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Optimization of the therapeutic efficacy of human umbilical cord blood–mesenchymal stromal cells in an NSG mouse xenograft model of graft-versus-host disease

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Abstract

Background aims. Although *in vitro* studies have demonstrated the immunosuppressive capacity of mesenchymal stromal cells (MSCs), most *in vivo* studies on graft-versus-host disease (GVHD) have focused on prevention, and the therapeutic effect of MSCs is controversial. Moreover, optimal time intervals for infusing MSCs have not been established. *Methods.* We attempted to evaluate whether human umbilical cord blood–MSCs (hUCB-MSCs) could either prevent or treat GVHD in an NSG mouse xenograft model by injection of MSCs before or after *in vivo* clearance. Mice were infused with either a single dose or multiple doses of 5×10^5 hUCB-MSCs (3- or 7-day intervals) before or after GVHD onset. *Results.* Before onset, hUCB-MSCs significantly improved the survival rate only when repeatedly injected at 3-day intervals. In contrast, single or repeated injections after GVHD onset significantly increased the survival rate and effectively attenuated tissue damage and inflammation. Furthermore, the levels of prostaglandin E_2 and transforming growth factor-β1 increased significantly, whereas the level of interferon-γ decreased significantly in all MSC treatment groups. *Conclusions.* These data establish the optimal time intervals for preventing GVHD and show that hUCB-MSCs effectively attenuated symptoms and improved survival rate when administered after the onset of GVDH.

Key Words: *graft-versus-host disease, human umbilical cord blood–mesenchymal stromal cells, NSG mice, therapeutic effect*

Introduction

Although allogeneic hematopoietic stem cell transplantation can cure certain malignant and non-malignant diseases, graft-versus-host disease (GVHD) represents the most common complication despite the use of transplants from human leukocyte antigen (HLA)-matched siblings (1,2). GVHD can be induced by multiple factors, including conditioning regimens, total-body irradiation, the bone marrow (BM) microenvironment, patient and donor age or sex, stem cell source and graft composition (3,4). However, most cases of GVHD are caused by the reaction of transplanted T cells with histoincompatible antigens of the recipient. The ensuing proliferation or activation of other immune cells leads to a wide variety of injuries to host tissues caused by the release of inflammatory cytokines (5).

Corticosteroids are generally used as primary treatment for acute GVHD and are more effective when combined with immunosuppressive agents such as cyclosporine or methotrexate (4). Primary treatment with steroids improves skin, liver or gastrointestinal tract lesions (4) and increases the probability of survival (1-year survival: approximately 50%) (6,7). Patients with steroid-resistant GVHD are usually offered second-line therapy such as anti-thymocyte globulin; however, 31% of patients show initial improvement in signs and symptoms, particularly involving the skin, and only 10% have long-term survival (12–60 months) (8). Therefore, new approaches for increased survival rate are required, and recent strategies have introduced the infusion of mesenchymal stromal cells (MSCs) to take advantage of their immunoregulatory properties (9–12).

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MSCs suppress T-cell proliferation or cytotoxicity induced by cellular or humoral stimuli in a dose-dependent manner (13,14) and mediate T-cell suppression by secreted factors, such as prostaglandin E_2 (PGE_2) (15), transforming growth factor-β1 (TGF-β1) and hepatocyte growth factor (16). Moreover, because there is no immunologic restriction on their ability to suppress T cells, MSCs provide an important tool for transplantation biology. Recent studies on MSCs *in vivo* have been facilitated by a GVHD animal model (17–20). However, despite the immunosuppressive capacity of MSCs, studies on GVHD focus on prevention, and the use of MSCs for treatment is controversial. Moreover, the therapeutic effect of human umbilical cord blood (hUCB)-MSCs in a xenograft model of GVHD (xeno-GVHD) has not been demonstrated (17) and the optimum dosing frequency has not been established. Therefore, the goal of the present study was to determine optimal time intervals for infusing MSCs and to assess the efficacy of hUCB-MSCs for preventing and treating GVHD.

Methods

Human peripheral blood mononuclear cells

Human peripheral blood mononuclear cells (hPBMCs) were acquired from Astarte Biologics (Redmond, WA, USA) for research use only after written informed consent was given by the donor. The hPBMCs were stored in their blood bank, in compliance with the Health Insurance Portability and Accountability Act. We purchased the hPBMCs used to induce GVHD from Lifeline Cell Technology (Frederick, MD, USA), which is the distributor for Astarte Biologics. We stored the hPBMCs at −196°C until use.

hUCB-MSCs

Culture conditions for hUCB-MSCs were the same as those described in our previous study (21). Human UCB was obtained after written informed consent was given by normal full-term pregnant women. The hUCB-MNCs were isolated with the use of a Histopaque (1.077 g/mL, Sigma-Aldrich, St Louis, MO, USA) density gradient centrifuge at 400 *g* for 30 min and were grown to a density of 5×10^6 cells/cm^2 in 175-cm^2 flasks (Nunc, Roskilde, Denmark) in α-minimum essential medium (α-MEM; Gibco/Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco/Life Technologies). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2, and half of the media was replaced with fresh media twice each week. After spindle-shaped colonies were observed, cells were harvested with the use of 0.25% trypsin–ethylenediaminetetraacetic acid (EDTA) (Gibco/Life Technologies) and grown to 80% confluence. Because the immunosuppressive activity of hUCB-MSCs differs for each donor, we selected the optimal lot that significantly reduced lymphocyte proliferation (mixed lymphocyte reaction assays) and secretion of interferon-γ (IFN-γ enzyme-linked immunosorbent spot assays kit [BD Biosciences, San Jose, CA, USA]) (Supplementary Figure 1). We confirmed the phenotype, differentiation ability and immunosuppressive activity of the UCB-MSC lots and show the respective results in Supplementary Figures 1 and 2. Additional information regarding hUCB-MSC preparation is provided in the Supplementary information. Cells were cryopreserved in liquid nitrogen until use.

Xenogeneic GVHD mouse model

NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ, 7 weeks old) male mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and allowed to acclimate for 2 weeks before the experiments. All animal experiments and use of hPBMCs and hUCB-MSCs were approved in advance by the Institutional Review Board and the Institutional Animal Care and Use Committee, College of Medicine, Hanyang University, Seoul, Republic of Korea, respectively (permit No. HY-IACUC-10–030, 10–046, 11–057, 12–019). Mice (9 weeks old) were irradiated by means of 2.0 Gy with the use of a Gammacell 1000/3000 irradiator (Best Theratonics Ltd, Ottawa, Ontario, Canada). The control (group 1) did not receive hPBMCs or irradiation, and group 2 only was irradiated. The viability of the hPBMCs after thawing was >95%, and hPBMCs (group 3, 0.5×10^6; group 4, 1×10^6 or group 5, 2.5×10^6) were transplanted intravenously within 24 h after irradiation to establish acute or chronic xenogeneic GVHD animal models. Survival, weight loss, fur texture, physical activity, skin integrity and hunched back were recorded every 3–4 days. The severity of GVHD was assessed by means of a clinical scoring system described by Cooke *et al.* (22). A clinical GVHD index was generated by summing the five criterion scores (0–10), and we evaluated acute and chronic GVHD according to overall survival rate, GVHD clinical score and histological analysis until day 100. Five mice per group were used, and experiments were performed three times. Two mice in each group were euthanized for histological analysis on day 24. Lungs, liver, kidneys and the small intestine were removed to observe morphological changes and lymphocyte infiltration. Paraffin embedding, sectioning, hematoxylin and eosin staining and analysis were performed at the Tissue and Cell (T & C) Pathology Centre (Seoul, Korea), and a clinical pathologist determined the score according to