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Enhancement of the immunoregulatory potency of mesenchymal stromal cells by treatment with immunosuppressive drugs

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Abstract

Background aims. Multipotent mesenchymal stromal cells (MSCs) are distinguished by their ability to differentiate into a number of stromal derivatives of interest for regenerative medicine, but they also have immunoregulatory properties that are being tested in a number of clinical settings. *Methods.* We show that brief incubations with rapamycin, everolimus, FK506 or cyclosporine A increase the immunosuppressive potency of MSCs and other cell types. *Results.* The treated MSCs are up to 5-fold more potent at inhibiting the induced proliferation of T lymphocytes *in vitro*. We show that this effect probably is due to adsorption of the drug by the MSCs during pre-treatment, with subsequent diffusion into co-cultures at concentrations sufficient to inhibit T-cell proliferation. MSCs contain measurable amounts of rapamycin after a 15-min exposure, and the potentiating effect is blocked by a neutralizing antibody to the drug. With the use of a pre-clinical model of acute graft-versus-host disease, we demonstrate that a low dose of rapamycin-treated but not untreated umbilical cord–derived MSCs significantly inhibit the onset of disease. *Conclusions.* The use of treated MSCs may achieve clinical end points not reached with untreated MSCs and allow for infusion of fewer cells to reduce costs and minimize potential side effects.

Key Words: *immunoregulation, immunosuppression, mesenchymal stromal cells, rapamycin, sirolimus*

Introduction

Mesenchymal stromal cells (MSCs) are defined by their ability to differentiate into osteoblasts, chondrocytes and adipocytes [1], but much of the current clinical interest is aimed at exploiting their immunoregulatory properties [2,3]. A prevailing hypothesis is that MSCs exert their beneficial effects on tissue regeneration, not through replacement of damaged cells, but by providing anti-inflammatory signals and growth factors that promote the regeneration process [4]. MSCs have been shown to actively suppress the function or differentiation of all immune cell types tested (monocytes, dendritic cells, B and T lymphocytes and natural killer cells), and multiple mechanisms appear to be involved, including cell-cell contact and secretion of agents such as prostaglandins, transforming growth factor, indoleamine oxidase, TSG6, or heme oxygenase [4–7].

Hundreds of clinical trials have been registered that involve infusion of autologous, second-party or third-party MSCs derived from bone marrow, adipose tissue or umbilical cord (Clinicaltrials.gov). Many of the applications are directed at inhibition of undesirable immune responses such as acute graft-versus-host disease (aGVHD) after hematopoietic stem cell transplantation, rejection of solid-organ transplants or autoimmune diseases such as multiple sclerosis and Crohn's disease [8,9]. Current protocols use relatively small numbers of MSCs per treatment, on the order of 1×10^6/kg body weight [10,11]. It is surprising that positive responses have been reported from such low doses, particularly because tracking experiments indicate that MSCs

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act in a transient manner, and few are detected even several days after infusion [12]. The ability of intravenously injected MSCs to localize at sites of tissue damage is one mechanism by which such limited cell numbers could promote repair [13], and the release of exosomes with immunoregulatory potential could allow for disseminated effects [14]. Although the properties of MSCs make them attractive for treating inflammatory conditions, they can also be co-opted by tumors, in which their trophic and immunosuppressive functions could promote disease [15].

There is interest in exploiting the homing ability of MSCs to use them as drug delivery systems and, to this end, they have been genetically modified to produce cytokines and enzymes for anti-cancer pro-drug conversion [16–18]. Although MSCs are not thought to persist long after infusion, any genetic manipulation introduces the potential for oncogenic or other undesirable changes and complicates their clinical application. In the current report, we describe a method by which the immunoregulatory potency of MSCs, as well as other cell types, can be increased without genetic modification simply by brief exposure to immunosuppressive drugs (ISDs). The ability to combine the homing and suppressive activities of MSCs with ISDs has the potential to increase the therapeutic potential of this experimental cell therapy and to reduce production costs if fewer MSCs are required per treatment.

Methods

MSCs and fibroblasts

Umbilical cord MSCs (UC-MSCs) were generated as described [19] from fresh cord segments collected from full-term births by NHS Cord Blood Bank (NHS-CBB) staff (Colindale, United Kingdom) after informed ethical consent was obtained. Cells were cultured at 37°C with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM):F12 (Lonza) with penicillin/streptomycin (Sigma) and 10% fetal calf serum (FCS) (Life Technologies) and were passaged with the use of 0.125 % trypsin (Sigma). Bone marrow (BM)-MSCs were generated through the use of standard methods from frozen aliquots of mononuclear cells (MNCs) purchased from DV Biologics. Briefly, the MNCs were thawed and plated in a tissue culture flask with the medium as above. After colonies of MSC-like cells were observed, they were passaged, and expanded and their phenotype was assessed by means of flow cytometry for the presence and absence of surface markers (cluster of differentiation [CD]73, CD90, CD45 and CD34; Biolegend) as described [19].

The HS27 human foreskin fibroblast cell line (ECACC) and primary human dermal fibroblasts (TCS Cellworks) were grown under the conditions used for MSCs. Human umbilical vein endothelial cells (HUVECs) were purchased from ECACC and Life Technologies and expanded in endothelial cell growth medium (TCS Cellworks).

Mononuclear cells

Adult peripheral blood (AB) MNCs from consenting platelet donors were prepared from apheresis cones [20] provided by NHSBT. The contents of the cones were diluted with calcium and magnesium-free phosphate-buffered saline (PBS) and were centrifuged over Lymphoprep (Axis-Shield); cells at the interphase were then subjected to a 200*g*, 12-min spin to deplete platelets. Aliquots were frozen in 10% dimethyl sulfoxide (DMSO), 20% FCS and 70% DMEM:F12 and stored in a −150° freezer (Panasonic). Purified CD4+ responder T-cell populations were prepared by means of incubation of MNCs with biotinylated antibodies against CD8, CD14, CD15, CD16, CD19, CD56 and HLA-DR (Biolegend); depletion with streptavidin-coated magnetic beads (Sigma) was then performed. CD4+ populations were depleted of T-regulatory cells (Tregs) with the use of magnetic beads to remove CD25+ cells (Miltenyi Biotec), with the efficiency tested by means of staining for CD25 and CD127, and for FoxP3 after perm/fix treatment (eBioscience) and incubation with PE-labeled anti-FoxP3 (eBioscience clone PCH101) (Supplementary Figure 1). Antigen-presenting cells (APC) were generated through magnetic bead depletion of MNCs with biotinylated anti-CD2 and anti-CD3 and used in a 1:1 ratio with CD4+ T cells as described [21]. For cell proliferation assays, MNCs and lymphocytes were labeled with carboxyfluorescein succinimidyl ester (CFSE) (Sigma) as described [21].

Drug treatment and suppression assays

Rapamycin was purchased as a 2.5 mg/mL DMSO solution; cyclosporine A (CsA), everolimus, azathioprine, mycophenolate mofetil and FK-506 monohydrate (all from Sigma) were dissolved in DMSO, with aliquots stored at −20° until use. For drug pretreatment of MSCs and fibroblasts, cells were cultured in T25 flasks with 5 mL of standard growth medium until near confluence. Drug stock solutions were diluted in DMSO such that they were added to the cultures at ≤10 μL. These volumes of DMSO were shown to have no effect on MSC function in control experiments (data not shown). At times indicated in the text, the medium was removed from