Characterization of the Cellular Responses of Dental Mesenchymal Stem Cells to the Immune System

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Abstract

Introduction: Dental stem cells have gained importance recently and are being used for various purposes in regenerative medicine and dentistry. Although much research has been done to show the various properties of these dental stem cells, the immunomodulatory properties of some of these stem cells are still unknown. This is important considering these cells are being used routinely. Therefore, the aim of this study was to investigate the interactions between the activated immune cells and 3 types of dental-derived mesenchymal stem cells: dental pulp stem cells, stem cells from human exfoliated deciduous teeth, and stem cells of the apical papilla (SCAP). **Methods:** SCAP, dental pulp stem cells, stem cells from human exfoliated deciduous teeth, and periodontal ligament fibroblasts were cultured, and various assays were performed including a proliferation assay, flow cytometric analysis, lactate dehydrogenase and chromium-51 cytotoxicity assays, and an enzyme-linked immunosorbent assay to evaluate the interactions of these dental stem cells when cocultured with either peripheral blood mononuclear cells or natural killer cells. **Results:** SCAPs were less resistant to immune cell–mediated cytotoxicity as seen from the results obtained from the LDH and chromium-51 cytotoxicity assays. The flow cytometric analysis showed a lower resilience of SCAP to cytotoxic compounds. The enzyme-linked immunosorbent assay results demonstrated that the SCAP induced high levels of proinflammatory cytokine secretion compared with the other dental stem cells. **Conclusions:** SCAP did not perform as well as the other dental stem cells. This could in turn affect their survival and differentiation abilities as well as their functionality. This may be an important aspect to consider when selecting dental stem cells for various regenerative procedures. *(J Endod 2018;■:1–6)*

Key Words

Cytokines, cytotoxicity, dental stem cells, immune cells, regeneration

Significance

This study characterized the interactions of different dental stem cells with the immune system, which will prove to be beneficial in future regenerative medicine and dentistry procedures.

A mesenchymal stem cell (MSC) is defined by its adherence to tissue culture plastic and its capacity to differentiate into multiple lineages with profiles of certain stem cell markers and the absence of other surface markers (1). MSCs were first isolated from the bone marrow, but more recently other sources of these cells have been identified for tissue engineering purposes. These sources include bone marrow stem cells, adipose tissue stem cells, umbilical cord stem cells, dental stem cells, and many others (2–4). These MSCs have attracted tremendous attention over the last few years as a source of cells necessary for tissue engineering and regeneration because of their multilineage differentiation capability. However, dental-derived MSCs have gained importance in the past several years because they are easier to obtain as compared to other stem cells (eg, bone marrow stem cells) by noninvasive routine clinical procedures (eg, extraction of third molars) and their ability to differentiate into several lineages including osteogenic, odontogenic, adipogenic, neurogenic, chondrogenic, myogenic, and neurogenic (5–9). This is 1 of the main reasons these cells are used in various tissue engineering procedures beyond the oral cavity in regenerative dentistry and medicine. These cells have been used in the treatment of neurotrauma, autoimmune diseases, myocardial infarction, acute lung injury, muscular dystrophy, and connective tissue damages and for corneal reconstruction (10–13). Dental MSCs are thought to originate from the cranial neural crest, expressing both MSC and neuroectodermal stem cell markers, such as CD105, CD73, and CD90 (14). Multiple sources for dental-derived MSCs have been reported in the literature, some of which include stem cells of the apical papilla (SCAP), dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), and stem cells from human exfoliated deciduous teeth (SHED) (15).

Previous studies have shown that some of these dental MSCs share many common characteristics and have distinct functional properties (16). However, their interaction with the immune system is still unknown. This is an important aspect because previous studies have shown that stem cells are more susceptible to immune cell–mediated cytotoxicity compared with differentiated cells (17). Furthermore, this will provide useful information related to the level of stemness of various stem cells, which has been shown

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to be a factor in immune cell–mediated cytotoxicity (18). Understanding the levels of stemness and the interaction of the cells with the immune system is important because these dental stem cells are used for many other regenerative purposes in medicine, as stated previously.

The immunomodulatory effects of other stem cells, like bone marrow stem cells, have been studied extensively in health and disease (19). Other research has shown the immunomodulatory properties of dental pulp and follicle stem cells (20, 21). However, there has been limited research that has evaluated the cellular responses of dental MSCs, like SCAP and SHED, with the immune system. Therefore, the aim of this study was to investigate the interactions of 3 types of dental-derived MSCs, namely DPSCs, SHED, and SCAP, with the activated immune system.

Methods and Materials

Cell Culture

SCAP and DPSCs were obtained from Dr Songtao Shi's laboratory at the University of Pennsylvania, Philadelphia, PA. SHED were obtained from Dr. Peter Murray's laboratory at Nova Southeastern University, Ft Lauderdale, FL. Periodontal ligament (PDL) fibroblast cells were obtained from Lonza Laboratories, Allendale, NJ. Peripheral blood mononuclear cells (PBMCs) were obtained from Dr Anahid Jewett's lab at the University of California, Los Angeles.

Human DPSCs, SHED, SCAP, and PDL fibroblast cells were maintained in alpha minimum essential medium (Invitrogen, Carlsbad, CA) supplemented with 15% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine (Invitrogen). Cells were incubated at 37°C in an atmosphere of 5% CO_2. Cells were used only until passage 5 because previous research has shown that these stem cells demonstrate a more differentiated phenotype after passage 5 (22).

Culture of PBMCs

Human PBMCs were cultured in RPMI 1640 (Thermo Fisher Scientific, Waltham, MA) supplemented with 1% sodium pyruvate, 1% nonessential amino acids, 1% glutamine, 1% penicillin-streptomycin (Life Technologies, Grand Island, NY), and 10% FBS (Irvine Scientific, Santa Ana, CA). Cells were incubated with and without recombinant IL-2 (r-IL2 [1000 U/mL; Peprotech, Rocky Hill, NJ]) for 24 hours.

Culture of Natural Killer Cells

Natural killer (NK) cells were isolated by our collaborators at the University of California, Los Angeles, as described previously and maintained in RPMI 1640 supplemented with 10% FBS, 1% penicillin, and 1% L-glutamine (Invitrogen) (23). Cells were incubated with and without r-IL2, 1000 U/mL for 24 hours before they were cocultured with the dental stem cells.

Cell Proliferation

This assay was performed using the WST-1 Cell Proliferation Assay Kit from Millipore (Billerica, MA). This colorimetric assay is based on the cleavage of a water-soluble tetrazolium salt (WST-1) to formazan by cellular mitochondrial dehydrogenases. Cells were cultured in 24-well plates, and proliferation rates were assessed on days 1, 4, and 7 for all cell types. At the indicated time point, 550 μL of the kit reagent WST-1 with media was added to each well. After 90 minutes, 100 μL was collected from each sample and placed in a 96-well plate. The optical density was measured using a microplate reader at an absorbance of 450 nm. Samples were run in triplicate.

Flow Cytometric Analysis

Cells were treated with 2 different concentrations of hydroxyethyl methacrylate (HEMA) in 12-well plates. After a 24-hour incubation period, the cells were analyzed for apoptosis using FITC-Annexin V and propidium iodide (PI) staining (Fisher Scientific, Pittsburg, PA) to determine the levels of apoptosis. After treatment with HEMA, the cells were washed twice and resuspended in binding buffer containing FITC-Annexin V/PI as suggested by the manufacturer. After 15 minutes of incubation on ice, Annexin V/PI–stained cells were analyzed by flow cytometry. Flow cytometric analysis was performed using LSR-2 (BD Biosciences, Franklin Lakes, NJ). Dead cell fragments and debris were gated out by forward and side scatter analysis. Analysis included 10,000 events. The numbers in the quadrants represent the percentages of the early and late apoptotic cells (Q4 and Q2) and the necrotic cells (Q1).

Chromium-51 Release Cytotoxic Assay

The chromium-51 release assay was performed as described previously (24). The conditions used were similar to those used for the LDH assay. Different numbers of purified NK cells (effector cells) that were treated or left untreated with r-IL2 were incubated with Cr-51–labeled target cells (different MSCs) at different effector (E):target (T) ratios (10:1, 5:1, 2.5:1, 1.25:1, and 0.625:1). After a 4-hour incubation period, the supernatants were harvested from each sample and counted for released radioactivity using a gamma counter. The percentage specific cytotoxicity was calculated as follows:

% cytotoxicity = experimental cpm − spontaneous cpm/total cpm − spontaneous cpm.

Lactate Dehydrogenase Assay

The CytoTox 96 nonradioactive lactate dehydrogenase (LDH) assay was used to quantitatively assess cell-mediated cytotoxicity. LDH is a stable cytosolic enzyme that is released upon cell lysis. This colorimetric assay is based on the conversion of a tetrazolium salt (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium) into a red formazan product. The amount of color is proportional to the number of cells lysed. PBMCs were left treated or untreated with r-IL2 (1000 U/mL) for 24 hours before they were cocultured with DPSCs, SHED, SCAP, or PDL fibroblast cells at E:T ratios of 10:1, 5:1, 2.5:1, 1.25:1, and 0.625:1 in a 96-well plate. After a 4-hour incubation period, the lysis reagent was added to each well and incubated for 30 minutes. The optical density was measured using a microplate reader at 490 nm, and percent cytotoxicity was calculated according to manufacturer's instructions.

Enzyme-linked Immunosorbent Assay

DPSCs, SHED, SCAP, and PDL cells were plated on 12-well plates for about 4 hours; after which, they were coincubated with PBMCs with and without r-IL2 (1000 U/mL) for 24 hours with T:E cell ratios of 2:1, 1:1, and 0.5:1. After the 24-hour incubation period, the supernatants were analyzed for the levels of various proinflammatory cytokines. Tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and interleukin (IL)-6 levels were determined using a Duo-set ELISA from R&D Systems (Minneapolis, MN) according to the manufacturer's recommendations.

Statistical Analysis

All experiments were performed in triplicate. SigmaPlot 11.0 (Systat Software, Inc, San Jose, CA) was used for all statistical testing. Data were analyzed by 1-way analysis of variance with further pair-wise