IMMUNOSUPPRESSIVE PROPERTIES OF PALATINE TONSIL-DERIVED MULTIPOTENT MESENCHYAL STEM CELLS

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INTRODUCTION

Mesenchymal stem cells (MSCs), originally discovered in bone marrow stroma, support hematopoiesis as progenitor cell lines along multiple mesenchymal lineages, including osteoblasts, chondrocytes, adipocytes, and myocytes [1, 2]. Due to their differentiation capacities, MSCs have emerged as a promising tool for therapeutic applications in tissue engineering, cell and gene therapy. Besides their multilineage potential, MSCs display immunoregulatory properties that have prompted consideration of their use in allogeneic transplantation. The capacity of MSCs to suppress T-cell proliferation stimulated by allogeneic lymphocytes, dendritic cells, and phytohemagglutinin (PHA) is well documented [3].

We recently isolated MPCs from the stroma of palatine tonsils, expanded them in the culture and showed their multipotent differentiation properties [4]. Current work explores immunosuppressive characteristics of T-MSCs and compares them with bone marrow-derived MSCs (BM-MSCs).

MATERIALS AND METHODS

Tonsil-derived MSCs (T-MSCs) and bone marrow derived MSCs (BM-MSCs) isolation

T-MSCs were obtained from patients (ages 4-15 years) undergoing tonsilectomy. Tonsils were minced and digested in RPMI medium containing collagenase type I and DNase. Mononuclear cells were obtained by density gradient centrifugation. Cells were plated over 24-48 hours and nonadherent cells were washed out with expansion medium changes.

BM-MSCs were obtained from patients undergoing lower extremity reconstructive surgery, and processed by direct plating [5].

Culture Expansion. Expansion medium for both T-MSCs and BM-MSCs consisted of DMEM supplemented with 10% fetal bovine serum and antibiotics. At 80% confluency, cells were detached using 0.25% trypsin-EDTA solution, washed, and replated at 1:3 under same culture conditions. Cells obtained at passages 2 to 5 were used in this study.

Primary mixed lymphocyte reaction (MLR)

Peripheral blood from healthy human donors was collected into heparinized containers, and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. Polymorphonuclear leukocytes were depleted by incubation in RPMI 1640 medium containing 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin, 25 mM HEPES, 100 µM 2-Mercaptoethanol. Splenocytes were seeded in triplicates. PHA was used at 5 µg/ml, as a positive control to induce T-cell proliferation. MSCs (5 x 104 cells) were added to obtain a final volume of 100 µL. After 3 days of incubation, 1 µCi/well [3H]-thymidine was added overnight and radioactivity incorporation was determined by liquid scintillation counting.

Indoleamine 2,3-dioxygenase (IDO) activity assay

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