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Research paper

Effects of mesenchymal stromal cells on human myeloid dendritic cell differentiation and maturation in a humanized mouse model

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ABSTRACT

Mesenchymal stromal cells (MSCs) have shown promise as cellular therapy in allogeneic transplantation, although the precise mechanisms underlying their benefit in clinical trials are difficult to study. We previously demonstrated that MSCs exert immunoregulatory effects in mouse bone marrow-derived dendritic cell (DC) culture. Since mouse studies do not reliably reproduce human events, we used a humanized mouse model to study the immunomodulatory effects of human MSCs on human DC immunobiology. Humanized mice were established by injection of cord blood $CD34^+$ cells into NOD.Cg-Prkdc^{scid} Il2rg$^{tm1Wjl/SzJ}$ (NOD *scid* gamma, NSG) mice. Human cells were detected in the mouse bone marrow, blood, and spleen 12 weeks after transplantation. Human DCs were differentiated from humanized mouse bone marrow cells during human MSC co-culture. MSCs inhibited DC differentiation and kept DCs in an immature state as demonstrated by phenotype and function. In conclusion, humanized mouse models represent a useful method to study the function of human MSCs on human DC immunobiology.

1. Introduction

Dendritic cells (DCs) constitute a heterogeneous population of professional bone marrow-derived antigen presenting cells with potential to induce both immunity and tolerance (Banchereau et al., 2000; Steinman et al., 2003). DCs can be isolated freshly from blood, lymphoid or non-lymphoid organs, or generated from hematopoietic or more immediate precursors from different sources. Because of their tolerogenic capacity, DCs have been proposed as potential immunotherapy for autoimmune disease and allotransplantation in humans.

Mesenchymal stromal cells (MSCs) are adult multi-potent cells located within the stroma of bone marrow (BM) but have also been isolated from virtually all postnatal organs. MSCs differentiate into cell types of mesenchymal origin, produce important growth factors and cytokines that may facilitate repair of damaged tissues, and in addition, have immunoregulatory function (McTaggart and Atkinson, 2007; Nauta and Fibbe, 2007). MSCs appear to be immunologically privileged, as they do not express MHC class II or T cell co-stimulatory molecules and therefore escape recognition and lysis by nature killer (NK) cells and cytotoxic lymphocytes, making them attractive candidates for cellular therapy in allogeneic transplantation. Infusion of BM MSCs successfully treated severe, steroid-resistant acute graft-versus-host disease (Le Blanc et al., 2008). More recently, compared to standard anti-IL-2 receptor antibody induction, the use of autologous MSCs resulted in lower risk of acute rejection, decreased risk of opportunistic infections, and better graft function in recipients of live donor kidney transplants (Tan et al., 2012). The precise immunologic mechanisms by which these outcomes were achieved, however, have not been fully elucidated.

Previously we demonstrated an immunoregulatory effect of mouse MSCs in mouse BM-derived DC culture (Huang et al., 2009, 2010), which may represent one of several potential mechanisms that explain the beneficial effects of MSCs in clinical trials. However, mouse studies do not always reliably reproduce human events, and humanized mouse models represent an alternative approach to study the mechanisms underlying successful immunotherapies in humans. The objective of this project was to develop a humanized mouse model for the study of the immunomodulatory effects of MSCs on human DC immunobiology.

2. Materials and methods

2.1. Generation of humanized mice

The detailed protocol has been published previously (Shultz et al., 2005; Ishikawa et al., 2005). Briefly, NOD.Cg-Prkdc^{scid} Il2rg$^{tm1Wjl/SzJ}$ (NOD *scid* gamma, NSG) mice were purchased from The Jackson Laboratory (stock 005557, Bar Harbor, ME) and bred in specific pathogen-free

Abbreviations: DCs, Dendritic cells; MSCs, Mesenchymal stromal cells; NSG, NOD *scid* gamma; BM, Bone marrow; mAbs, Monoclonal antibodies; HSCs, Hematopoietic stem cells; LPS, Lipopolysaccharide; PBMCs, Peripheral blood mononuclear cells; NK cells, Nature killer cells.

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conditions. Human umbilical cord blood mononuclear cells were enriched by density gradient centrifugation (Fercoll; Sigma-Aldrich, St. Louis, MO). Hematopoietic stem cells (HSCs) expressing CD34 were isolated using the CD34 MicroBead kit (Miltenyi Biotech, San Diego, CA) according to the manufacturer's instructions. Four-week-old NSG male mice were irradiated at 250 cGy, and 2×10^5 purified human $CD34^{pos}$ cells were administered intravenously by tail vein injection to generate humanized NSG mice (4-week old). Twelve weeks after transplantation, cell populations were monitored by flow cytometry in conjunction with the following monoclonal antibodies (mAbs): human CD45, Lineage 1 (Lin1), CD3, CD4, CD19, CD11c, CD123, HLA-DR, and mouse CD45. Protocols were approved by the Johns Hopkins Animal Care and Use Committee.

2.2. Mesenchymal stromal cell culture

Human BM MSCs were purchased under the auspices of the NIH grant "Preparation and Distribution of Adult Stem Cells" (P40 RR017447, PI-Darwin Propock, MD PhD) and expanded with the mesenchymal stem cell growth kit (ATCC, Manassas, VA). The final concentrations for each component in complete MSC growth medium were rh FGE basic (125 pg/ml), rh IGF-1 (15 ng/ml), fetal bovine serum (7%) and L-alanyl-L-glutamine (2.4 mM). MSCs were cultured according to the methods previously reported for culturing kidney-derived mesenchymal stromal cells, with slight modification (Huang et al., 2010). MSCs were plated in 25-cm^2 flasks until confluence in standard MSC medium. Each passage was 5 days in duration. MSCs within 20 passages were used for experimentation.

2.3. Bone marrow-derived DC culture

BM-derived DCs were generated as described previously (Inaba et al., 1992). Briefly, BM cells from NSG mice were cultured for 6 days in RPMI 1640 medium containing 10% heat-inactivated FBS, 50 μM 2-Mercaptoethanol (Sigma-Aldrich), 2 mM glutamine, 10 mM HEPES, 1% non-essential amino acids 4 ml and 100 U/ml penicillin and 100 μg/ml streptomycin supplemented with 50 ng/ml human GM-CSF and 10 ng/ml human IL-4 (R&D, Minneapolis, MN). Half of the supernatant was replaced with fresh cytokine-containing medium every 2 days. Lipopolysaccharide (LPS; 1 μg/ml) was added for an additional 24 h to induce DC activation/maturation. To study the effect of MSCs on DC differentiation and maturation, MSCs were first treated with mitomycin (50 μg/ml) at 37 °C for 30 min to inhibit their proliferation, washed, and added into DC culture (MSC:DC = 1:10) using Corning Transwell (pore size 0.4 μm; no cell contact; Corning T, Lowell, MA) or mixed chamber systems (cell–cell contact). DCs were identified as $CD11c^{pos}$ cells, with apoptosis/necrosis (7-AAD, Annexin V), differentiation (absolute cell number), and activation/maturation (MHC class II, CD80, CD86) analyzed by flow cytometry.

2.4. Antigen uptake assay

Quantitative analysis of DC endocytosis was performed as described previously (Huang et al., 2009), with minor modifications. Briefly, 5×10^5 DCs with 0.1 mg/ml FITC-dextran (MW 42000; Sigma-Aldrich) were incubated at either 37 °C or 4 °C (as negative control) for 1 h. Endocytosis was stopped by cold wash in 0.1% sodium azide/1% FBS/PBS. Cells were stained with anti-CD11c, followed by flow cytometric analysis. Positive FITC-dextran fluorescence indicated DC antigen uptake.

2.5. Cytokine TNFα measurement

Seven-day supernatants from DCs cultured alone, DCs cultured directly with MSCs, and DCs cultured with MSCs in transwell plates were subjected to TNF-α cytokine analysis by Luminex multiplex cytokine detection system, according to the manufacturer's protocol (Millipore, Darmstadt, Germany).

2.6. Statistical analysis

Data are presented as mean ± standard deviation. Results shown are representative of at least three independent experiments. Statistical comparisons between groups were performed by student's *t* and ANOVA tests, where appropriate. $P < 0.05$ was deemed significant. Analysis was accomplished using Prism 4.0 (GraphPad software, San Diego, CA).

3. Results

3.1. Human immune cells detected in the bone marrow, blood and spleen of humanized NSG mice

Compared with control NSG mice, human $CD45^{pos}$ cells were detected in the BM (3.8% vs. 34.6%; Fig. 1A, B), blood (0.03% vs. 57.5%; Fig. 1C, D), and spleen (0.6% vs. 63.7%, Fig. 1E, F) of humanized NSG mice (control vs. humanized NSG mice, respectively). Mouse $CD45^{pos}$ cells were also detected in the BM (60.7% vs. 27.4%; Fig. 1A, B), blood (89.6% vs. 37.5%; Fig. 1C, D) and spleen (76.0% vs. 3.5%; Fig. 1E, F) of control and humanized NSG mice, respectively. Human $CD4^{pos}$ T cells were 13.9% of total human $CD45^{pos}$ cells in the spleen (Fig. 2A, C), while human $CD8^{pos}$ T cells were 16.3% (Fig. 2B, C). Gated on lineage 1^{neg} and $HLA\text{-}DR^{pos}$ cells (Fig. 2D), $CD11c^{pos}$ myeloid DCs were 13.4% and $CD123^{pos}$ plasmacytoid DCs were 2.7% of the total population in the spleen (Fig. 2E, F).

3.2. Generation of human DCs from humanized NSG mouse bone marrow and effects of MSCs on DC generation

After cells from BM of humanized NSG mice were cultured *in vitro* for 6 days with human recombinant GM-CSF and IL-4, flow cytometry revealed $CD11c^{pos}$ cells (myeloid DCs) to be 79.5% of the lineage 1^{neg} and $HLA\text{-}DR^{pos}$ population (Fig. 3A), consistent with numbers achieved during mouse BM-derived DC culture. DC generation was decreased with MSC co-culture in both direct and transwell culture systems ($p < 0.05$; Fig. 3B). DC viability was improved with direct MSC co-culture ($p < 0.05$, Fig. 3C) before or after LPS stimulation, although no such effect was observed in transwell MSC co-culture. The higher DC viability was due to lower early apoptosis in direct DC-MSC co-culture ($p < 0.05$, Fig. 3D). There were no effects of MSC-DC co-culture on late apoptosis (Fig. 3E). Thus, human myeloid DCs can be generated successfully from humanized NSG mouse BM, and direct addition of MSCs to culture results in decreased DC generation but increased DC viability through reduction of early apoptosis.

3.3. MSC-DC co-culture altered DC phenotype and function

After direct MSC-DC co-culture, DC surface expression of CD80 was significantly increased both before and after LPS stimulation, while statistical significance was only demonstrated prior to LPS stimulation in the transwell plate co-culture system (Fig. 4A). DC surface HLA-DR expression was significantly decreased compared to control but only after direct MSC-DC co-culture and only after LPS stimulation (Fig. 4B). MSC-DC co-culture resulted in significantly less TNF-α production after LPS stimulation in both culture systems (Fig. 4C). TNF-α production was lower from DCs co-cultured with MSCs in transwell plates compared to that from DCs co-cultured with MSC directly, although this difference did not achieve statistical significance. DC antigen uptake was significantly increased after MSC-DC co-culture ($p < 0.0001$, Fig. 4D). Thus, MSC co-culture resulted in DCs with increased antigen uptake ability and CD80 expression, yet blunted HLA-DR expression and TNF-α production upon LPS stimulation.