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Regulation of CD28 expression on umbilical cord blood and adult peripheral blood CD8$^+$ T cells by interleukin(IL)-15/IL-21

Yu-Han Chen [a,1], Ming-Ling Kuo [b,1], Po-Jen Cheng [c], Hsiu-Shan Hsaio [a], Pei-Tzu Lee [a], Syh-Jae Lin [a,*]

[a] Division of Asthma, Allergy, and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC
[b] Department of Microbiology and Immunology, Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan, ROC
[c] Department of Obstetrics/Gynecology, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC

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ABSTRACT

Interleukin (IL)-15 and IL-21, both belonging to common γ-chain-signaling cytokine family, have an important role to maintain homeostatic proliferation of CD8$^+$ T cells. CD28, an essential co-stimulatory molecule on T cells, may be a marker of replicative senescence. We investigated the effect of IL-15 and IL-21, alone or in combination, on activation, apoptosis, cytokine production and cytotoxic function of magnetic bead purified umbilical cord blood (UCB) and adult peripheral blood (APB) CD8$^+$ T cells with regards to their CD28 expression. We established that (1) IL-15-induced CD8$^+$ T cell proliferation was associated with a preferential expansion of CD28$^-$ population in UCB, which could be partially counteracted by IL-21; (2) UCB CD8$^+$ T cells were more readily responsive to IL-15 compared to their adult counterparts in terms of CD69 expression, with the majority of CD69-bearing CD8$^+$ T cells were CD28$^-$; (3) IL-21 further promoted interferon-gamma, but not tumor necrosis factor-alpha production from IL-15 treated CD8$^+$ T cells; (4) IL-21 also synergized with IL-15 to enhance perforin and granzyme B expression of CD8$^+$ T cells, especially in APB CD8$^+$CD28$^-$ subsets; (5) IL-21 resulted in CD8$^+$ T cells apoptosis both in APB and UCB cells, mainly in CD8$^+$CD28$^-$ subsets. Taken together, we demonstrate differential IL-15/IL-21 response in UCB CD8$^+$ T cells with regards to CD28 expression. Our results suggest that combining IL-21 and IL-15 immunotherapy may be better than IL-15 alone to ameliorate graft-versus-host disease while preserving antitumor effect in the post-UCB transplantation period.

1. Introduction

CD8$^+$ T cells are cytotoxic T lymphocytes which play an important role in adaptive immunity, responsible for destroying intracellular pathogens and cancerous cells [1]. CD8$^+$ T cell may decrease with aging, due mainly to the decline in production of naive T cells in the bone marrow and thymus and the expansion of incompetent memory lymphocytes [2], resulting in increased incidence of infections, cancer and autoimmunity. CD28, an important co-stimulatory molecule expressed on naïve T cells may be an important marker of immune senescence, as T cells gradually lose CD28 expression in the process of aging [2,3].

UCB has been increasingly used as a source of hematopoietic stem cells for treatment of various hematological malignancies. The incidence of acute and chronic GVHD in recipients of UCB transplants is lower than recipients of BMT [4]. UCB CD8$^+$ T cells are predominantly CD45RA "naïve" T cells, with lower activation state, cytokine production, and diminished cytotoxicity compared to APB [5,6].

Previous work have shown that IL-15, a γ_c signaling cytokine that promote CD8$^+$ T cells and NK cells proliferation, down regulate CD28 expression on UCB CD8$^+$ T cells to a greater extent than that on APB [3,5,7]. Thus, IL-15 used as an immune enhancing agent may have the disadvantage of accelerating CD8$^+$ T cells aging, especially in the setting of UCB transplantation. IL-21, also a γ_c signaling cytokine produced by activated CD4$^+$ T cells, enhances the proliferation, IFN-gamma production, and cytotoxicity of NK cells and T cells [8,9]. IL-21 has been well tolerated in clinical trial, and may therefore be considered a candidate for immunotherapy [10].

Recent studies revealed that IL-21 can prevent the IL-15-mediated CD28 down-regulation in both in naïve and memory CD8$^+$ T

Abbreviations: APB, adult peripheral blood; BMT, bone marrow transplantation; CFSE, carboxyfluorescein succinimidyl ester; CTL, cytotoxic T lymphocytes; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; GVHD, graft-versus-host disease; IFN-γ, interferon-gamma; IL-15, interleukin-15; MNC, mononuclear cells; PI, propidium iodide; γ_c, common gamma chain; TNF-α, tumor necrosis factor-alpha; UCB, umbilical cord blood.

* Corresponding author. Address: Division of Asthma, Allergy, and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, 5 Fu-Hsing Street, Kweishan, Taoyuan, Taiwan. Tel.: +886 3 3281200x8245; fax: +886 3 3288957.
E-mail address: sjlino@adm.cgmh.org.tw (S.-J. Lin).

[1] These authors contributed equally to this work.

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cells [3,7]. We postulate that co-administration of IL-15 and IL-21 may be a better regimen for immunotherapy than IL-15 alone in UCB transplantation. The aim of the present study therefore is to study the effect of IL-15/IL-21 on activation, apoptosis, cytotoxic function and cytokine production of UCB and APB $CD8^+$ T cells with regards to CD28 expression. We sought to determine whether IL-21 would preferentially counteract the IL-15 mediated CD28 down-regulation in UCB.

2. Materials and method

2.1. Cell preparation

UCB were obtained immediately after delivery of full term infants (Department of Obstetrics, Chang Gung Memorial Hospital). APB were obtained from healthy adult volunteers. Informed consent was obtained from each subject, with the pre-approval of the study by Medical Ethics and Human Clinical Trial Committee of the Chang Gung Memorial Hospital. UCB and APB mononuclear cells (MNCs) were isolated using Ficoll–Hypaque (GE Healthcare, Uppsala, Sweden) gradient separation.

2.2. Isolation of $CD8^+$ T cells

$CD8^+$ T cells were purified either from total APB MNCs or UCB MNCs by positive selection anti-CD8-coated magnetic beads (MACS system, Miltenyi Biotec Inc, USA). First, MNCs were stained with Biotin-Antibody Cocktail for 10 min at 8 °C, than stained with $CD8^+$ T Cell Microbeads Cocktail for 15 min. After washing, cells were resuspended in incubation buffer and enrichment was performed with the MACS magnetic separator. The purity of the enriched $CD8^+$ T cells was assessed by flow cytometry or fluorescence microscopy. The cells obtained were more than 98% $CD3^+/CD8^+$.

2.3. Cell culture

APB and UCB $CD8^+$ T cells were cultured at a concentration 1×10^6 cells/ml for 7 days at 37 °C in 5% CO_2 atmosphere, in RPMI 1640 with 10% FCS, in the presence or absence of IL-15 (10 ng/ml, Pepro Tech Inc., Rocky Hill, NJ, USA) and/or IL-21 (25 ng/ml, Pepro Tech Inc., Rocky Hill, NJ, USA) on 24-well flat-bottom plates. For assessment of proliferation, $CD8^+$ T cells were pre-stained with carboxyfluorescein succinimidyl ester (CFSE) (Sigma, St Louis, USA) in serum-free RPMI-1640 for 15 min before culture.

2.4. Flow cytometric analysis

Both control and cytokine-stimulated $CD8^+$ T cells were stained with FITC- or PE-conjugated mouse anti-human monoclonal antibodies including anti-CD8, anti-CD28 or anti-CD69 from Becton–Dickinson (Worldwide Inc., Taiwan Branch, Taiwan) for flow cytometric analysis. CD8 T cell apoptosis was assessed using a annexin-V(FITC)/propidium iodie(PI) apoptosis detection kits (BD Biosciences). To assess cytotoxic function of $CD8^+$ T cells, intracellular perforin and granzyme B expression in $CD8^+$ T cells was investigated after permeabilization of the cell membrane with Cytofix/Cytoperm kit (BD Pharmingen) using FITC-conjugated anti-perforin (Biolegend, San Diego, CA, USA), and anti-granzyme-B(BD pharmingen, San Jose, USA) according to the manufactor's instruction. Electronic gates were set to enable analysis of the fluorescence of the viable cell population according to FSC/SSC histograms following cytokine stimulation. The percentage of cells stained with each monoclonal antibody was determined by comparing each histogram with one from control cells stained with FITC- or PE-labeled isotype control monoclonal antibodies. Cells were analyzed on a FACS Calibur (BD Biosciences) with CellQuest software.

2.5. Measurement of cytokines

Cytokines-producing capacity was examined by collecting supernatants of previously cytokine-stimulated $CD8^+$ T cells after 7 days of culture. Secreted TNF-α and IFN-γ was quantitated in cell-free supernatants using a human TNF-α and IFN-γ ELISA kit (Quantikine, R&D Systems, Inc. Minneapolis, MN, USA), performed according to the manufacturer's instruction.

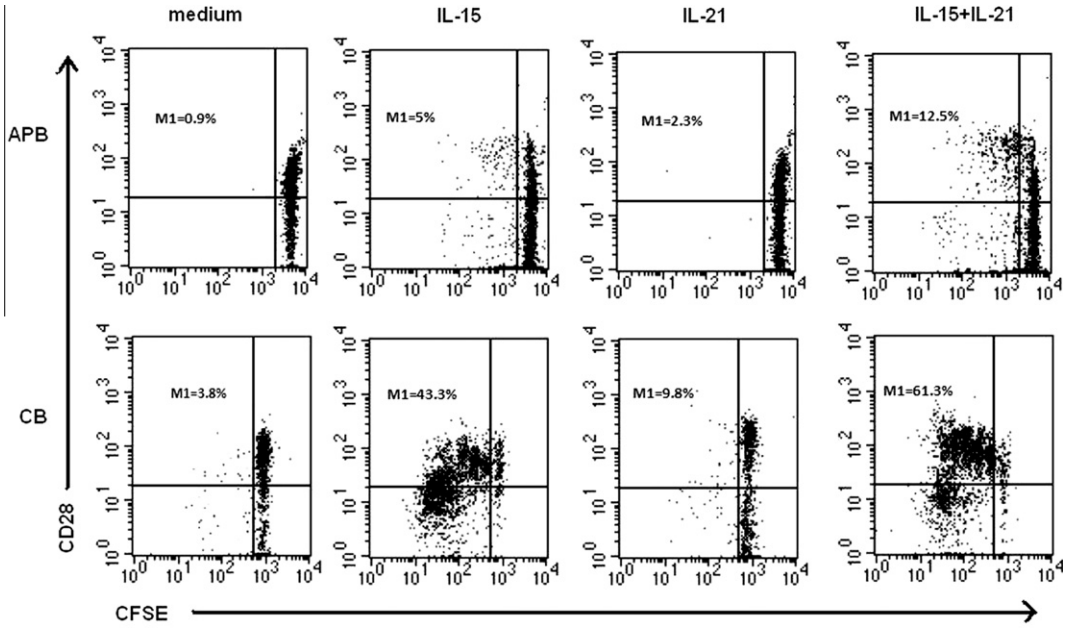

Fig. 1. (A) Representative profile of dot-plot analysis by flow cytometry of CD28 versus CFSE staining on adult peripheral blood (APB, $n = 5$) and umbilical cord blood (CB, $n = 6$) $CD8^+$ T cells. $CD8^+$ T cells were labeled with CFSE and cultured without cytokines (medium) or in presence of IL-15 (10 ng/ml) or IL-21 (25 ng/ml), alone or in combination (IL-15 + IL-21) for 7 days. The cultured cells were then harvested and stained for CD28 and analyzed by FACS. M1 represents the percentage of proliferating $CD8^+$ T cells.