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Effect of cyclosporin A on interleukin-15-activated umbilical cord blood natural killer cell function

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Background

Interleukin (IL)-15-activated natural killer (NK) cells may provide a graft-versus-leukemia (GvL) effect post-umbilical cord blood (CB) transplantation. The effect of cyclosporin A (CsA), a calcineurin-inhibitor used for prophylaxis of graft-versus-host disease (GvHD), on IL-15-mediated activation, cytotoxic function and target-induced apoptosis of CB NK cells, was examined in comparison with adult peripheral blood (APB) NK cells.

Methods

CsA was added to anti-CD3±IL-15-stimulated CB and APB mononuclear cells (MNC) for a 5-day incubation. $CD3^-$ $CD56^+$ NK cell recovery was determined by flow cytometric analysis. Magnetic bead-purified CB and APB NK cells were stimulated with IL-15 for 18 h under the influence of CsA. NK activation (CD69), K562 cytotoxicity and NK–K562 interactions (CD54, perforin and annexin-V expression 4 h following contact with K562 cells) were assessed by flow cytometry.

Results

CsA decreased $CD3^-$ $CD56^+$ NK cell recovery in anti-CD3-stimulated CB MNC 5-day cultures, an effect that could be counteracted by IL-15; comparable effects were observed with APB. Short-term (18-h) experiments revealed that CsA down-regulated K562 cytotoxicity of IL-15-activated ($P = 0.018$) but not resting ($P = 0.268$) purified CB NK cells. IL-15-induced CB NK CD69 expression showed increased CsA sensitivity over APB ($P = 0.012$). CsA down-regulated K562 cell-induced CD54 ($P = 0.028$) but not perforin ($P = 0.416$) expression of IL-15-activated CB NK cells. Target-induced apoptosis of IL-15-activated CB ($P = 0.043$) but not APB ($P = 0.144$) NK cells was decreased by CsA.

Discussion

We have demonstrated differential CsA sensitivity of IL-15-activated CB and APB NK cells. These results may be used to improve the design of IL-15-activated NK cell adoptive immunotherapy in cancer patients receiving CsA post-CB transplantation.

Keywords

cyclosporin, interleukin-15, natural killer cells, umbilical cord blood.

Introduction

Natural killer (NK) cells, a distinct lineage of lymphoid cells defined as being membrane $CD3^-$, $CD16^+$ and/or $CD56^+$, are responsible for killing tumor and virus-infected cells without major histocompatibility restriction [1,2]. Decreased graft-versus-host disease (GvHD) and easier accessibility encourage the use of umbilical cord blood (CB) stem cells for transplant in patients with hematologic malignancies [3,4]. Increased infectious complications [5] and delayed engraftment [6] following umbilical CB transplantation may be attributed in part to the deficient CB NK function. Compared with adult NK cells, CB NK cells are deficient in CD57 expression [7] and have decreased NK and antibody-dependent cell-mediated cytotoxicity (ADCC) [8–10] and adhesion molecule expression [7,11].

Because of the limited amount of NK cells in CB, adoptive immunotherapy using cytokine-expanded CB

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NK cells may further the immune recovery and enhance the graft-versus-leukemia (GvL) effect following CB transplantation [12]. *In vitro* expansion of cytotoxic $CD3^-$ $CD56^+$ NK cells could be efficiently enhanced using cultures supplemented with monoclonal anti-CD3 antibodies (Ab) and interleukin(IL)-2 [13,14]. IL-15, an IL-2-like gamma-chain signaling cytokine with even more potent NK-activating capability, may serve as an promising candidate to expand NK cells with enhanced function [15,16].

As in conventional bone marrow transplantation, CB transplant recipients require cyclosporin A (CsA) immunosuppressive therapy as GvHD prophylaxis and treatment. CsA is a potent inhibitor of cytokine (IL-2–IL-6 and interferon-gamma) production by $CD4^+$ T lymphocytes stimulated via the T-cell antigen receptor pathway. Increased CsA sensitivity of CB T cells has been proposed to explain the lessened GvHD observed in CB transplantation [17]. Previous studies on the effect of CsA on adult human and murine NK activity are contradictory because of different experimental designs [18–21]. A recent study detailed the differential effect of CsA on $CD16^+$ $CD56^-$ and $CD16^+$ $CD56^+$ subpopulations [22]. Little is known about whether CsA would adversely affect CB NK function, relevant to GvL in the setting of umbilical CB transplantation.

This study aimed to examine the effect of CsA on IL-15-mediated CB NK expansion and activation using bulk mononuclear cell (MNC) cultures as well as magnetic bead-purified NK cells. We also sought to determine how CsA affects activation and cytotoxic function of IL-15-activated CB NK cells and NK–target cell interactions in comparison with their adult counterparts. We show that IL-15-mediated NK expansion is resistant to CsA in CB and adult peripheral blood (APB) MNC alike. We also demonstrate differential CsA sensitivity between IL-15-activated CB and APB NK cells in terms of CD69 expression and target-induced apoptosis.

Methods

MNC and NK cell preparation

MNC were isolated using Ficoll–Hypaque density gradient centrifugation from both heparinized umbilical CB samples and APB with informed consent and under pre-approval of the study by the Medical Ethics and Human Clinical Trial Committee of the Chang Gung Memorial Hospital (Taoyuan, Taiwan). Blood samples were collected in sterile tubes and processed within 24 h of collection. NK cells were enriched from MNC by depleting $CD3^+$ cells (T lymphocytes), $CD19^+$ cells (B lymphocytes) and $CD14^+$ cells (macrophage/monocytes) using the MACS cell isolation kits for NK cells (Miltenyi Biotec, Bergisch Gladbach, Germany), including bead-coupled monoclonal antibodies (MAb) specific for CD3, CD4, CD19 and CD33. With a MACS magnetic separator (Miltenyi Biotec), NK cells were enriched phenotypically to $>90\%$ $CD3^-$ $CD56^+$, as determined by flow cytometry.

Cell culture

MNC were resuspended in RPMI with 10% fetal calf serum (FCS) at a concentration of 1×10^6/mL and placed in 6-well plates pre-coated with or without anti-CD3 MAb (1 μg/mL; BD Biosciences, San Diego, CA, USA) in the presence or absence of IL-15 (10 ng/mL; PeproTech Inc., Rocky Hill, NJ, USA) for a 5-day incubation. CsA (Prograf, Fujisawa Pharmaceutical Co., Osaka, Japan) at different concentrations (1 and 5 μg/mL) was added at the beginning of the cultures. Cell count and viability were assessed by trypan blue exclusion. The absolute NK cell count was calculated as viable MNC cell count × percentage $CD3^-$ $CD56^+$ cells. Percentage NK cell recovery = viable $CD3^-$ $CD56^+$ NK cell count on day 5/viable $CD3^-$ $CD56^+$ NK cell count on day 0×100. MACS-purified CB and APB NK cells were cultured in RPMI + 10% FCS in the presence or absence of IL-15 (10 ng/mL) with or without CsA for 18 h.

Ab and flow cytometry

Cultured MNC were washed in cold phosphate-buffered saline (PBS) with 2% FCS and 0.1% sodium azide and then stained with fluorescein isothiocyanate (FITC)- or phycoerythin (PE)-conjugated mouse anti-human MAb. (Becton-Dickinson, San Jose, CA, USA). The FITC/PE paired Ab, anti-CD3/CD56, were used in two-color flow cytometric analysis. Purified unstimulated or IL-15-treated (for 18 h) NK cells were washed and stained with anti-CD69 to see the effect of CsA on NK activation. To examine the effect of CsA on adhesion molecule expression following contact with K562 cells, NK cells were incubated for another 4 h with or without K562 cells at an effector to target (E:T) ratio of 1:1 in a U-bottomed microtiter plate (Costar, Cambridge, MA, USA) and then stained with anti-CD54, anti-CD18, anti-CD11b and anti-CD44. Target-induced early NK apoptosis was assessed using an FITC–annexin-V/propidium iodide (PI)