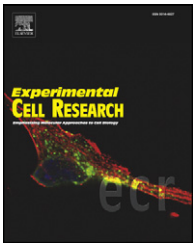
EXPERIMENTAL CELL RESEARCH 317 (2011) 2210–2221

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yexcr



Research Article

Interleukin-4 enhances trafficking and functional activities of GM-CSF-stimulated mouse myeloid-derived dendritic cells at late differentiation stage☆

Shu-Yi Yin[a,b,c], *Chien-Yu Wang*[a], *Ning-Sun Yang*[a,b,c,*]

[a]*Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan, ROC*
[b]*Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan, ROC*
[c]*Taiwan International Graduate Program (TIGP), Molecular and Biological Agricultural Sciences Program, Academia Sinica, Taipei, Taiwan, ROC*

ARTICLE INFORMATION

Article Chronology:
Received 15 March 2011
Revised version received 20 June 2011
Accepted 21 June 2011
Available online 30 June 2011

Keywords:
Interleukin-4
Bone marrow-derived dendritic cells
Trafficking
Temporal control

ABSTRACT

Mouse bone marrow-derived dendritic cells (BMDCs) are being employed as an important model for translational research into the development of DC-based therapeutics. For such use, the localization and specialized mobility of injected BMDCs within specific immune tissues are known to define their immunity and usefulness in vivo. In this study, we demonstrate that IL-4, a key driving factor for in vitro propagation and differentiation of BMDCs, when added during a late culture stage can enhance the in vivo trafficking activity of granulocyte-macrophage colony-stimulating factor (GM-CSF)-induced BMDCs. It suggests that the temporal control of IL-4 stimulation during the in vitro generation of DCs drastically affects the DC trafficking efficiency in vivo. With this modification of IL-4 stimulation, we also show that much less cytokine was needed to generate BMDCs with high purity and yield that secrete a high level of cytokines and possess a good capacity to induce proliferation of allogeneic $CD4^+$T cells, as compared to the conventional method that uses a continuous supplement of GM-CSF and IL-4 throughout cultivation. These results provide us with an important know-how for differentiation of BMDCs from myeloid stem cells, and for use of other immune cells in related medical or stem cell applications.

Introduction

Since dendritic cells (DCs) are specialized antigen-presenting cells (APC) known to be important for mediating various immune responses, including innate and T-cell-mediated immune responses [1], antigen-loaded BMDCs have been used as cell-based vaccines to improve immunity [2–5]. With the increasing demands for clinical applications, it is highly desirable that the quality of DCs should be assessed before they can be applied in clinical settings. In this regard, the use of GM-CSF and IL-4 in primary cultures of BMDCs has made a technical contribution to promote the differentiation of myeloid precursor cells into immature DCs [6–8]. Previously, IL-4-induced DCs were proven to be effective in eliciting melanoma-specific T-cell responses in human patients [9]. In addition, such ex vivo-generated DCs were shown to induce functionally superior $CD8^+$ T cells and polarize $CD4^+$ T cells towards IFN-γ production [10]. Together, these investigations indicated the important role of IL-4 in functional maturation and utility of the in vitro-generated DCs. However,

☆ This work was supported by grants (94F002-1) from the Genomics and Proteomics Program and the Investigator Award of Academy to N.S. Yang, Academia Sinica, Taiwan, Republic of China.

* *Corresponding author at:* Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan, ROC.
E-mail addresses: in_shuyi@hotmail.com (S.-Y. Yin), sallywang1973@hotmail.com (C.-Y. Wang), nsyang@gate.sinica.edu.tw (N.-S. Yang).

0014-4827/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2011.06.013

whereas the effects of IL-4-induced DCs on promoting T-cell immunity have been evaluated, little is known with regard to the in vivo targeting efficiency of IL-4-indued BMDCs to specific immune organs. Therefore, extensive research of IL-4 application on homing efficiency of BMDCs to immune tissue systems is recognized as a critical component for developing DC-based medical biotechnology.

Previously, the ligand-receptor interaction-mediated DC migration activity has been extensively described [11–13]. However, recent studies focused on the effect or manipulation of different cytokines or chemokines on DCs at a "steady state". To evaluate the possible role of IL-4 in DC trafficking activity during in vitro differentiation processes, our present study has aimed to compare the effect of IL-4 on mouse DCs during a specific period of the in vitro cultivation and to determine their homing/trafficking activity, after being translocated back for potential cell-based therapeutic effect in vivo. We considered that this experimental approach not only could provide us with useful information on the temporal control of IL-4 stimulation on differentiation of myeloid-derived DC precursor cells, but it might also generate technical knowhow on high quality control and mass production of BMDCs.

In general, myeloid DCs are potent APCs and can stimulate naive T cells against specific antigens [14]. Inaba and co-workers have shown that murine bone marrow (BM) cells cultured in GM-CSF for 6 to 8 days can generate large numbers of mature DCs [6]. These GM-CSF-derived DCs can be further activated and enriched by supplementing IL-4 [7,8] in the culture medium. DCs derived in the presence of GM-CSF plus IL-4 express surface antigens typically associated with DCs, including DEC205, MHC class II, CD80, and CD86, and demonstrate potent allostimulatory activity [8]. Although a number of studies have reported technical procedures for isolation of large quantities of mouse myeloid-derived DCs [15–17], the use of IL-4 is still controversial. The present study also demonstrates that our treatment regime with IL-4 can effectively improve the yield, purity and functional properties of GM-CSF-stimulated BMDCs. We therefore further explored the action mode of IL-4 stimulation and the specific protocol for sequential supplementation of GM-CSF and IL-4 to cultivate DCs. This manipulation protocol should be valuable for research on DC-based therapeutics.

Materials and methods

Culture of DCs from bone marrow

BMDCs were generated by the method reported previously [6,7,16] with some modifications. Six-week-old female C57BL/6JNarl mice were purchased from the National Laboratory Animal Center and kept under specific pathogen-free (SPF) conditions in accordance with the requirements of the institutional animal care and use committee. Femurs and tibiae were removed after euthanasia and the marrow was flushed out from the bone lumen with RPMI-1640 using a syringe with a 0.45 mm needle. Cell clusters within the marrow suspension were disassociated by vigorous pipetting and filtered through a 45 μm cell strainer. Red blood cells in suspension were lysed with ACK lysing buffer (150 mM NH_4Cl, 1.0 mM $KHCO_3$, 0.1 mM EDTA) for 5 min. Approximately $4–5\times10^7$ BM cells were obtained per mouse. BM cells were suspended in 30 mL complete medium (CM, RPMI-1640 supplemented with 10% fetal bovine serum (FBS) (Gibco BRL), 2 mM L-glutamine, 1% of nonessential amino acids, 100 U/mL penicillin and 100 μg/mL streptomycin) containing the cytokines listed below. They were cultured for up to 8 days with either 200 U/mL GM-CSF (PeproTech) or 200 U/mL GM-CSF plus 200 U/mL IL-4 (PeproTech) at 37 °C under 5% CO_2. On day 2, half of the medium was removed and 20 mL fresh medium containing the same amount of cytokines was added. On day 5, culture plates were gently swirled, and floating and loosely adherent cells were discarded. Subsequently, 75% of the medium was replaced with fresh culture medium with the same amount of cytokines. On day 8, non-adherent cells were collected in six-well plates and used as an immature DC population for subsequent analyses. In some experiments, the concentration of GM-CSF was reduced at later stages when combined with IL-4. To induce further maturation, some DCs were cultured in the presence of 1 μg/mL of lipopolysaccharide (LPS, Sigma) for 24 h in six-well plates.

BMDC maturation was analyzed in the presence of GM-CSF and/or IL-4 under the following conditions: 1) [GM-CSF]: 200 U/mL; 2) [GM-CSF + IL-4]: both 200 U/mL; 3) [GM-CSF + IL-4(0–5)]: 200 U/mL IL-4 from day 0 to day 5; 4) [GM-CSF + IL-4 (5–8)]: 200 U/mL IL-4 from day 5 to day 8; 5) [GM-CSF + IL-4 (7–8)]: 200 U/mL IL-4 from day 7 to day 8; 6) [GM-CSF(0–5) + IL-4(5–8)]: 200 U/mL GM-CSF from day 0 to day 5 and then 200 U/mL IL-4 from day 5 to day 8; and 7) [GM-CSF(0–5)(50%, 5–8) + IL-4(5–8)]: 200 U/mL GM-CSF from day 0 to day 5 and then 200 U/mL IL-4 and 100 U/mL (50%) GM-CSF from day 5 to day 8.

DC trafficking in vivo

To analyze the migration of in-vitro cultured DCs under in vivo experimental conditions in test mice, 1×10^6 BMDCs generated from transgenic GFP mice [C57BL/6J-Tg(*Pgk1-EGFP*)03Narl] were injected into the foot pad of non-transgenic C57BL mice. After 24 h, the draining inguinal lymph node (LN), spleen and liver tissues were harvested. Frozen tissue sections of 12 μm in thickness were mounted on precleaned microscope slides (Superfrost/Plus; Fisher Scientific, Pittsburgh, PA), and stored at −80 °C. To maintain good fluorescent signal, the tissue sections were pretreated with 4% paraformaldehyde at room temperature. Tissue sections were then incubated with blocking solution (2% fetal calf serum in PBS) for 10 min, stained by incubation with 4,6-diamidino-2-phenylindole (DAPI), and washed with PBS (2×10 min each). Tissue sections were then mounted with 50% glycerol in H_2O with coverslips. Fluorescence microscopy evaluation of immunostained tissue sections was performed using a Zeiss Axiovert 200 M microscope (Carl Zeiss, Heidelberg, Germany). Microscopy images were acquired with a digital camera (Orca ER; Hamamatsu) and processed using Axiovision 4.6.3 (Carl Zeiss). For comparing DC targeting efficiency, some LN and spleen tissues were harvested and applied over a 40-μm cell strainer. Single-cell suspensions were then stained with phycoerythrin (PE)-conjugated anti-CD11c antibody and analyzed by flow cytometry. DC mobility was then assayed by detecting the percentage of $CD11c^+/CFSE^+$ double-positive cells in LN and spleen tissue samples.

Flow cytometric analysis

We used fluoresce in isothiocyanate (FITC)- or PE-labeled monoclonal antibodies for the staining of MHC class II (I-Ab, mouse IgG2a, 2G9), CD40 (mouse IgG, HM40-3), CD80 (mouse IgM, 16-10A1), CD86 (mouse IgG, GL-1), and CD11c (mouse IgG, N418) proteins in test cells. These antibodies were purchased from Biolegend (San Diego, CA). The PE-labeled monoclonal antibodies