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

Ke Chen [a,c,1], Ding Wang [a,c,1], Wei Ting Du [a], Zhi-Bo Han [a,c], He Ren [b], Ying Chi [a,c], Shao Guang Yang [a], Delin Zhu [b], Francis Bayard [d], Zhong Chao Han [a,b,c,*]

[a] *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, 288 Nanjing Road, Tianjin 300020, China*
[b] *National Engineering Research Center of Cell Products, AmCellGene Co. Ltd, TEDA, Tianjin, China*
[c] *TEDA Life and Technology Research Center, Institute of Hematology, Chinese Academy of Medical Sciences, TEDA, Tianjin, China*
[d] *Institut de Médecine Moléculaire de Rangueil, Institut National de la Santé et de la Recherche Médicale, Unité 858, Université Toulouse III Paul Sabatier, 31432 Toulouse, France*

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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.

* Corresponding author. 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, 288 Nanjing Road, Tianjin 300020, China. Fax: +86 22 66211430.
E-mail address: tihzchan@public.tpt.tj.cn (Z.C. Han).
[1] These authors contributed equally to this work.

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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