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MESENCHYMAL STROMAL CELLS

Quantitative activation suppression assay to evaluate human bone marrow–derived mesenchymal stromal cell potency

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Abstract

Background aims. With the increasing use of cell therapies involving immune modulatory cells, there is a need for a simple standardized method to evaluate and compare the suppressive potency of different cell products. We used the Karpas 299 (K299) cell line as the reference suppressor cell to develop a standardized suppression assay to quantify the immune-modulatory capacity of bone marrow–derived mesenchymal stromal cells (BM-MSCs). *Methods.* Healthy donor CD4 T cells were co-cultured with the K299 cell line or with third-party BM-MSCs. After stimulation with anti-CD3/CD28 beads, CD154 activation and proliferation of CD4 T cells were measured to calculate suppression. *Results.* The K299 cell line reproducibly suppressed both the activation and proliferation of healthy donor CD4 T cells in a dose-dependent manner. A rapid (16-h) assay that was based on activation-suppression was selected for development. In replicate testing, there was an inherent variability of suppression of 11% coefficient of variation between different responder T cells. Suppression by BM-MSCs on different responders correlated with suppression by K299. We therefore used K299 suppression as the reference to define suppression potency of BM-MSCs in K299 Suppression Units. We found that inter-donor variability, passage number, method of manufacture and exposure of BM-MSCs to steroids or interferon-γ all affected BM-MSC potency of suppression. *Conclusions.* This method provides a platform for standardizing suppressor function to facilitate comparisons between laboratories and for use as a cell product release assay.

Key Words: *immunosuppression, suppression potency assay, mesenchymal stromal cells, K299*

Introduction

Immunologic tolerance is a critical homeostatic function to protect the self from auto-immunity [1–3]. Various immune-regulatory cells, including regulatory T cells (T_{regs}) [4], myeloid-derived suppressor cells (MDSCs) [5], regulatory B cells (B_{regs}) [6], tolerogenic dendritic cells and mesenchymal stromal cells (MSCs) [7] are responsible for orchestrating this tolerance [8]. Immune-regulatory cells play a central role in the pathophysiology of different diseases. For example, recent evidence suggests that cancer cells can hijack immune tolerance and impair cancer immunity through recruitment of MDSCs and T_{regs}, creating an immunosuppressive cancer micro-environment [9]. Conversely, auto-immune diseases and graft-versus-host disease

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(GVHD) can originate from the qualitative and quantitative deficiencies of immune-regulatory cells such as T_{regs} [10] and B_{regs} [11]. Most recently, adoptive cellular therapies with MSCs [12,13] and T_{regs} [14,15] have been used in clinical trials to prevent or treat GVHD, with promising outcomes.

Immune-regulatory cells have classically been characterized by their *in vitro* ability to suppress lymphocyte proliferation in a mixed lymphocyte reaction (MLR). However, because of inherent variability in the MLR, this approach does not allow quantitative comparisons from one experiment to the next or between laboratories. Also, peripheral blood mononuclear cells (PBMCs) instead of purified T cells are often used as responder cells in MLRs, which can produce additional unpredictable variability. Furthermore, the conventional proliferation suppression assay [16] requires up to 96 h, allowing for significant proliferation of the responder cells [17]. In the setting of adoptive cellular therapy, a rapid potency assay is needed to release functionally validated cell products in a timely fashion [18]. In addition, a standardized quantitative assay to permit objective comparisons of suppression among cell products and laboratories is essential. Rapid suppression assays (7−16 h) measuring T-cell activation markers CD154 [19] and CD69 on CD4 T cells have been developed for assessing T_{reg} function [20,21]. We sought to develop a rapid and easily applicable assay that uses a widely available suppressor cell line to serve as a standard suppressor cell for interlaboratory comparisons. Karpas 299 (K299), a human cell line derived from high-grade non-Hodgkin lymphoma, expresses CD4, CD25 and FoxP3. This unique cell line is both phenotypically and functionally similar to T_{regs}; it has been shown to suppress T-cell proliferation similar to T_{regs} [22,23]. For these reasons, we selected this cell line as a reference standard for a quantitative suppression assay. We describe the development and characteristics of an assay to evaluate the suppression potency of bone marrow−derived mesenchymal stromal cells (BM-MSC), with the use of the K299 cell line as a reference standard.

Methods

CD4 T-cell isolation and storage

PBMCs from healthy volunteers were prepared by use of Ficoll-Hypaque density gradient centrifugation (Organon Teknika). CD4 T cells were isolated from healthy donor PBMCs by use of an automated cell separator (RoboSep: Stem Cell Technologies). Healthy donor PBMCs and isolated CD4 T cells were cryopreserved in Roswell Park Memorial Institute (RPMI)-1640 medium (Life Technologies) with 20% fetal bovine serum (FBS) and a final concentration of 10% dimethyl sulfoxide, according to standard protocol; vials were stored in liquid nitrogen until further use. Written informed consent from all subjects was obtained in accordance with the Declaration of Helsinki for the use of samples for research protocol approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute.

BM-MSC isolation and expansion

BM-MSCs were expanded from BM aspirates collected from healthy volunteers at the Hematology Branch, National Heart, Lung, and Blood Institute, or in the Department of Transfusion Medicine, National Institutes of Health. The BM aspirates were plated in 75-cm^2 flasks in MSC medium consisting of minimum essential medium-α (Life Technologies) supplemented with 20% FBS (Sigma-Aldrich). Nonadherent cells were removed after 24 h, and the adherent cells were cultured for approximately 14 days with twice-weekly MSC medium changes. The cells were harvested with the use of 0.05% trypsin-ethylene diamine tetra-acetic acid (EDTA) (Life Technologies) when 70% confluence was achieved and used for further expansion. The cells were plated at a density of $4 \times 10^3/cm^2$ in four-layer cell factory flasks (Thermo Scientific Nunc Cell Factory Systems) in MSC medium. Serial passages were obtained once the cells reached 70% confluence in successive MSC expansions. At the third or fourth passage, cells were cryopreserved in freezing medium according to standard protocol and stored in liquid nitrogen until further use. Written informed consent from all subjects was obtained in accordance with the requirement of the Institutional Review Board of the National Heart, Lung, and Blood Institute and Department of Transfusion Medicine (DTM), National Institutes of Health. We also evaluated the clinical-grade BM-MSCs provided by DTM, Clinical Research Center, National Institutes of Health and Children's National Medical Center. The clinical-grade BM-MSCs at Children's National Medical Center were manufactured through the use of the Quantum Cell Expansion System (Terumo BCT), according to the protocol as previously reported [24,25]. Briefly, 4 to 18 h before the initiation of BM-MSC expansion, a cell expansion set was loaded onto the device and the system was primed with phosphate-buffered saline, and 5 mg of fibronectin was loaded into the system. Either 25 mL of filtered bone marrow or 2.0 to 3.5×10^7 of expanded cells were then loaded into the bioreactor. Cells were allowed to adhere and were then perfused in 5%