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DENTRITIC CELLS

Generation of potent dendritic cells with improved migration ability through p-cofilin and sarco/endoplasmic reticulum Ca^{2+} transport ATPase 2 regulation

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Abstract

Background aims. It is important to improve the migratory ability of dendritic cells (DCs) and to increase DC potency for successful DC-based cancer immunotherapy. The intracellular Ca^{2+} signaling pathway has an important role on the regulation of DC migration. Our preliminary studies revealed that sarco/endoplasmic reticulum Ca^{2+} transport ATPase 2 (SERCA2) expression was inversely related to DC migratory capacity, and the expression level of p-cofilin and SERCA2 on mature DCs showed a counter-trend. *Methods.* We selected the appropriate six maturation cocktails on the basis of the expression levels of SERCA2 and p-cofilin and investigated the functional characteristics and migratory capacity of mature DCs. Among the these six maturation cocktails, $DC_{IFN\text{-}\gamma/IL\text{-}1\beta/Poly\text{-}I:C}$ showed potent type 1 immune response with interleukin (IL)-12p70 production and strong Th1-polarization, and this DC elicited strong antigen-specific cytotoxic T-lymphocyte responses. *Results.* Interestingly, $DC_{IFN\text{-}\gamma/IL\text{-}1\beta/Poly\text{-}I:C}$ showed lower expression of SERCA2 and higher expression of p-cofilin compared with those matured with the use of other cocktails. *In vitro* migration assay showed that DCs matured with the use of this maturation cocktail had significantly increased migratory ability compared with αDC1s and other DCs. *Conclusions.* Interferon-γ, IL-1β and Poly-I:C maturation cocktail may be used in the field of cancer immunotherapy to generate potent immune-stimulatory DCs with improved type 1 immune response and migration capacity.

Key Words: *chemotaxis, dendritic cell, migration, SERCA2*

Introduction

Cellular immunotherapy with the use of dendritic cells (DCs) has been investigated as a promising treatment for a wide range of cancers [1–3]. DCs for cancer immunotherapy have been generated by the maturation of monocyte-derived immature DCs (imDCs) with tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and prostaglandin (PG) E(2) [4]. This maturation procedure was considered a conventional method for standard DC (sDC) maturation and has been used in clinical studies [5,6]. However, sDCs show a lower IL-12p70 production, which is important for the induction of effective Th1 responses and cytotoxic T-lymphocyte (CTL) responses [7]. To increase DC potency with the use of cytokine combinations, alpha-type 1-polarized DCs (αDC1s) that are induced to mature with the use of the αDC1-polarizing cytokine cocktail, such as IL-1β, TNF-α, interferon (IFN)-α, IFN-γ and polyinosinic: polycytidylic acid [poly(I:C)], have been developed to generate strong functional CTLs compared with sDCs [8,9]. Although αDC1s may provide increased IL-12p70 production and potent CTL generation, αDC1s show a lower migratory ability due to the relatively low expression of CCR7 compared with sDCs [10]. Therefore, it is important to improve the migratory ability of DCs and to increase DC potency for successful DC-based cancer immunotherapy.

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Calcium (Ca^{2+}) is an important second messenger in the phospholipase C signal transduction pathway. Intracellular Ca^{2+} released from store in cooperation with entry Ca^{2+} through Ca^{2+} release-activated Ca^{2+} channel modify membrane potential by diverse antigens, including Toll-like receptor ligands, intact bacteria and microbial toxins. Subsequently, Ca^{2+} activates nuclear factor-κB (NF-κB) and nuclear factor of activated T cells that regulate DC activation, maturation and immunological synapses formation with T cells. Interaction between chemokine and the receptor leads to Ca^{2+} mobilization and actin polarization and finally induces DC migration toward ATP released from injures or infected sites [11,12]. Sarco/endoplasmic reticulum Ca^{2+} transport ATPase 2 (SERCA2) is a unique endoplasmic reticulum Ca^{2+} channel for Ca^{2+} translocation from the cytoplasm to sarco/endoplasmic reticulum (sER) and is involved in the regulation of cytosolic Ca^{2+} concentrations [13]. Increased cytosolic Ca^{2+} concentration improves the migratory ability of DCs.

Rho/Pyk2/cofilin has been known as regulator for the migratory speed of DCs independently of the presence of chemokines [14]. Our previous study showed that mDCs exposed to a secondary lymphoid organ chemokine (SLC/CCL21) dramatically enhanced cytolytic activity in CTL response by Th1-attracting chemokine IP-10 (CXCL-10) expression without any significant alterations on expression of cell surface markers or on the production of cytokines [15]. Also, we demonstrated that SERCA2 is directly associated with the migration capacity of CCL21-pretreated monocyte-derived DCs, and the DC migration is coordinated by SERCA2 expression and cofilin phosphorylation. Along this line, we investigated whether the potent DCs are generated or not with improved migration ability by using maturation cocktails (several pro-inflammatory cytokines, IFNs and Toll-like receptor [TLR] agonists) with the regulation of SERCA2 expression and cofilin phosphorylation.

On the basis of the expression level of p-cofilin and SERCA2 on mature DCs by pro-inflammatory cytokines (TNF-α, IL-1β) TLR agonists (Poly-I:C, R848) or cytokines (IFN-α, IFN-γ), we selected six maturation cocktails. We then evaluated the functional characteristics of DCs matured with specific maturation cocktails in terms of their ability to induce type 1 immunity. In addition, we investigated the effect of each cocktail on the expression of SERCA2 and p-cofilin and the migratory capacity of mature DCs.

Methods

Generation of DCs

Peripheral blood was obtained from healthy donors with HLA-A*0201$^+$ according to the protocol by the Institutional Ethics Committee of the Chonnam National University Hwasun Hospital. Peripheral blood mononuclear cells were isolated by means of density gradient centrifugation with Lymphoprep (AXIS-SHIELD), and monocytes were then isolated by positive selection with CD14$^+$-conjugated microbeads and magnetic activated cell separation (MACS; Miltenyi Biotec). Isolated monocytes (purity >95%) were cultured in IMDM (Iscove's modified Dulbecco's medium) with 10% heat-inactivated fetal bovine serum (FBS) and 1% PS (penicillin-streptomycin; Gibco-BRL) for 6 days in six-well plates (FALCON) at 2×10^6 cells per well in the presence of 50 ng/mL rhGM−colony-stimulating factor and 20 ng/mL rhIL-4 (PEPROTECH). Freshly prepared cytokines were added every 2 days by changing half of the volume of culture medium. On day 6, imDCs were matured with the use of TLR agonists and cytokines for an additional 2 days in 24-well plates (FALCON) at 3×10^5 cells per well. αDC1s were generated with the use of αDC1-polarizing cocktails containing rhTNF-α (50 ng/mL), IFN-γ (100 ng/mL), IL-1β (25 ng/mL) (PEPROTECH), IFN-α (3000 units/mL) (LG Life Science) and poly-I:C (20 μg/mL; Sigma-Aldrich), and sDCs were generated with the use of an sDC maturation cocktail containing rhTNF-α (50 ng/mL), IL-1β (25 ng/mL), IL-6 (10 ng/mL) and PGE_2 (1 μg/mL). In addition, R848 (TLR7 and 8 agonist, 1 μg/mL) (InvivoGen) was used for DC maturation.

Phenotypic analysis of DCs

Fluorescence-activated cell-sorting (FACS) analysis was performed with the use of FACSCalibur (BD Bioscience Immunocytometry Systems), followed by labeling of single-cell suspensions with mAbs against HLA-A2-fluorescein isothiocyanate (FITC), CD38-phycoerythrin (PE), CD80-PE, CD83-FITC, CD86-PE, CD74-FITC (BD Biosciences), CCR7-FITC (R&D Systems) and CD8-APC for 15 min at 4°C. In addition, isotype-matched controls (mouse IgG1 and mouse IgG2, BD Biosciences) were used in parallel. The expression of cytokine and protein in activated CD4$^+$ T cells and CD3$^+$ T cells was assessed with the use of mAbs against human IFN-γ−FITC, IL-4−PE, Granzyme B−FITC, Perforin-PE and CD8-APC, and isotype-matched controls were used in parallel. The acquired data were analyzed with the use of Win MDI version 2.9 (Biology Software Net). The results are presented as mean fluorescence intensity indices (MFI), calculated as the ratio of the MFI of the sample to that of the isotype-matched control.