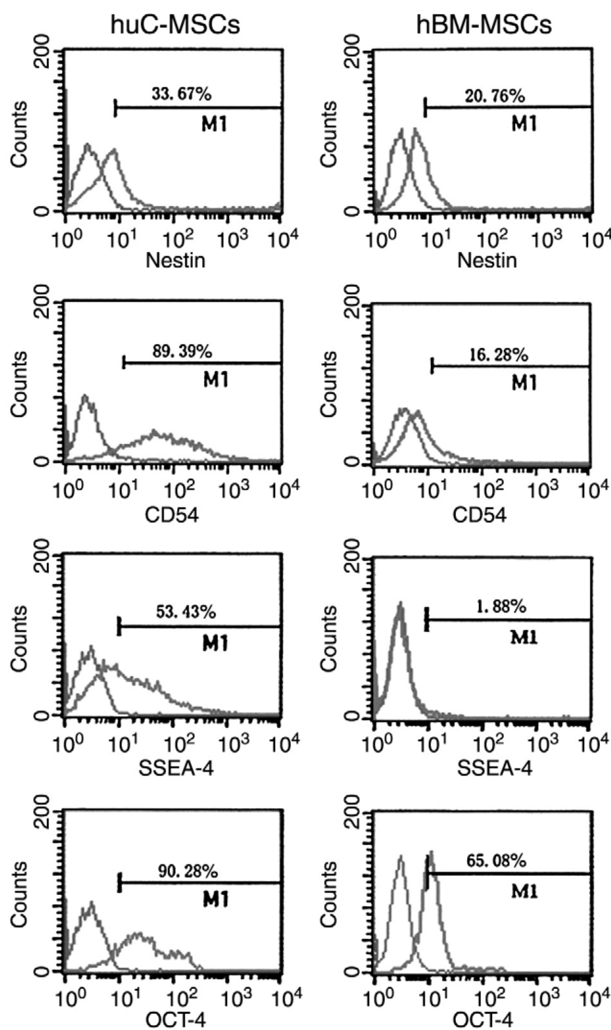


Figure 1 Differential marker expression by UC-MSCs and hBM-MSCs. hUC-MSCs and hBM-MSCs were stained with surface antibodies and analyzed by flow cytometry. The figure shows one representative results from five independent experiments. *P* value was analyzed by the two-tailed *t*-test.

Previous reports have suggested that TGF-β, IDO, NO, and PGE_2 might be relevant mediators of BM-MSCs-induced inhibition [18–21]. To investigate the possible soluble factors that may be involved in the immunosuppressive effect of hUC-MSCs, co-culture experiments were performed in the presence or absence of specific inhibitors or neutralizing antibodies. Blocking or neutralization of TGF-β, IDO, and NO had no effects on the suppressive activities of hUC–MSCs (Supplement Fig. 2). By contrast, inclusion of the PGE_2 synthesis inhibitors, indomethacin or NS-398, in the co-culture experiments almost completely abrogated the inhibitory effects of hUC-MSCs, as the proliferation of hPBMCs following mitogenic, superantigenic, or allogeneic stimulations was restored to levels comparable to those obtained when they were cultured without hUC-MSCs (Fig. 3B). Similar results were also obtained by BrDU ELISA colorimetric assay (data not shown). To further investigate the importance of PGE_2 in the immunosuppression, the levels of PGE_2 produced by hUC-MSCs and by mitogen- or alloantigen-stimulated hPBMCs were measured. Very low levels of PGE_2 were produced by either hUC-MSCs or hPBMCs when they were cultured separately. Remarkably, however, the PGE_2 concentrations were dramatically increased in the supernatants of co-cultures of hUC-MSCs and mitogen- or alloantigen-stimulated hPBMCs (>300-fold when hPBMCs: hUC-MSCs ratio was 5:1), but inclusion of indomethacin in the co-cultures completely blocked the increase (Fig. 3C). Moreover, addition of exogenous PGE_2 could reproduce the suppressive effects of hUC-MSCs on hPBMCs proliferation and IFN-γ production in response to mitogen, superantigen, or allogeneic activation (Fig. 3D). Collectively, the above data strongly suggest that PGE_2 is a major player in the hUC-MSCs-mediated immunosuppression.

Inflammatory cytokines induce the PGE_2 production by hUC-MSCs

The finding that PGE_2 production by hUC-MSCs was elevated when they were co-cultured with activated hPBMCs suggested that there was a requirement for some inducing signal(s) from hPBMCs delivered through direct cell contact and/or through secretion of soluble factors. To determine the possible mechanism(s) of upregulation of PGE_2 production, cell-free conditioned culture media (CM) of hPBMCs stimulated with PHA, SEB, or allogeneic hPBMCs were prepared and tested for their effects on PGE_2 production by hUC-MSCs. As shown in Fig. 4A, only low levels of PGE_2 (2.107±0.46 ng/ml) were secreted by hUC-MSCs cultured in the regular medium, but by contrast, significantly increased production of PGE_2 was induced when hUC-MSCs were cultured in PHA-CM (98.50± $32# 4.02 ng/ml), SEB-CM (135.95±7.65 ng/ml), or MLR-CM (97.04±5.93 ng/ml). The data indicate that soluble factors in the CM are responsible for the increased PGE_2 production.

Next, whether the proinflammatory cytokines, IFN-γ, TNF-α, IL-1β, and IL-6, could induce the production of PGE_2 by hUC-MSCs was investigated, since previous studies have shown evidence that suppression of T cell proliferation by MSCs requires the presence of IFN-γ [26], and TNF-α, IL-1β, and IL-6 are the potential inducers of PGE_2 production [22,25]. Hence, these four inflammatory cytokines were quantified in these CM (data are not shown), then,

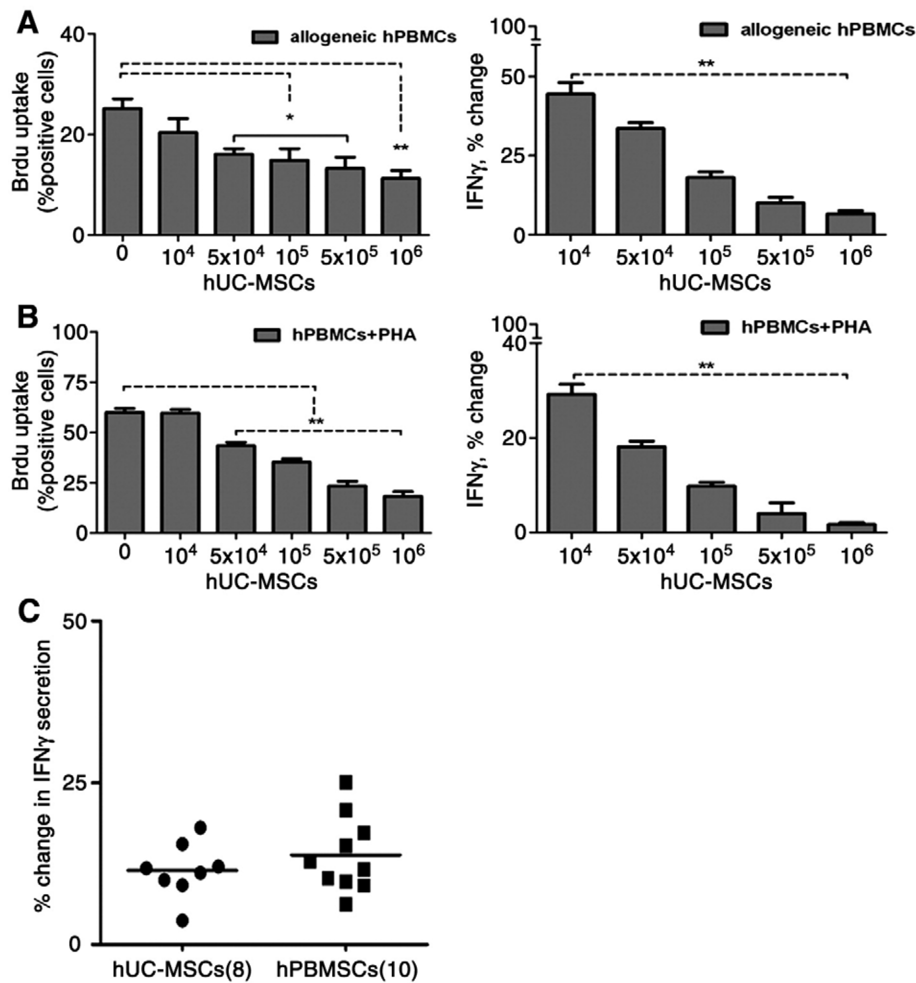
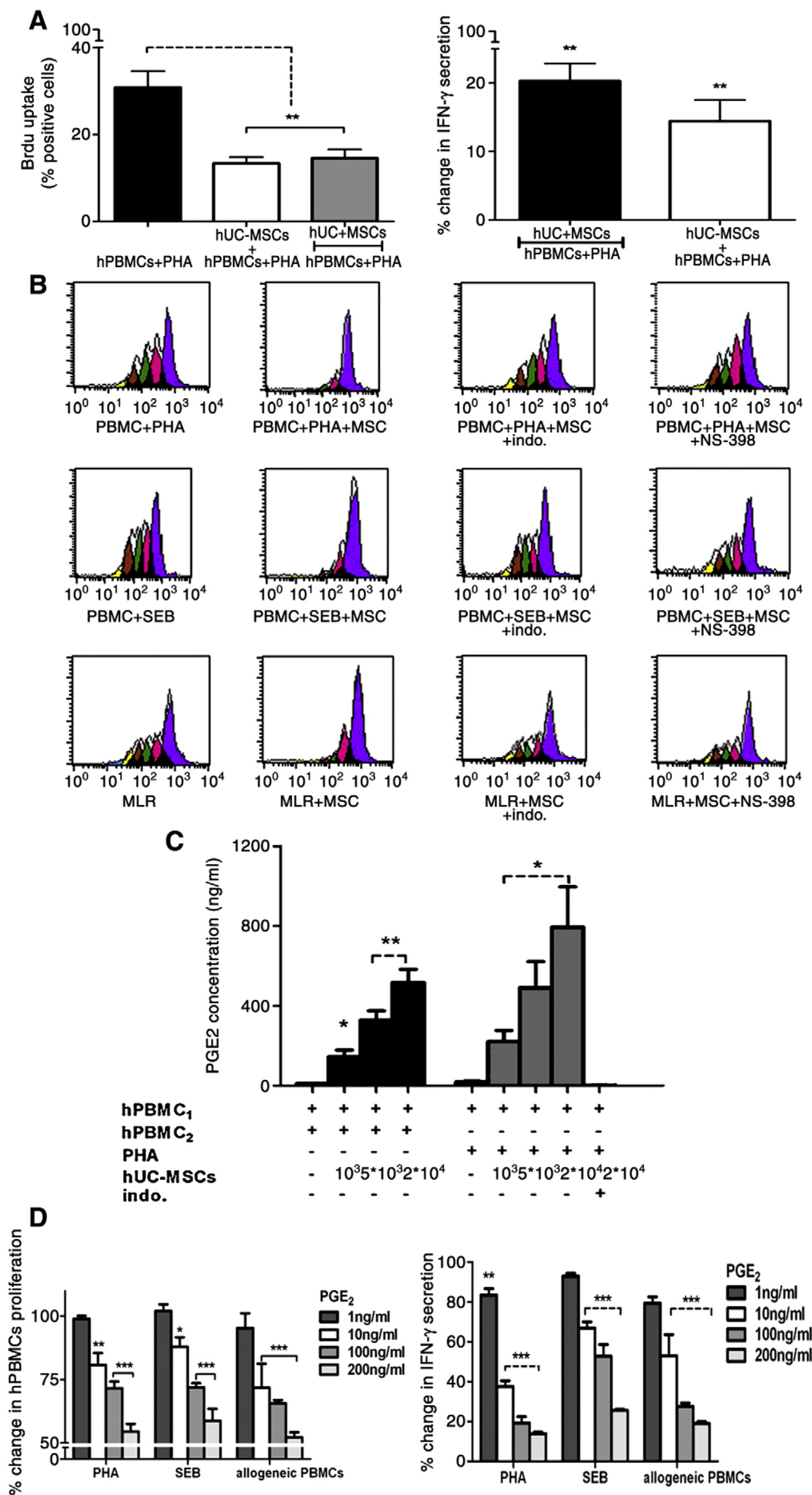


Figure 2 Suppression of hPBMCs proliferation and IFN-γ secretion by hUC-MSCs. (A) hPBMCs (10^6 cells per well) were activated by 10 μg/ml PHA in absence or presence of hUC-MSCs at different ratios, and (B) hPBMCs (10^6 cells per well) were co-cultured with allogenetic hPBMCs (10^6 cells per well) in absence or presence of hUC-MSCs at different ratios. Proliferation data were evaluated by BrDU Flow kits and corresponding cell-free supernatants were analyzed by IFN-γ ELISA kits. Each data represent the mean ± SEM of three independent experiments, each performed in triplicate. *$p<0.05$; **$p<0.01$; (C) hUC-MSCs (8) from eight donors were co-cultured with PHA-activated hPBMCs at ratio of 1/1 or PHA-activated hPBMCs (10) from ten donors were co-cultured with one source of hUC-MSCs. IFN-γ concentrations in each culture were measured by ELISA and data represent the mean of single experiment, each performed in triplicate.

recombinant IFN-γ (30 ng/ml), TNF-α (10 ng/ml), IL-1β (10 ng/ml), and IL-6 (20 ng/ml) were added to the culture system of hUC-MSCs. As shown in Fig. 4B, IFN-γ and IL-1β both significantly stimulated the production of PGE_2 ($p<0.01$ vs. unstimulated hUC-MSCs), whereas TNFα and IL-6 had no obvious effect ($p>0.05$ vs. unstimulated hUC-MSCs). Furthermore, consistent with the results of PGE_2 measurement, quantitative real-time RT-PCR analysis demonstrated that the mRNA expression of COX-2, a key enzyme in the biosynthesis of PGE_2, was dramatically augmented by treatment with IFN-γ (90.77 ± 25.96-fold) and by IL-1β treatment (120 ± 29.37-fold) (Fig. 4C).

Figure 3 The suppressive effects of hUC-MSCs are mediated by PGE_2. (A) The importance of soluble factors in immunosuppressive activities of hUC-MSCs was evaluated by hUC-MSCs and hPBMCs co-culture experiments in transwell plates. Results of cell proliferation and IFN-γ concentrations in the cell-free supernatants were analyzed by the BrDU Flow kits and the ELISA kits, respectively. (B) Irradiated hUC-MSCs were co-cultured with hPBMCs (at ratio of 1/3) activated by PHA, SEB, allogenic hPBMCs in the presence or absence of PGE_2 inhibitors, indomethacin (Indo.) or NS-398. hPBMCs proliferation was detected by Carboxy Fluorescein Diacetate Succinimidyl Ester (CFSE). One representative of three separated experiments is shown. (C) hUC-MSCs were co-cultured with activated hPBMCs at different ratios. Cell-free supernatant of each well was harvested and measured by PGE_2 ELISA (D) Activated hPBMCs were cultured with increasing amounts of exogenous PGE_2 for 4 days. Proliferation was evaluated by BrDU ELISA colorimetric assay and corresponding IFN-γ concentration of cell-free supernatants was presented. Data represent the mean ± SEM of three independent experiments, each performed in triplicate. *$p<0.05$; **$p<0.01$.


A
Brdu uptake (% positive cells)
hPBMCs+PHA
hUC-MSCs + hPBMCs+PHA
hUC+MSCs hPBMCs+PHA
% change in IFN-γ secretion
B
PBMC+PHA
PBMC+PHA+MSC
PBMC+PHA+MSC +indo.
PBMC+PHA+MSC +NS-398
PBMC+SEB
PBMC+SEB+MSC
PBMC+SEB+MSC +indo.
PBMC+SEB+MSC +NS-398
MLR
MLR+MSC
MLR+MSC +indo.
MLR+MSC+NS-398
C
PGE2 concentration (ng/ml)
hPBMC1
hPBMC2
PHA
hUC-MSCs
indo.
D
% change in hPBMCs proliferation
PGE2
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allogeneic PBMCs
% change in IFN-γ secretion

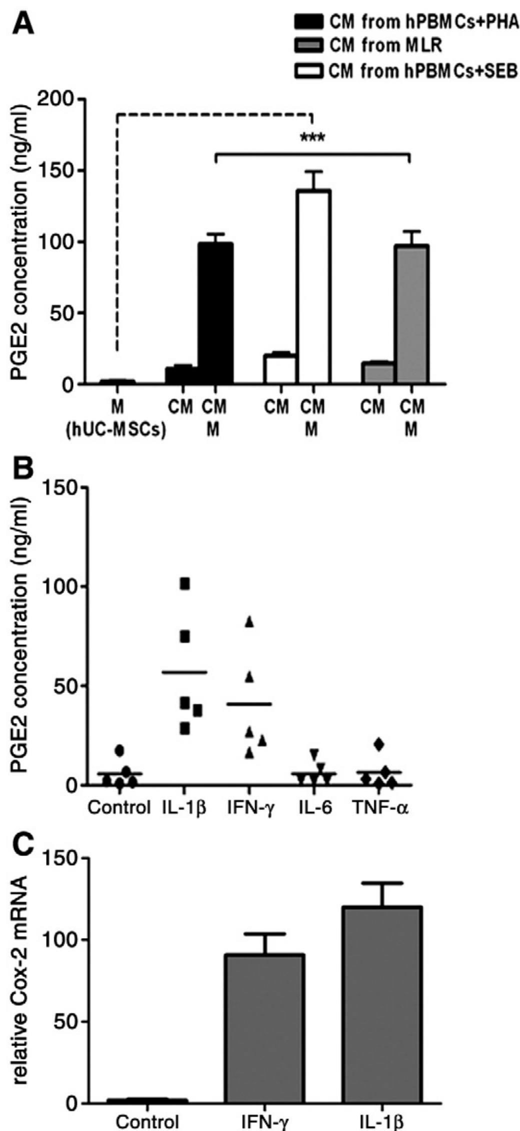

Figure 4 IL-1β and IFN-γ upregulate PGE_2 production of hUC-MSCs. (A) Condition culture media (CM) were prepared by activating hPBMCs with PHA, SEB, or allogeneic hPBMCs for 2 days in 24-well plate. hUC-MSCs were cultured in the three CM for 3 days and then the PGE_2 concentrations in each culture were measured by ELISA (**$p<0.01$). (B) hUC-MSCs were stimulated by exogenous IFN-γ, IL-1β, IL-6, and TNF-α separately for 3 days and the PGE_2 concentrations were determined by ELISA (**$p<0.01$). (C) The levels of COX-2 mRNA expression were measured by quantitative real-time RT-PCR. The data are expressed as the mean ± SEM from three different experiments, each performed in triplicate.

The immunomodulatory activities of hUC-MSCs are not related to the apoptosis of T cells

There is inconsistency in the literature with regard to the role of MSCs on T cell apoptosis, as one report suggested that MSCs may exert their immunomodulatory activities by triggering early death of activated T cells [14], but a later study showed that MSCs may in fact promote T cell survival [15]. Therefore, the effect of hUC-MSCs on survival of activated T cells was evaluated. hPBMCs were stimulated with PHA and cultured with or without the presence of hUC-MSCs for 3 days, and the portions of apoptotic cells were analyzed by annexin V staining and flow cytometry. As shown in Fig. 5A, there was a significant reduction of apoptosis when hPBMCs were cultured in the presence than in the absence of hUC-MSCs (53.8% vs. 70%, $p<0.01$), indicating that hUC-MSCs protected the activated hPBMCs from activation-induced apoptosis. Because of the importance of PGE_2 in the immunosuppression, apoptosis of activated cells was also examined by adding the PGE_2 synthesis inhibitor indomethacin to assess whether PGE_2 was related to the protective effect of hUC-MSCs. This experiment showed that blocking of PGE_2 production with indomethacin completely abolished the protective effect on apoptosis (Figs. 5B and C). The data in this study thus demonstrated that hUC-MSCs inhibited rather than induced apoptosis, and PGE_2 was required for the inhibitory effect.

Discussion

Although the clinical studies of MSCs reported in the literature so far have almost exclusively used BM-derived MSCs, there is growing interest in finding alternative sources of MSCs and exploring their therapeutic potential. Several studies including our own have demonstrated that cells that share most characteristics of BM-MSCs can be readily isolated from human umbilical cord and culturally expanded to large numbers in vitro [27]. Compared with BM-MSCs, hUC-MSCs have several advantages such as abundant supply, painless collection procedure, less chance of microbial contamination, and better expandability [27].

In this study, we prepared hUC-MSCs and analyzed the expression of a number of cell surface and intracellular markers. The expression profile of hUC-MSCs is similar to that of BM-MSCs and consistent with the definition proposed by ISCT [28]. However, some difference was also noted as Nestin, CD54 (ICAM-I), SSEA-4, and OCT-4 were expressed at higher levels on hUC-MSCs than on hBM-MSCs (Fig. 1). Higher expression of nestin, which was initially identified as a marker of neural stem and progenitor cells [29], may explain the potential of hUC-MSCs to differentiate into neuron. ICAM-1 as an adhesion molecule [30] may mediate the effect of binding to leukocytes. In our culture conditions, ~50% of hUC-MSCs express SSEA-4, whereas <2% of BM-MSCs are positive for SSEA-4 expression. It appears also that OCT-4 is expressed by most hUC-MSCs (~90%) at higher levels in comparison with BM-MSCs. SSEA-4 and OCT-4 are initially identified as human embryonic stem cell-specific antigens that are involved in the self-renew and maintenance of pluripotency of embryonic stem cells [31,32]. Expression of SSEA-4, OCT-4, and several other pluripotent stem cell markers by umbilical cord-derived MSCs was also reported by Jo et al. [33]. Expression of some of the embryonic stem cell-specific markers by hUC-MSCs is perhaps not surprising since umbilical cord is a fetal organ, and this may explain the better passagability and higher proliferative capacity of hUC-MSCs compared with the adult BM-MSCs [1].

One of the clinical applications of BM-MSCs showing encouraging results is in the treatment of severe, steroid-resistant, acute GVHD frequently occurred after allogeneic

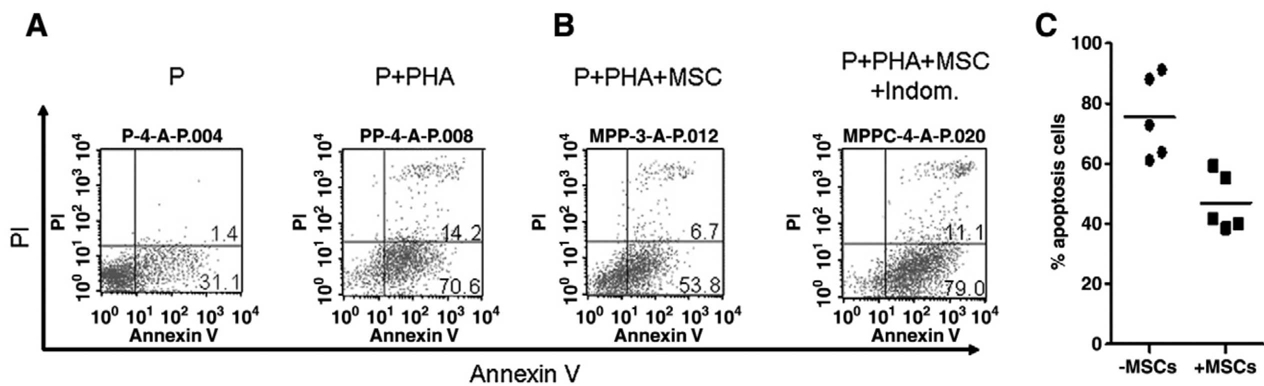

Figure 5 hUC-MSCs protect activated T cells from apoptosis. Cell death of T cells activated by PHA for 3 days in the presence or absence of hUC-MSCs was evaluated by annexin V staining and propidium iodide incorporation. (A)(B) FACS profiles of one representative experiments. (C) Scatter plot of the results of five separate experiments. The means were shown with a dash (–) and the presence of hUC-MSCs significantly protected T cells from apoptosis ($p<0.01$).

hematopoietic stem cell transplantation [34]. The finding that HLA-matching is apparently not required for the therapeutic efficacy of donor BM-MSCs is of particular interest as it implies that the infusion of BM-MSCs may become a readily applicable therapeutic option for GVHD treatment. Similar to the low immunogenecity of BM-MSCs described by previous studies [35,36], we found that the hUC-MSCs prepared by our procedure did not express HLA-DR or the costimulatory molecules (CD80 and CD86) and did not elicit a proliferative response of allogeneic hPBMCs. These results were in line with those obtained by Weiss et al., who also showed that human umbilical cord Wharton's jelly-derived cells (referred to as umbilical cord matrix stromal cells) are hypoimmunogenic in vitro and in vivo in both allogeneic and xenogeneic settings [10]. Although there are some differences in preparation protocols, the data from us and from Weiss et al. have clearly indicated that the low immunogenic cells derived from the human umbilical cord tissue should be applicable in allogeneic transplantations.

The immunomodulatory properties BM-MSCs have been intensively investigated by a number of groups in the last few years [37,38]. Our data here show that umbilical-cord-derived MSCs are able also to potently suppress hUC-MSCs proliferation and IFN-γ secretion in response to mitogenic or allogeneic stimulation. Moreover, the finding that physical separation of hUC-MSCs and hPBMCs in the transwell experiments has little effects on the immunosuppression demonstrates unequivocally that hUC-MSCs exert their suppressive effects mainly through secretion of soluble factor(s). This result is not unexpected as most studies on BM-MSCs have reached similar conclusion [18,19]. However, there are still controversies with regard to the nature of the predominant factor(s) involved, although a number of candidates, such as TGF-β, HGF, PGE_2, NO, and IDO, have been put forward. Our data in this study clearly identify PGE_2 as the critical mediator of the immunosuppressive activities of hUC-MSCs: (1) production of large amounts of PGE_2 was induced upon co-culturing of activated hPBMCs and hUC-MSCs; (2) blocking of PGE_2 biosynthesis by specific inhibitors completely abolished the immunosuppression activities of hUC-MSCs, whereas neutralization of TGF-β, IDO, and NO had no effects; and (3) addition in the hPBMCs culture of exogenous PGE_2 had similar inhibitory effects on hPBMCs activation. It is noteworthy that PGE_2 is equally effective in suppressing hPBMCs that have been stimulated with mitogen (PHA), bacterial superantigen (SEB), or alloantigens (MLR) according to our results. In contrast, Rasmusson et al. have previously shown that inhibition of PGE_2 synthesis has no effect on the MSC-induced inhibition of allogeneic T cell response [39,40]. Moreover, when injected into the spleen, multipotent adult progenitor cells (MAPCs) resulted in an unfavorable in vivo environment for supporting T cell activation via the elaboration PGE_2 in situ, which reduced GVHD [41]. Although we did not investigated the exact mechanism through which PGE_2 modulates T cell responses, several studies have revealed that PGE_2 inhibits T cell proliferation by a protein kinase A (cAMP)-dependent mechanism [42,43] through interaction with the G-protein-coupled receptors EP2 and EP4 [44,45].

Several previous studies have found that the supernatants from BM-MSCs have no inhibitory effect unless they have been co-cultured with lymphocytes, suggesting a requirement of some sort of activation signal(s) for the inhibitory activity of BM-MSCs. In line with those reports, we found that hUC-MSCs produced low levels of PGE_2 when cultured in the regular growth medium, but co-culturing with activated hPBMCs resulted in dramatically increased PGE_2 production. Furthermore, some soluble factor(s) is sufficient for the induction of PGE_2 production as culturing hUC-MSCs in the conditioned supernatants from allo- or mitogen-stimulated hPMCs led to similar increase in PGE_2 concentrations. We tested several inflammatory cytokines for their potential role in stimulating PGE_2 production and identified IFN-γ and IL-1β as strong inducers because both the mRNA expression of Cox-2 (a key enzyme in the biosynthesis of PGE_2) and production of PGE_2 were augmented by IFN-γ or IL-1β treatment. The role of IFN-γ in the activation of BM-MSCs has been described by several reports. Aggarwal and Pittenger [21] demonstrated that treatment of BM-MSCs with IFN-γ resulted in enhanced levels of PGE_2. Ryan et al. [46] showed that IFN-γ treatment led to significantly increased expression of

TGF-β and HGF by BM-MSCs, although the authors did not show whether IFN-γ would also enhance the production of PGE_2 despite its apparent importance in immunosuppression. Jarvinen et al. have reported that IL-1β increases PGE_2 production by lung resident mesenchymal stem cells [22], and the similar effect from porcine maternal placenta was described by Jana et al. [47]. Apart from IFN-γ and IL-1β, it is possible that other inflammatory cytokines such as TNF-α may also induce MSCs to produce PGE_2 [21]. These results may offer an explanation for the clinical observations that MSCs are more effective in treating severe ongoing GVHD [34] than in preventing the onset of GVHD [48], because the extent of alloreactive T cell responses (e.g., the amounts of inflammatory cytokines produced) may be an important factor for the activation of MSCs and therefore their efficacy. Polchert et al. have recently directly tested this hypothesis using a mouse model of acute GVHD and found that only IFN-γ pretreated, but not untreated MSCs, are effective in prevention of GVHD development [49]. These data therefore have important implications in the optimization of clinical applications of MSCs such as in the management of GVHD and in various autoimmune disorders.

Induction of apoptosis of CD3-activated T cells was proposed by Plumas et al. as a possible mechanism of hBM-MSCs-mediated immunosuppression [14], but a later study by Benvenuto et al. showed that hBM-MSCs prevented cell death when T cells were overstimulated through TCR engagement (OKT3) [15]. We found in this study that hUC-MSCs could significantly reduce rather than promote apoptosis of T cells activated with PHA. The differences in the sources of T cells, the stimulators used for T cell activation and the sources or preparations of MSC may explain the discrepancy. Moreover, PGE_2 also appeared to be an important factor in protecting hPBMCs from apoptosis by hUC-MSCs because inhibition of PGE_2 biosynthesis reversed the anti-apoptosis effect. One possible mechanism of PGE_2-mediated protection may be through downregulation of Fas receptor and Fas ligand expression by T cells as shown by Porter and Malek [50]. Interestingly, downregulation of Fas receptor and Fas ligand expression was also noted in the study of Benvenuto et al., although whether PGE_2 was responsible was not directly examined in the study.

In summary, we here demonstrate that hUC-MSCs possess potent immunomodulatory properties, and PGE_2 produced in large amounts by inflammatory cytokine-activated hUC-MSCs is the principal mediator of the immunomodulation. Our data suggest that hUC-MSCs could potentially be used for the treatment of various disorders caused by dysregulated immune reactions.

Authorship

Contribution:

Ke Chen and Ding Wang designed research, performed experiments, analyzed data, and wrote the article;

Wei-Ting Du, Zhi-Bo Han, Ying Chi, and Shao-Guang Yang performed experiments;

He Ren corrected the article;

Delin Zhu analyzed the data and wrote the article;

Francis Bayard designed research; and

Zhong Chao Han designed research, evaluated the data, corrected the article.

Conflict-of-interest disclosure: the authors declare no competing financial interests.

Acknowledgments

This study 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), and Jerome Lejuene Foundation, France.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.clim.2010.01.015.

References

[1] L.L. Lu, Y.J. Liu, S.G. Yang, Q.J. Zhao, X. Wang, W. Gong, Z.B. Han, Z.S. Xu, Y.X. Lu, D. Liu, Z.Z. Chen, Z.C. Han, Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials, Haematologica 91 (2006) 1017–1026.

[2] S. Karahuseyinoglu, O. Cinar, E. Kilic, F. Kara, G.G. Akay, D.O. Demiralp, A. Tukun, D. Uckan, A. Can, Biology of stem cells in human umbilical cord stroma: in situ and in vitro surveys, Stem Cells 25 (2007) 319–331.

[3] H.S. Wang, S.C. Hung, S.T. Peng, C.C. Huang, H.M. Wei, Y.J. Guo, Y.S. Fu, M.C. Lai, C.C. Chen, Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord, Stem Cells 22 (2004) 1330–1337.

[4] A. Can, S. Karahuseyinoglu, Concise review: human umbilical cord stroma with regard to the source of fetus-derived stem cells, Stem Cells 25 (2007) 2886–2895.

[5] R.D. Lund, S. Wang, B. Lu, S. Girman, T. Holmes, Y. Sauve, D.J. Messina, I.R. Harris, A.J. Kihm, A.M. Harmon, F.Y. Chin, A. Gosiewska, S.K. Mistry, Cells isolated from umbilical cord tissue rescue photoreceptors and visual functions in a rodent model of retinal disease, Stem Cells 25 (2007) 602–611.

[6] M.L. Weiss, S. Medicetty, A.R. Bledsoe, R.S. Rachakatla, M. Choi, S. Merchav, Y. Luo, M.S. Rao, G. Velagaleti, D. Troyer, Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease, Stem Cells 24 (2006) 781–792.

[7] W. Liao, J. Xie, J. Zhong, Y. Liu, L. Du, B. Zhou, J. Xu, P. Liu, S. Yang, J. Wang, Z. Han, Z.C. Han, Therapeutic effect of human umbilical cord multipotent mesenchymal stromal cells in a rat model of stroke, Transplantation 87 (2009) 350–359.

[8] P.C. Tsai, T.W. Fu, Y.M. Chen, T.L. Ko, T.H. Chen, Y.H. Shih, S.C. Hung, Y.S. Fu, The therapeutic potential of human umbilical mesenchymal stem cells from Wharton's jelly in the treatment of rat liver fibrosis, Liver Transpl. 15 (2009) 484–495.

[9] R. Friedman, M. Betancur, L. Boissel, H. Tuncer, C. Cetrulo, H. Klingemann, Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation, Biol. Blood Marrow Transplant. 13 (2007) 1477–1486.

[10] M.L. Weiss, C. Anderson, S. Medicetty, K.B. Seshareddy, R.J. Weiss, I. VanderWerff, D. Troyer, K.R. McIntosh, Immune properties of human umbilical cord Wharton's jelly-derived cells, Stem Cells 26 (2008) 2865–2874.

[11] P.S. Cho, D.J. Messina, E.L. Hirsh, N. Chi, S.N. Goldman, D.P. Lo, I.R. Harris, S.H. Popma, D.H. Sachs, C.A. Huang, Immunogenicity of umbilical cord tissue derived cells, Blood 111 (2008) 430–438.
[12] M.L. Weiss, K.E. Mitchell, J.E. Hix, S. Medicetty, S.Z. El-Zarkouny, D. Grieger, D.L. Troyer, Transplantation of porcine umbilical cord matrix cells into the rat brain, Exp. Neurol. 182 (2003) 288–299.
[13] S. Medicetty, A.R. Bledsoe, C.B. Fahrenholtz, D. Troyer, M.L. Weiss, Transplantation of pig stem cells into rat brain: proliferation during the first 8 weeks, Exp. Neurol. 190 (2004) 32–41.
[14] J. Plumas, L. Chaperot, M.J. Richard, J.P. Molens, J.C. Bensa, M.C. Favrot, Mesenchymal stem cells induce apoptosis of activated T cells, Leukemia 19 (2005) 1597–1604.
[15] F. Benvenuto, S. Ferrari, E. Gerdoni, F. Gualandi, F. Frassoni, V. Pistoia, G. Mancardi, A. Uccelli, Human mesenchymal stem cells promote survival of T cells in a quiescent state, Stem Cells 25 (2007) 1753–1760.
[16] M. Krampera, S. Glennie, J. Dyson, D. Scott, R. Laylor, E. Simpson, F. Dazzi, Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide, Blood 101 (2003) 3722–3729.
[17] S. Beyth, Z. Borovsky, D. Mevorach, M. Liebergall, Z. Gazit, H. Aslan, E. Galun, J. Rachmilewitz, Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness, Blood 105 (2005) 2214–2219.
[18] M. Di Nicola, C. Carlo-Stella, M. Magni, M. Milanesi, P.D. Longoni, P. Matteucci, S. Grisanti, A.M. Gianni, Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli, Blood 99 (2002) 3838–3843.
[19] R. Meisel, A. Zibert, M. Laryea, U. Gobel, W. Daubener, D. Dilloo, Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation, Blood 103 (2004) 4619–4621.
[20] K. Sato, K. Ozaki, I. Oh, A. Meguro, K. Hatanaka, T. Nagai, K. Muroi, K. Ozawa, Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells, Blood 109 (2007) 228–234.
[21] S. Aggarwal, M.F. Pittenger, Human mesenchymal stem cells modulate allogeneic immune cell responses, Blood 105 (2005) 1815–1822.
[22] L. Jarvinen, L. Badri, S. Wettlaufer, T. Ohtsuka, T.J. Standiford, G.B. Toews, D.J. Pinsky, M. Peters-Golden, V.N. Lama, Lung resident mesenchymal stem cells isolated from human lung allografts inhibit T cell proliferation via a soluble mediator, J. Immunol. 181 (2008) 4389–4396.
[23] T. Lahiri, J.D. Laporte, P.E. Moore, R.A. Panettieri Jr., S.A. Shore, Interleukin-6 family cytokines: signaling and effects in human airway smooth muscle cells, Am. J. Physiol. Lung Cell Mol. Physiol. 280 (2001) L1225–L1232.
[24] X.X. Jiang, Y. Zhang, B. Liu, S.X. Zhang, Y. Wu, X.D. Yu, N. Mao, Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells, Blood 105 (2005) 4120–4126.
[25] K. English, F.P. Barry, C.P. Field-Corbett, B.P. Mahon, IFN-gamma and TNF-alpha differentially regulate immunomodulation by murine mesenchymal stem cells, Immunol. Lett. 110 (2007) 91–100.
[26] M. Krampera, L. Cosmi, R. Angeli, A. Pasini, F. Liotta, A. Andreini, V. Santarlasci, B. Mazzinghi, G. Pizzolo, F. Vinante, P. Romagnani, E. Maggi, S. Romagnani, F. Annunziato, Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells, Stem Cells 24 (2006) 386–398.
[27] D. Baksh, R. Yao, R.S. Tuan, Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow, Stem Cells 25 (2007) 1384–1392.
[28] M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, R. Deans, A. Keating, D. Prockop, E. Horwitz, Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, Cytotherapy 8 (2006) 315–317.
[29] E. Cattaneo, R. McKay, Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor, Nature 347 (1990) 762–765.
[30] A. Clayton, R.A. Evans, E. Pettit, M. Hallett, J.D. Williams, R. Steadman, Cellular activation through the ligation of intercellular adhesion molecule-1, J. Cell Sci. 111 (Pt. 4) (1998) 443–453.
[31] K. Noaksson, N. Zoric, X. Zeng, M.S. Rao, J. Hyllner, H. Semb, M. Kubista, P. Sartipy, Monitoring differentiation of human embryonic stem cells using real-time PCR, Stem Cells 23 (2005) 1460–1467.
[32] H. Niwa, Molecular mechanism to maintain stem cell renewal of ES cells, Cell Struct. Funct. 26 (2001) 137–148.
[33] C.H. Jo, O.S. Kim, E.Y. Park, B.J. Kim, J.H. Lee, S.B. Kang, H.S. Han, S.H. Rhee, K.S. Yoon, Fetal mesenchymal stem cells derived from human umbilical cord sustain primitive characteristics during extensive expansion, Cell Tissue Res. 334 (2008) 423–433.
[34] K. Le Blanc, F. Frassoni, L. Ball, F. Locatelli, H. Roelofs, I. Lewis, E. Lanino, B. Sundberg, M.E. Bernardo, M. Remberger, G. Dini, R.M. Egeler, A. Bacigalupo, W. Fibbe, O. Ringden, Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study, Lancet 371 (2008) 1579–1586.
[35] C.D. Porada, E.D. Zanjani, G. Almeida-Porad, Adult mesenchymal stem cells: a pluripotent population with multiple applications, Curr. Stem Cell Res. Ther. 1 (2006) 365–369.
[36] D. Noel, F. Djouad, C. Bouffi, D. Mrugala, C. Jorgensen, Multipotent mesenchymal stromal cells and immune tolerance, Leuk. Lymphoma 48 (2007) 1283–1289.
[37] Z. Selmani, A. Naji, I. Zidi, B. Favier, E. Gaiffe, L. Obert, C. Borg, P. Saas, P. Tiberghien, N. Rouas-Freiss, E.D. Carosella, F. Deschaseaux, Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells, Stem Cells 26 (2008) 212–222.
[38] A. Uccelli, V. Pistoia, L. Moretta, Mesenchymal stem cells: a new strategy for immunosuppression? Trends Immunol. 28 (2007) 219–226.
[39] W.T. Tse, J.D. Pendleton, W.M. Beyer, M.C. Egalka, E.C. Guinan, Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation, Transplantation 75 (2003) 389–397.
[40] I. Rasmusson, O. Ringden, B. Sundberg, K. Le Blanc, Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms, Exp. Cell Res. 305 (2005) 33–41.
[41] S.L. Highfill, R.M. Kelly, M.J. O'Shaughnessy, Q. Zhou, L. Xia, A. Panoskaltsis-Mortari, P.A. Taylor, J. Tolar, B.R. Blazar, Multipotent adult progenitor cells can suppress graft-versus-host disease via prostaglandin E2 synthesis and only if localized to sites of allopriming, Blood 114 (2009) 693–701.
[42] E.D. Anastassiou, F. Paliogianni, J.P. Balow, H. Yamada, D.T. Boumpas, Prostaglandin E2 and other cyclic AMP-elevating agents modulate IL-2 and IL-2R alpha gene expression at multiple levels, J. Immunol. 148 (1992) 2845–2852.
[43] R. Minakuchi, M.C. Wacholtz, L.S. Davis, P.E. Lipsky, Delineation of the mechanism of inhibition of human T cell activation by PGE2, J. Immunol. 145 (1990) 2616–2625.
[44] C. Nataraj, D.W. Thomas, S.L. Tilley, M.T. Nguyen, R. Mannon, B.H. Koller, T.M. Coffman, Receptors for prostaglandin E(2)

that regulate cellular immune responses in the mouse, J. Clin. Invest. 108 (2001) 1229–1235.

[45] N. Kvirkvelia, I. Vojnovic, T.D. Warner, V. Athie-Morales, P. Free, N. Rayment, B.M. Chain, T.W. Rademacher, T. Lund, I.M. Roitt, P.J. Delves, Placentally derived prostaglandin E2 acts via the EP4 receptor to inhibit IL-2-dependent proliferation of CTLL-2 T cells, Clin. Exp. Immunol. 127 (2002) 263–269.

[46] J.M. Ryan, F. Barry, J.M. Murphy, B.P. Mahon, Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells, Clin. Exp. Immunol. 149 (2007) 353–363.

[47] B. Jana, M. Koszykowska, A. Andronowska, The effect of tumor necrosis factor-alpha (TNF-alpha, interleukin (IL)-1beta and IL-6 on prostaglandins (PG)F2alpha and E2 secretion from maternal placenta in pigs, Pol. J. Vet. Sci. 11 (2008) 315–322.

[48] H.M. Lazarus, O.N. Koc, S.M. Devine, P. Curtin, R.T. Maziarz, H.K. Holland, E.J. Shpall, P. McCarthy, K. Atkinson, B.W. Cooper, S.L. Gerson, M.J. Laughlin, F.R. Loberiza Jr., A.B. Moseley, A. Bacigalupo, Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients, Biol. Blood Marrow Transplant. 11 (2005) 389–398.

[49] D. Polchert, J. Sobinsky, G. Douglas, M. Kidd, A. Moadsiri, E. Reina, K. Genrich, S. Mehrotra, S. Setty, B. Smith, A. Bartholomew, IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft versus host disease, Eur. J. Immunol. 38 (2008) 1745–1755.

[50] B.O. Porter, T.R. Malek, Prostaglandin E2 inhibits T cell activation-induced apoptosis and Fas-mediated cellular cytotoxicity by blockade of Fas-ligand induction, Eur. J. Immunol. 29 (1999) 2360–2365.