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Fibroblasts feeder niche and Flt3 Ligand as a novel inducer of plasmacytoid dendritic cells development in vitro

Mohammad Taher Tahoori [a], Ali Akbar Pourfathollah [a,*], Masoud Soleimani [b,c], Ebrahim Vasheghani-Farahani [d], Adel Mohammadzadeh [e], Afshin Amari [f], Seyed Mahmoud Hashemi [g], Majid mossahebi-mohammadi [b]

[a] Department of Immunology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran
[b] Department of Stem Cell Biology, Stem Cell Technology Research Center, Tehran Iran
[c] Department of Hematology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran
[d] Department of Chemical Engineering, Biotechnology Division, Tarbiat Modares University, Tehran, Iran
[e] Department of Microbiology, Immunology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
[f] Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[g] Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Plasmacytoid dendritic cell (pDC), plays central role in antiviral immunity. The aim of this study was to assess the effect of Flt3 ligand (FL) alone or with L929 fibroblast feeder or L929 conditioned media on differentiation of mouse bone marrow (BM) cells into pDC in vitro. Murine BM cells were cultured with FL or with L929 or conditioned media for 9 days. The differentiated cells were analyzed using flow cytometry for PDCA-1, B220 and CXCR4. The relative expression of Stat3, CXCR4, CXCR7, IFN-β, TGF-β and Runx2 in differentiated cells determined by real time PCR. The development of pDC showed up to 19% increase after co-culture of BM cells with fibroblast feeder. Upregulation of Stat3, Runx2 and CXCR4 due to the presence of fibroblast feeder with FL in culture results in improved pDC development. Furthermore, 30% L929 supernatant along with Flt3 ligand was able to derive pDC up to 8.9% in comparison with FL alone, which was 6.6% in vitro. Thus, for the first time we introduced L929 fibroblast feeder as a niche producer of M-CSF and probably other growth factors and chemokines, which promotes the development of pDC in vitro along with FL, similar to in vivo niche.

1. Introduction

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that modulate the outcome of immune response toward immunity or tolerance. There are a large variety of DC subsets according to surface phenotype, function, and tissue distribution [1]. DC can be classified into 2 classes: classical or conventional DC (cDC) and plasmacytoid DC (pDC), but the plasticity and origin of them remains unclear [2].pDC or Type 1 interferon-(α, β, ω)-producing cells (IPCs) are a haematopoietic cell population with a characteristic plasma cell-like morphology found in many tissues in mouse, including blood, thymus, bone marrow, liver, and the T-cell areas of lymphoid organs [3,4]. Due to the critical role of pDC in immunity against viral infections, autoimmunity and tolerance, they have recently been the focus of attention. IPCs selectively express toll-like receptors (TLR-7 and TLR9), and quickly release high levels of type 1 interferon in contact with viral stimulation [5]. pDC can be directly identified in lymphoid and non-lymphoid organs by the specific expression of mPDCA-1 (murine plasmacytoid dendritic cell antigen-1) [6]. PDC have been recently considered as immunomodulating cells with a potential to induce tolerance [7]. PDC also function as tolerogenic cells by expressing indoleamine 2,3-dioxygenase (IDO), inducible costimulator ligand (ICOS-L), and/or programmed death 1 ligand (PD-L1), which can provoke the development of regulatory T-cells (Treg) and suppression of self- and alloreactive cells [1]. Hereby, we propose a method to study pDC development from bone marrow (BM) cultures. However, precise regulation mechanism of pDC development is not fully understood. Thus, the in vitro generation of murine pDC is a useful tool to further investigate pDC biology and function in the immune system.

1.1. Developmental biology of pDC

As mentioned in the literature, the use of FL in vitro can derive DCs plus pDC [8]. Investigation of progenitor cells generating M-CSF–dependent DC revealed that common lymphoid progenitors (CLPs) were efficient producers of pDC. Common myeloid progenitors (CMPs)

* Corresponding author. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, P.O. Box 14115-331, Tehran, Iran.
E-mail address: pourfa@modares.ac.ir (A.A. Pourfathollah).

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