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Galectin-1 is a local but not systemic immunomodulatory factor in mesenchymal stromal cells

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) have powerful immunosuppressive activity. This function of MSCs is attributed to plethora of the expressed immunosuppressive factors, such as galectin-1 (Gal-1), a pleiotropic lectin with robust anti-inflammatory effect. Nevertheless, whether Gal-1 renders or contributes to the immunosuppressive effect of MSCs has not been clearly established. Therefore, this question was the focus of a complex study. *Methods.* MSCs were isolated from bone marrows of wild-type and Gal-1 knockout mice and their *in vitro* anti-proliferative and apoptosis-inducing effects on activated T cells were examined. The *in vivo* immunosuppressive activity was tested in murine models of type I diabetes and delayed-type hypersensitivity. *Results.* Both Gal-1-expressing and -deficient MSCs inhibited T-cell proliferation. Inhibition of T-cell proliferation by MSCs was mediated by nitric oxide but not PD-L1 or Gal-1. In contrast, MSC-derived Gal-1 triggered apoptosis in activated T cells that were directly coupled to MSCs, representing a low proportion of the T-cell population. Furthermore, absence of Gal-1 in MSCs did not affect their *in vivo* immunosuppressive effect. *Conclusions.* These results serve as evidence that Gal-1 does not play a role in the systemic immunosuppressive effect of MSCs. However, a local contribution of Gal-1 to modulation of T-cell response by direct cell-to-cell interaction cannot be excluded. Notably, this study serves a good model to understand how the specificity of a pleiotropic protein depends on the type and localization of the producing effector cell and its target.

Key Words: *apoptosis, delayed type hypersensitivity, galectin-1, immunosuppression, mesenchymal stromal cells, type I diabetes*

Introduction

Mesenchymal stromal cells (MSCs) represent multipotent adult stem cells. Because of their plasticity in differentiation to mesodermal lineages such as bone, cartilage and adipose tissue and because of their powerful immunosuppressive function [1], MSCs have recently been implicated in various therapeutic approaches [2]. Various immunological pathologies can be efficiently treated by MSC transplantation [3–6], and therefore, the exact nature of their anti-inflammatory function must be correctly defined. Allogeneic and autoimmune/inflammatory responses are dampened [7] by identified soluble and cell-bound factors, such as transforming growth factor (TGF)-β, nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), hepatocyte growth factor (HGF), human leukocyte antigen (HLA)-G, programmed death ligand 1 (PD-L1) [8,9]; however, the precise role of the individual factors and other mechanisms in the immunosuppressive activity has not yet been suitably clarified.

Recently, the production and secretion of an immunosuppressive factor, galectin-1, has also been identified in MSCs [10–14]. Although Gal-1 has been suggested to contribute to MSCs' immunosuppressive potential [14–16], its true function in their anti-inflammatory activity has been poorly examined.

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Nevertheless, the immunoregulation by recombinant, soluble Gal-1 has been confirmed in multiple *in vitro* and *in vivo* experimental systems [17]. It has been revealed that Gal-1 reverses the clinical symptoms in animal models of autoimmune and chronic inflammation [18] by various mechanisms, affecting T cells [19], dendritic cells [20], macrophages [21] and extravasation of leucocytes [22]. Gal-1 induces cell death of activated T cells either in soluble [23–25] or in cell-bound form [26], with an increased selectivity toward Th1 and Th17 cells *in vitro* [27], a phenomenon that explains its therapeutic effect in autoimmune inflammatory animal models.

Despite the tremendous *in vitro* and *in vivo* data confirming the immunoregulatory functions of Gal-1, the exact role of Gal-1 in MSCs' immunosuppressive functions has not been thoroughly determined. In this study, we have clearly defined that Gal-1 does not contribute to the systemic immunoregulatory functions of MSCs because the *in vitro* inhibition of T-cell proliferation was not affected by the presence or absence of Gal-1 in MSCs. Moreover, we show that the therapeutic impact of MSCs on autoimmune diabetes and delayed-type hypersensitivity (DTH) in animal models does not depend on their Gal-1 expression. Nevertheless, activated T cells in an intimate cell-to-cell interaction with MSCs undergo apoptosis via a Gal-1-dependent mechanism. Our results may serve an explanation for an important biological problem: how a pleiotropic protein (Gal-1) exerts its specific function (immunosuppression) depending on the type (MSCs) and localization (systemic or tissue) of the producing effector cell and communication with its target (T cells).

Methods

Cells

Bone marrow MSCs were isolated from male C57BL/6 or Gal-1 knockout (strain: B6.Cg-*Lgals1*^{tm1Rob}/J, 006337, Jackson Laboratory) mice as described previously [28]. Briefly, bone marrow cells were collected from femurs and tibias of 8- to 10-week-old male mice and seeded in plastic flask in Dulbecco's Modified Eagle's Medium/Ham's F-12 medium (Invitrogen), complemented with 10% fetal bovine serum (FCS, Invitrogen), 10% horse serum (Invitrogen), 50 U/mL penicillin, 50 μg/mL streptomycin (Sigma-Aldrich), and 2 mmol/L L-glutamine (Invitrogen). Non-adherent cells were removed after 72 h, and then the cell culture medium was changed twice a week. All MSC cultures were free from hematopoietic contamination and used after 10–15 passages. Wild-type and Gal-1-deficient MSCs were designated as wtMSCs and MSC$^{gal1-/-}$, respectively. The cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco, Invitrogen) supplemented with fetal calf serum (10% FCS, Gibco, Invitrogen), penicillin (100 IU/mL), streptomycin (100 μg/mL) and L-glutamine (2 mmol/L) and characterized as described previously [28].

Murine lymphocytes were isolated from the inguinal and mesenteric lymph nodes or the spleen. Homogenized lymphoid tissues were cleared up from erythrocytes by lysis with ammonium-chloride-potassium buffer (150 mmol/L NH_4Cl, 10 mmol/L $KHCO_3$, 0.1 mmol/L ethylenediaminetetraacetic acid). T cells were purified with magnetic bead separation using BD IMag Mouse T Lymphocyte Enrichment Set-DM and BD IMagnet Cell Separation Magnet (BD Biosciences) according to the manufacturer's instructions and were then tested for purity with flow cytometry using anti-CD3 fluorescein isothiocyanate (FITC; BioLegend). The enriched cell population contained 98% of $CD3^+$ T cells (Supplementary Figure S1), which were then cultured in RPMI-1640 (Gibco, Invitrogen) supplemented with FCS (10%), penicillin (100 IU/mL), streptomycin (100 μg/mL), L-glutamine (2 mmol/L) and β-mercaptoethanol (50 μmol/L). All cells were kept in a humidified incubator with 5% CO_2 at 37°C.

Proliferation assay

MSCs were plated into 96-well cell culture plates (Costar, Corning) at different densities and allowed to adhere to the plastic surface. After 3 h, lymph node cell-suspension (2×10^5/well) or enriched T cells (10^5/well) were added to the wells. Lymph node cells and T cells were stimulated in the MSCs/lymphocyte co-cultures for 72 h with 7.5 μg/mL Concanavalin A (Con A) and with Dynabeads Mouse T-activator CD3/CD28 (2 μL beads/well, Invitrogen), respectively. For the thymidine incorporation assay, cells were pulsed with 1 μCi of ^{3}H-thymidine (American Radiolabeled Chemicals) for 16 h before harvesting, and then the incorporated ^{3}H-thymidine was measured with liquid scintillation counter. Alternatively, cell proliferation was measured based on labeling the T cells with CellTrace CFSE Cell Proliferation Kit (2.5 μmol/L, Molecular Probes, Life Technologies) before co-cultures according to the manufacturer's instruction. T cells blocked with mitomycin C (8 μg/mL) served as non-proliferating control cells. Fluorescence intensity of the cellular carboxyfluorescein succinimidyl ester (CFSE) was analyzed with flow cytometry.

MSC-derived factors responsible for the inhibition of T-cell proliferation were analyzed as follows: (i) For the analysis of the anti-proliferative effect of MSC-derived soluble factors, Transwell inserts (pore size 0.4 μm, Costar, Corning) were used in 24-well plates (Costar, Corning). (ii) The inhibition of PD-L1 was carried out by adding neutralizing antibody

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