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Inducible indoleamine 2,3-dioxygenase 1 and programmed death ligand 1 expression as the potency marker for mesenchymal stromal cells

QINGDONG GUAN[1,2,3,4], YUN LI[1], TANNER SHPIRUK[1,3], SWAROOP BHAGWAT[2,3] & DONNA A. WALL[1,2,3,5]

[1]*Department of Pediatrics & Child Health, University of Manitoba, Winnipeg, Canada,* [2]*Department of Immunology, University of Manitoba, Winnipeg, Canada,* [3]*Cellular Therapy Laboratory, CancerCare Manitoba, Winnipeg, Canada,* [4]*The Institute of Clinical Research and Translational Medicine, Gansu Provincial Hospital, Lanzhou, China, and* [5]*Blood and Marrow Transplant/Cellular Therapy, Division of Haematology/Oncology, The Hospital for Sick Children and the University of Toronto, Toronto, Canada*

Abstract

Aim. Establishment of a potency assay in the manufacturing of clinical-grade mesenchymal stromal cells (MSCs) has been a challenge due to issues of relevance to function, timeline and variability of responder cells. In this study, we attempted to develop a potency assay for MSCs. *Methods.* Clinical-grade bone marrow–derived MSCs were manufactured. The phenotype and immunosuppressive functions of the MSCs were evaluated based on the International Society for Cellular Therapy guidelines. Resting MSCs licensed by interferon (IFN)-γ exposure overnight were evaluated for changes in immune suppression and immune-relevant proteins. The relationship of immune-relevant protein expression with immunosuppression of MSCs was analyzed. *Results.* MSC supressed third-party T-lymphocyte proliferation with high inter-donor and inter-test variability. The suppression of T-lymphocyte proliferation by IFN-γ–licensed MSCs correlated with that by resting MSCs. Many cellular proteins were up-regulated after IFN-γ exposure, including indoleamine 2,3-dioxygenase 1 (IDO-1), programmed death ligand 1 (PD-L1), vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) and bone marrow stromal antigen 2 (BST-2). The expression levels of IDO-1 and PD-L1 on licensed MSCs, not VCAM-1, ICAM-1 or BST-2 on licensed MSCs, correlated with MSC suppression of third-party T-cell proliferation. *Conclusion.* A flow cytometry–based assay of MSCs post–IFN-γ exposure measuring expression of intracellular protein IDO-1 and cell surface protein PD-L1 captures two mechanisms of suppression and offers the potential of a relevant, rapid assay for MSC-mediated immune suppression that would fit with the manufacturing process.

Key Words: *cell manufacturing, mesenchymal stromal cells, potency assay*

Introduction

Mesenchymal stromal cells (MSCs), also called multipotent mesenchymal stromal cells or stromal progenitors of mesodermal cells, are being tested widely as a cellular therapy for autoimmune diseases and other immunologic diseases [1–4]. However, a lack of a reliable, rapid and relevant potency assay to evaluate the immunologic function of MSC products slows standardization of MSC cell products and ultimately slows development of new treatments based on these cells.

MSCs are found *in situ* within all mammalian supportive stromal tissue compartments, including bone marrow and adipose tissue [5,6], and are thought to be a key cell source for tissue repair and regeneration. MSCs can secrete/express several immunoregulatory factors that can mediate suppression of T-cell function by both cell contact-dependent and -independent mechanisms [5,7,8]. MSCs cultured *in vitro* maintain a stable phenotype and allow for the propagation of large numbers of cells [5,7]. MSCs have potential clinical utility in suppressing uncontrolled immune responses by providing negative regulation through multiple pathways, including cell contact (e.g., programmed death ligand 1 [PD-L1], CD200 and intercellular adhesion molecule 1 [ICAM-1]) and release of mediators (e.g., indoleamine 2,3-dioxygenase 1 [IDO-1], interleukin [IL]-10 and prostaglandin E2

Correspondence: **Qingdong Guan**, PhD, MD, Cellular Therapy Laboratory, CancerCare Manitoba, MS773M, 820 Sherbrook St, Winnipeg, MB R3A 1R9, Canada. E-mail: qdguan@gmail.com; Correspondence: **Donna A. Wall**, MD, 9210 Black Building, 555 University Ave, Toronto, ON M5G 1X8, Canada. E-mail: donna.wall@sickkids.ca

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[PGE2]). These pathways lead to suppression of both the proliferation and function of effector $CD4^+$ and $CD8^+$ T cells, while promoting of the development of regulatory T cells (Tregs) [1,6].

Manufacturing of cell therapeutic products requires a potency assay to confirm efficacy and to standardize cell preparations. In the case of MSCs, this has been a challenge [9]. The benchmark potency assay for MSC-mediated immunosuppression is the inhibition of mitogen/antigen-stimulated third-party responder T-cell proliferation. This assay involves a titration of MSCs co-cultured with carboxyfluorescein succinimidyl ester (CFSE)–labeled responder T lymphocytes for 4–5 days. Although this assay directly measures the immunosuppressive abilities of MSC, it has poor inter- and intra-patient reproducibility, due to not only differences in products but also variable healthy donor T-cell responses to stimulation [9]. This test is time- and resource-intensive, usually requiring 4–5 days and high technologist time requirements [9]. The International Society for Cellular Therapy (ISCT) recommended a standardized approach of immunologic characterization of MSCs in 2013 [10], which included IDO-1 expression as part of an *in vitro* licensing MSC assay by interferon (IFN)-γ ± tumor necrosis factor (TNF). In 2016, the ISCT outlined 50 candidate proteins that could potentially serve as potential "universal" markers of strength for MSCs developed for their suppressive functionalities, including HLA-DR, ICAM-1, vascular cell adhesion molecule 1 (VCAM-1), CD40, C-X-C motif chemokine ligand 10 (CXCL10) and vascular endothelial growth factor (VEGF) [8]. However, it is unknown whether the expression of IDO-1 and other mediators involved in MSC immunosuppression correlates with MSC suppression of the inflammatory immune response, thus preventing their use as potency markers. To address this gap, we analyzed the changes in protein expression of resting MSCs and IFN-γ–licensed MSCs to identify potential candidate molecules for potency testing. Our previous work using liquid chromatography–mass spectrometry (LC-MS) of whole cell pellets identified 169 proteins dramatically upregulated in IFN-γ–licensed MSCs [11]. In this work, we have now correlated the expression of a number of proteins identified in our screening study [11] or suggested by the ISCT with the MSC ability to suppress third-party T-lymphocyte proliferation through working on a panel of clinical-grade MSCs [8].

Methods

MSC expansion

Bone marrow aspirates were obtained from the posterior iliac crest of 13 healthy volunteers following informed consent, according to guidelines approved by institution Research Ethics Board. Bone marrow–derived MSC cultures were established and maintained in a Good Manufacturing Practice (GMP) facility as described previously [12]. Briefly, fresh bone marrow cells were seeded into T175 flasks at 1000–3000 cells/cm^2 in complete growth culture media (Dulbecco's Modified Eagle's Medium [DMEM; Lonza] with 5% human platelet lysate [Mill Creek Life Sciences], 1% glutamax [Life Technologies] and gentamicin [5 μg/mL; Life Technologies]) for 3 days. Then unattached cells were removed and flasks were washed with phosphate-buffered saline (PBS) twice. The adherent cells were further cultured until confluence reached 70–80%, and then they were harvested with Tryple Select (Life Technologies).

Licensing MSCs

MSCs were seeded into the cell flasks in the complete media at 2000–3000 cells/cm^2. When the cell confluence reached 70–80%, human recombinant IFN-γ (eBioscience) was added into the MSC culture media at 30 ng/mL for 20 h. Then MSCs were harvested for analysis.

Phenotyping of MSCs

The immunophenotype of expanded MSC was characterized using flow cytometry as we described previously [11], and showed positive expression (≥95%) of CD90, CD73 and CD105, and negative expression (<2%) of CD45, CD34, CD14, CD19 and HLA-DR, which met the ISCT standard [13] for cell identity (Supplementary Figure S1). Using the same methods, phycoerythrin-cyanine 7 (PE-Cy7), fluorescein isothiocyanate (FITC), allophycocyanin-cyanine 7 (APC-Cy7) or Peridinin chlorophyll-Cyanine 5.5 (Percp-Cy5.5) labeled antibodies against PD-L1, bone marrow stromal antigen 2 (BST-2), ICAM-1 and VCAM-1 (eBioscience) were used to evaluate the expression of PD-L1, BST-2, ICAM-1 and VCAM-1 on the cell surface of MSCs. To evaluate the expression of intracellular proteins IDO-1, MSCs were fixed and permeabilized using eBioscience intracellular fixation/permeabilization buffer. Cells were then stained with PE-labeled anti-IDO-1 for 20 min. After staining, cells were acquired and analyzed using flow cytometry (FACS Canto II, BD Biosciences) and FlowJo software (TreeStar).

T-cell proliferation and expansion assay

The suppressive effect of MSCs on lymphocyte proliferation and T-cell expansion was confirmed as described previously [14]. Briefly, third-party peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors and labeled with CFSE. Then the