Biochemical and Biophysical Research Communications 476 (2016) 15–20

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Contact-dependent abrogation of bone marrow-derived plasmacytoid dendritic cell differentiation by murine mesenchymal stem cells

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ARTICLE INFO

Article history:
Received 18 May 2016
Accepted 21 May 2016
Available online 24 May 2016

Keywords:
Dendritic cells
Toll-like receptor
Interferon-a
Mesenchymal stromal cell
Inflammation

ABSTRACT

Plasmacytoid dendritic cells (pDCs) are rare central regulators of antiviral immunity and unsurpassed producers of interferon-α (IFN-α). Despite their crucial role as a link between innate and adaptive immunity, little is known about the modulation of pDC differentiation by other bone marrow (BM) cells. In this study, we investigated the modulation of pDC differentiation in Flt-3 ligand (Flt3L)-supplemented BM cultures, using highly purified mesenchymal stem cells (MSCs) that were FACS-isolated from murine BM based on surface marker expression and used after in vitro expansion. Initial analysis revealed an almost complete inhibition of BM-derived pDC expansion in the presence of >2% MSC. This inhibition was cell contact-dependent and soluble factor-independent, as indicated by trans-well experiments. The abrogation of functional pDC development by MSCs was confirmed after TLR9 stimulation, revealing a complete, contact-dependent suppression of the IFN-a producing capacity of pDCs in Flt3L MSC BM co-cultures. MSC selectively inhibited pDC development in contrast to myeloid DC development, as indicated by the significantly increased numbers of myeloid DC in Flt3L-supplemented BM cultures. The absence of significant MSC-mediated inhibitory effects on myeloid DC differentiation was confirmed by additional experiments in GM-CSF/IL-4-supplemented BM cultures. In summary, we describe a novel contact-dependent immunomodulatory mechanism of MSC that targets the BM-derived expansion of functional pDCs.

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1. Introduction

Dendritic cells (DC) comprise a heterogeneous population of professional antigen-presenting cells and represent the most important cellular link between the innate and adaptive immune systems [1,2]. Plasmacytoid dendritic cells (pDCs) are unique cells of the hematopoietic system with respect to their capacity to produce unsurpassed amounts of interferon-α (IFN-α) upon Toll-like receptor 7 and 9 (TLR7 and 9) ligation [3,4]. IFN-α released by pDCs not only plays key roles in antiviral and tumoricidal immunity but is also a critical component in the pathogenic courses of various autoimmune diseases, such as systemic lupus erythematosus and psoriasis [4].

Functionally, IFN-α promotes the maturation and survival of myeloid DCs (mDCs), subsequent activation of NK cells and T lymphocytes, and differentiation of monocytes into cytotoxic cells, thus promoting cytotoxic immunity against tumors and viral pathogens [5,6]. Clinically, the TLR7 agonist imiquimod has demonstrated efficacy for the treatment of basal cell carcinoma, the most common form of skin cancer [7–9]. IFN-α is among the few cytokines that have been successfully established in routine clinical medicine, and it is used for the treatment of chronic viral hepatitis and different malignancies [10–13]. Thus, both pDCs and IFN-α play key roles in innate and adaptive immune responses against viruses and tumors as well as in certain autoimmune diseases.

Mesenchymal stem or stromal cells (MSCs) of the bone marrow (BM) are spindle-shaped stromal cells that can differentiate into adipocytes, chondrocytes, and osteocytes [14]. Moreover, MSCs also

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http://dx.doi.org/10.1016/j.bbrc.2016.05.108
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exhibit potent immunomodulatory and anti-inflammatory effects [15–17]. With respect to the interactions of MSCs with DCs, most reports have focused on the interactions of MSCs with mDCs or monocyte-derived DCs. In this context, MSCs have been reported to inhibit mDC maturation and T-cell stimulatory activity by reducing costimulatory surface molecule expression and promoting immunoregulatory IL-10 expression [16,18–20]. However, Aggarwal and Pittenger have reported reduced IL-10 production from pDCs in the presence of MSC [21]. Interestingly, most of the immunomodulatory effects mediated by MSCs are related to soluble MSC-derived factors such as prostaglandin E2, indoleamine 2,3-dioxygenase, soluble HLA-G, or IL-10; little is known about the potential contact-dependent immunomodulatory effects of these cells [15,22,23,23].

Currently, MSCs are of clinical interest as advanced therapy medicinal cellular therapeutics (ATMP) because of the potential for therapeutic applications in the areas of tissue regeneration as well as immunomodulation in the context of inflammatory and autoimmune diseases [15,24,25]. Although most conventional MSC isolation protocols rely on plastic adherence [26], such methods are limited by factors such as the presence of contaminating cells and reduced proliferative potential consequent to long-term expansion *in vitro* [26]. In the present study, we have incorporated a prospective MSC isolation method based on the co-expression of CD140a (PDGFR-a) and stem cell antigen 1 (SCA-1) and CD45/Ter119 negative BM to isolate highly-purified, defined MSCs for further experiments in accordance with a protocol originally developed by Morikawa et al. [27,28]. We recently demonstrated in a murine experimental cellular therapy study that these prospectively defined MSCs could inhibit *Klebsiella pneumoniae*-induced acute lung injury and improve survival from pneumonia [29].

In the present study, we have investigated the effects of prospectively purified murine MSCs on BM-derived pDC differentiation. We report herein a novel contact-dependent immunomodulatory mechanism by which MSCs can suppress functional pDC generation.

2. Materials and methods

2.1. Mice

Specific-pathogen-free C57BL/6 (C57BL/6NCrl) mice (body weight, 20–25 g) were purchased from Charles River Laboratories, GmbH (Sulzfeld, Germany) and maintained under specific-pathogen-free conditions. BM preparation from murine femurs and tibiae was approved by the regional authority board (#453_M).

2.2. MSC purification and in vitro expansion

Femora and tibiae were prepared as described previously, with minor modifications [27,29]. Bone fragments were collected and digested for 1 h at 37 °C in alpha-Modified Eagle's Medium (MEM) supplemented with L-Glutamine (PAN Biotech GmbH, Aidenbach, Germany), 10% fetal bovine serum (FBS; PAA Laboratories, GmbH, Cölbe, Germany), 1% penicillin/streptomycin (PAN Biotech), 3.92 U/ml collagenase (Wako Chemicals, Osaka Japan), 10 mM Hepes (Gibco, Darnstadt, Germany) and 3 mM $CaCl_2$. The resulting cell suspension was filtered through a 70-μm cell strainer (BD Falcon, Heidelberg, Germany) and collected by centrifugation at 400*g* for 5 min at 4 °C. Red blood cells were lysed using 155 mM NH_4Cl/10 mM $KHCO_3$ buffer (pH 7.4) and washed with Hanks's buffered saline solution (HBSS; PAN Biotech).

After digestion, leucocytes were depleted from the cell suspension using CD45 magnetic beads (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany) and remaining cells were stained with fluorochrome-labeled monoclonal antibodies, and sorted on a BD ARIA III cell sorter (Becton Dickinson, San Jose, CA, USA). PαS MSCs, which were defined as positive for CD140a and Sca-1 and negative for CD45 and TER119, were sorted to a final purity of >98% and expanded in PureCoat Amine plates/flasks (BD Falcon) with MesenPRO RS™ Medium (Gibco). The medium was changed every 3–7 days depending on cell growth, and MSCs were expanded for 20–40 days prior to use in co-culture assays.

2.3. DC generation

DCs were generated as previously described with minor modifications [30,31]. Briefly, BM cells were removed from the femurs and tibiae of mice via flushing with RPMI 1640 (PAA Laboratories) supplemented with 10% FCS (Invitrogen, Darnstadt, Germany), filtered through nylon mesh, and depleted of RBC by hypotonic lysis with 0.83% w/v ammonium chloride (Sigma-Aldrich GmbH, Schnelldorf, Germany). BM cells were cultured for 5 days in DC medium with either 100 ng/ml murine FLT-3 ligand (Miltenyi Biotec) or 1000 U/mL murine granulocyte-macrophage colony stimulating factor (GM-CSF; Peprotech GmbH, Hamburg, Germany) plus 1000 U/mL murine IL-4 (Peprotech). DC medium was composed of RPMI 1640 supplemented with glutamine (PAA Laboratories), 10% heat-inactivated FCS (Invitrogen), 0.1 mM nonessential amino acids (Sigma-Aldrich), 0.1 mM sodium pyruvate (Gibco), 1% penicillin-streptomycin solution (PAA Laboratories), 0.1 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; Invitrogen, Germany), and 5 μM 2-mercaptoethanol (2-ME; Sigma-Aldrich).

2.4. Flow cytometry and staining procedure

Cellular phenotyping was performed on a FACSCanto II flow cytometer (Becton Dickinson). FITC-, PE-, PE-Cy7-, or APC-conjugated monoclonal antibodies (mAbs) against the following markers, as well as the appropriate isotype controls, were used for surface staining according to the manufacturer's instructions: CD11b, CD11c, CD45, CD86, MHC-class II (I-Ab), CD140a (PDGFR-α), TER-119, and SCA-1 (Biolegend GmbH, Fell, Germany). The pDC/IpC mAb 120g8 was obtained from Dendritics (Lyon, France). Cells were stained for 30 min on ice and subsequently washed with staining buffer (1x phosphate-buffered saline [PBS]/5% FBS; PAA) at 400 g for 5 min at room temperature (RT) before analysis. More than 30,000 flow cytometric events were acquired per sample.

2.5. Transwell co-culture

For co-culture experiments, cells in DC generation medium were cultured in 24-well plates containing transwell inserts (thincerts, pore size: 0.4 μM; Greiner Bio-One, Kremsmünster, Austria). MSCs were plated directly in the wells (5×10^3–2×10^4/well in 600 μL), and BM cells were plated in the inserts (2.5×10^5/well in 200 μL). Cells were incubated at 37 °C in an atmosphere containing 5% CO_2.

2.6. In vitro stimulation

Cells were cultured in DC medium in 96-well round-bottom plates (Greiner; 2×10^5 cells/well) and stimulated for 24 h with 5 mg/ml TLR9 ligand CpG oligodeoxyribonucleotides (ODN 2216; InvivoGen, Toulouse, France). IFN-α in the cell-free supernatants from these cultures was quantitated on a FACS-Canto II flow cytometer along with recombinant cytokine standards by cytometric bead assay technology (FlowCytomix; eBioscience, Frankfurt am Main, Germany) as previously described [32]. In some experiments, DC maturation was induced for 24 h with Klebsiella pneumonia lysate and CD86 expression was analyzed by flow cytometry. Klebsiella pneumonia lysate (Serotype 2, ATCC 43816) was

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