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Human adipose stromal cells expanded in human serum promote engraftment of human peripheral blood hematopoietic stem cells in NOD/SCID mice

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Abstract

Human mesenchymal stem cells (hMSC), that have been reported to be present in bone marrow, adipose tissues, dermis, muscles, and peripheral blood, have the potential to differentiate along different lineages including those forming bone, cartilage, fat, muscle, and neuron. Therefore, hMSC are attractive candidates for cell and gene therapy. The optimal conditions for hMSC expansion require medium supplemented with fetal bovine serum (FBS). Some forms of cell therapy will involve multiple doses, raising a concern over immunological reactions caused by medium-derived FBS proteins. In this study, we cultured human adipose stromal cells (hADSC) and bone marrow stroma cells (HBMSC) in human serum (HS) during their isolation and expansion, and demonstrated that they maintain their proliferative capacity and ability for multilineage differentiation and promote engraftment of peripheral blood-derived CD34(+) cells mobilized from bone marrow in NOD/SCID mice. Our results indicate that hADSC and hBMSC cultured in HS can be used for clinical trials of cell and gene therapies, including promotion of engraftment after allogeneic HSC transplantation.
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Adult mesenchymal stem cells (hMSC) can be obtained from human bone marrow, adipose tissue, and muscle [1], and are expandable in culture [2,3], and can be genetically modified by viral and nonviral methods [4,5]. hMSC have a multipotent population of cells capable of differentiating into a number of mesodermal lineages; adipocytes, osteoblasts, and other mesodermal pathways [6,7]. Bone marrow stromal cells also support the proliferation and differentiation of hematopoietic stem cells (HSC) [8]. Adult stem cells may differentiate into tissues that during normal embryonic development would arise from a different germ layer. For example, bone marrow-derived mesenchymal stem cells may differentiate into neural tissue, which is derived from

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embryonic ectoderm [9,10]. The usefulness of hMSC administration has been demonstrated for the treatment of some genetic diseases such as osteogenesis imperfecta (brittle bone disease) [11,12], metachromatic leukodystrophy, and Hurler syndrome [13], and for hematopoietic recovery following high-dose chemotherapy in cancer patients [14]. Therefore, several characteristics of hMSC make them potentially useful for cell and gene therapy [15].

hMSC expanded in fetal bovine serum (FBS) have been used for all reported clinical trials [11–14]. hMSC themselves are not highly immunogenic [16–19]. However, residual FBS in hMSC is likely to generate immune responses, particularly in patients that repeated administrations are required. In fact, anaphylactic reactions have been noted in several patients who received repeated administrations of dendritic cells or lymphocytes cultured in FBS [20–22]. Therefore, FDA requires reduction of FBS exposure for cell culture and extensive washing before administration to patients.

Allogeneic stem cell transplantation (SCT) is being used increasingly in the treatment of hematologic and nonhematologic diseases of neoplastic and nonneoplastic origin. The transplant procedure involves infusion of HSC into the circulation, using the capacity of these cells to home to the marrow and to dock at specific sites in the marrow microenvironment [23]. Maintenance of hematopoiesis depends on the self-renewal and multilineage differentiation capacity of HSC that is thought to be regulated and controlled by the bone marrow microenvironment [24]. Marrow stroma is composed of a heterogeneous population of cells, including reticular endothelial cells, fibroblasts, adipocytes, and osteogenic precursor cells, which provide growth factors, cell-to-cell interactions, and matrix proteins [25–27]. MSC could be used either to replace host cells in the marrow microenvironment that have been damaged by chemotherapy or irradiation, or as vehicles for gene therapy. In fact, coinfusion of bone marrow-derived MSC with HSC has been reported to increase frequency and levels of HSC engraftment [28,29]. However, availability of autologous bone marrow can be limited in patients with hematologic malignancies.

The studies for replacing FBS with human serum (HS) in hMSC culture have been reported [30–33]. Kuznetsove et al. [32] reported that culture of hMSC in HS decreased their proliferation rate and differentiation potentials. Other reports showed that human serum (HS) can support short-term growth and increased spontaneous osteogenic differentiation of MSC [30,34].

Zuk et al. [35] reported that MSC from adipose tissue were cultured and differentiated in vitro into adipogenic, chondrogenic, myogenic, and osteogenic cells under appropriate culture conditions. A recent study has shown that adipose-derived-stromal cells (ADSC) can be induced to expression of neural cells in vitro [36]. We have reported that adipose tissue also contains multipotent progenitor cells, and that they have similar characteristics with bone marrow stromal cells (BMSC) in vitro and in vivo [37–39]. Therefore, human adipose tissue presents an alternative source of multipotent stromal cells.

In this study, we established the culture condition of hMSC without exposure to FBS while maintaining the proliferation and differentiation capacity necessary to generate clinically relevant numbers of cells, and examined their effects on engraftment of HSC in NOD/SCID mice to determine whether hMSC grown in HS still maintain their characteristics in vivo.

Materials and methods

Isolation and culture of stem cells hADSC and hBMSC. After informed consent, leftover materials (heparinized bone marrow cells and adipose tissues) were obtained from eight individuals undergoing total hip arthroplasty and elective plastic surgery (ages 16–54 years). hBMSC and hADSC were isolated according to the methods described in the previous studies [37,38]. Isolated cells were cultured at 37 °C/5% CO_2 in α-MEM containing 10% FBS. One week later, when the monolayer of adherent cells had reached confluence, cells were trypsinized (0.25% trypsin; Sigma) and resuspended in MD media (60% DMEM-LG (Life Technologies, Grand Island, NY), 40% MCDB-201 (Sigma) with 1× insulin–transferrin–selenium, 10^{-9} M dexamethasone (Sigma), 10^{-4} M ascorbic acid 2-phosphate (Sigma), 100 U/mL penicillin, 100 μg/mL streptomycin (Invitrogen, USA), and 10 ng/mL hEGF (Daewoong Pharmaceuticals, Korea) on fibronectin (Sigma) with 5% FBS at a concentration of 2000 cells/cm^2. Before using cells for experiment, cells were cryopreserved in expansion media containing 25% HS and 10% DMSO before use in experiment.

To prepare HS, whole blood was taken from each of four consenting donors. The protocol was approved by an Institutional Review Board. The blood was recovered in the absence of anti-coagulants and allowed to clot for 4 h at room temperature. The serum was aspirated from the clot and centrifuged at 500*g* for 20 min. The supernatant was then centrifuged for a further 20 min at 2000*g*. The cleared serum was stored at −80 °C. To culture and expand cells in HS, isolated cells from collagenase treatment in adipose tissue or from Ficoll centrifugation in bone marrow were plated in α-MEM containing 10% HS. When cells reached a confluent state, cells were plated at a density of 2000 cells/cm^2 in MD or MF media containing 10% HS. In MF media, 40% MCDB-201 in MD media was replaced with 40% F-12. Cells were cryopreserved in expansion media containing 25% HS and 10% DMSO before use in experiment. To obtain sufficient amount of HS for experiment, two samples were combined to two lots. The effects of two different lots of HS were tested for each MSC sample.

Adipogenic and osteogenic differentiation. Adipogenic differentiation was induced by culturing hMSC for 2 weeks in adipogenic medium (10% FBS or HS, 1 μM dexamethasone, 100 μg/mL of 3-isobutyl-1-methylxanthine, 5 μg/mL insulin, and 60 μM indomethacin in α-MEM) and assessed using an Oil Red O stain as an indicator of intracellular lipid accumulation. In order to obtain quantitative data, 1 ml of isopropyl alcohol was added to the stained culture dish and optical density was measured at 510 nm by a spectrophotometer.

Osteogenic differentiation was induced by culturing hMSC for a minimum of 2 weeks in osteogenic medium (10% FBS or HS, 0.1 μM dexamethasone, 10 mM β-glycerophosphate, and 50 μg/mL ascorbic acid in α-MEM) and examined for extracellular matrix calcification by alizarin red stain. Osteogenic differentiation was quantified by measurement of alizarin red-stained area and density in six-well dishes by

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