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ORIGINAL ARTICLE

Bone marrow mesenchymal stem cells altered the immunoregulatory activities of hepatic natural killer cells

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Summary We explored the biological characteristics of bone marrow-derived mesenchymal stem cells (BMSCs) and their immunological effects in vivo. To establish the characteristics of BMSCs, we first examined their morphology, differentiation ability, phenotype, and growth patterns. We further explored the effects of intravenous infusion of BMSCs on the immunological activities in vivo and the possible mechanism involved in it. The results showed that BMSCs displayed a fibroblast-like morphology and could differentiate into bone, fat and cartilage cells. Phenotypic analysis indicated the cells were negative for CD34 and CD31 but positive for Flk1, CD29, CD44 and CD105. In addition, BMSC culture supernatants could not improve the resistance against H_2O_2-induced apoptosis in L02 cells. We also found that infusion of BMSCs suppressed the activity of intrahepatic natural killer T cells. In summary, BMSCs are an ideal candidate for therapeutic application because they are relatively easy to harvest, easily expandable in vitro, and can be isolated from adult bone marrow while retaining their differentiation potential. BMSCs have stem cell properties, and BMSC therapy is an alternative treatment for acute liver disease.

Introduction

Mesenchymal stem cells (MSCs) are non-hematopoietic stem cells with the capacity to differentiate into tissues of both mesenchymal and non-mesenchymal origin [1]. A large number of studies have shown that this kind of bone marrow-derived adherent cell can differentiate into a variety of cell types such as esteoblasts, fat cells, nerve cells, muscle cells, and endothelial cells [2—7]. Numerous studies have demonstrated that MSCs avoid allorecognition, interfere with dendritic cell and T cell functions, and generate a local immunosuppressive microenvironment by secreting

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cytokines [8,11,14]. Low immunogenicity allows MSCs to be possibly used for allografts, indicating that these cells may become ideal seed cells for tissue engineering and immune regulation, which brings new hope for the treatment of autoimmune diseases. Some researchers have reported that MSCs cultured and expanded in vitro have immune regulatory effects on a variety of immune cell types, such as dendritic cells, T and B cells, and natural killer T (NKT) cells [9—13], and this inhibition is not major histocompatibility complex (MHC) restricted. Bone marrow MSCs (BMSCs) from a donor, recipient or a third party have similar immunosuppressive effects. In addition, MSCs cultured in vitro can play a role in immune regulation in vivo. For example, MSCs can extend the survival time of allogeneic skin grafts [14] and promote the proliferation of allogeneic tumor cells [15]. MSC treatment of graft-versus-host disease (GVHD) has been proved to be effective not only in animal studies but also in clinical application[16].

The main function of transplanted BMSCs is the suppression of disease development rather than the repair of post-traumatic defects, which can explain the proportion of differentiated BMSCs in the corresponding tissues. We used ConA-induced acute liver injury as a model to confirm the role of BMSCs in disease development. The advantage of this model is that the liver damage caused by inflammation occurs quickly and the liver rapidly undergoes self-repair in 10 days. Therefore, infused BMSCs will not differentiate into liver cells and affect the MSC treatment.

Materials and methods

Materials

Collagenase II was obtained from Sigma (St Louis, MO). Dispase was purchased from Gibco (Gibco Life Technologies, Paisley, UK). For flow cytometric analyses, the anti-human CD29, CD31, CD34, CD44, CD45, CD105, CD106, CD184, HLA-ABC, Flk1 and control antibodies were purchased from BD Bioscience. For the analysis of endocytosis, DiI-labeled acetylated LDL was provided by Molecular Probes. C57bl/6 mice were obtained from the animal center of Capital Medical University.

Cell preparation and culture

Isolation and culture of BMSCs were performed as described previously with some modifications [16]. Briefly, mononuclear cells (MNCs) were separated by Ficoll-Paque gradient centrifugation (specific gravity 1.077 g/mL; Nycomed Pharma AS, Oslo, Norway), and the isolated cells were plated at a concentration of 1 cell/well by limiting dilution in a total of 10 × 96-well plates coated with fibronectin (Sigma) and collagen (Sigma). The cells were cultured in DMEM/F-12 containing 40% MCDB-201 medium complete with trace elements (Sigma), 2% fetal calf serum (FCS; Gibco Life Technologies, Paisley, UK), 1 × insulin transferrin selenium (Gibco Life Technologies), 10^{-9} M dexamethasone (Sigma), 10^{-4} M ascorbic acid 2-phosphate (Sigma), 20 ng/mL IL-6 (Sigma), 10 ng/mL epidermal growth factor (Sigma), 10 ng/mL platelet-derived growth factor BB (Sigma), 50 ng/mL fetal liver tyrosine kinase 3 ligand (Sigma), 30 ng/mL bone morphogenetic protein-4 (Sigma), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco Life Technologies, Paisley, UK) at 37 °C in a humidified atmosphere with 5% CO_2. The culture medium was changed every 4—6 days.

Western blotting

MSCs were harvested at specific times after treatment with regents as indicated in each experiment. Cells were mixed with loading buffer and subjected to electrophoresis. After electrophoresis, proteins were transferred to polyvinyl difluoride membranes (Pall Filtron) using a semi-dry blotting apparatus (Pharmacia) and probed with mouse monoclonal antibodies, followed by incubation with peroxidase-labeled secondary antibodies. Detection was performed by a chemiluminescence system (Amersham) according to the manufacturer's instructions. Then membrane was striped with elution buffer and reprobed with antibodies against the non-phosphorylated proteins as a measure of loading. Controls for the immunoprecipitation used the same procedure, except that the agarose beads contained only mouse IgG.

Flow cytometry

For immunophenotype analysis, expanded clonal cells were stained with antibodies against Flk1, CD29, CD31, CD34, CD44, CD45, CD105, HLA-ABC, and vWF (all purchased from Becton Dickinson Immunocytometry Systems, Mountain View, CA). For intracellular antigen detection, cells were fixed in 2% paraformaldehyde (Sigma) for 15 minutes at 4 °C, and then permeabilized with 0.1% saponin (Sigma) for 1 hour at room temperature. Cells were washed and labeled with fluorescein isothiocyanate (FITC)-conjugated secondary goat anti-mouse, goat anti-rabbit, or sheep anti-goat antibodies (Sigma), and then washed and analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA).

Multilineage differentiation

Cells were induced in the presence of 10^{-6} mol/L dexamethasone, 10 mmol/L β-glycerol phosphate, 0.05 mmol/L dexamethasone, 10 mmol/L β-glycerol phosphate, 0.05 mmol/L ascorbic acid and 10% FCS in IMDM. The culture medium was changed every 3—5 days. Calcification matrix deposition was stained by Von Kossa, and then fixed with neutral formalin for 1 hour. Samples were washed with deionized water and then a 2% silver nitrate solution was added, followed by incubation at 37 °C in the dark for 10 minutes. Then, the samples were washed with deionized water, and the calcified matrix precipitation was observed under a light microscope. Control cells grew in culture in the expansion of BMSCs.

Adipose differentiation

Cells were induced in the presence of 10^{-6} mol/L dexamethasone, 0.5 mmol/L 3-isobutyl-1-methylxanthine, 0.1 mmol/L ascorbic acid and 10% FCS in IMDM. Half of the medium volume was changed every 3 days. Lipid droplets