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A Low Effective Dose of Interleukin-7 Is Sufficient to Maintain Cord Blood T Cells Alive without Potentiating Allo-Immune Responses

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Slow reconstitution of T cell immunity remains a critical issue after umbilical cord blood (CB) transplantation. Although this may be a consequence of the low cell dose, it may also reflect the propensity of naïve T cells, which predominate in CB, to undergo apoptotic cell death. Exogenous interleukin 7 (IL-7) can prevent apoptosis of naïve T cells, but at high concentrations, IL-7 may also expand alloreactive T cells, thereby aggravating the risk of graft-versus-host disease. We evaluated the survival of CB T cells from 34 healthy full-term pregnancies, and we found wide interdonor variation, from 17.4% to 79.7%, of CB T cells that were still alive after being rested for 4 days in culture medium without cytokine supplementation. The viability of CB T cells was negatively correlated to infant birth weight (Spearman's $\rho = .376$; $P = .031$) and positively correlated to venous CB pH ($\rho = .397$; $P = .027$); both associations were confirmed by multivariate analysis ($P = .023$ and $P = .005$, respectively). A low supplemental concentration (100 pg/mL) of recombinant human IL-7 was sufficient to maintain the viability of cryopreserved/thawed CB T cells, with most (>80%) cells remaining in a quiescent state and without significant changes in their CD4/CD8 ratio and the proportion of $CD4^+$ $CD31^+$ $PTK7^+$ recent thymic emigrants. IL-7 at 100 pg/mL did not lead to any significant enhancement of the alloreactive response of CB T cells, as evaluated by proliferation rates (thymidine incorporation and carboxyfluorescein diacetate succinimidyl ester dilution) and interferon-gamma production (ELISPOT). This effective concentration of IL-7 is far lower than that obtained in vivo after pharmacological administration of the cytokine. This study suggests that administration of lower doses of recombinant human IL-7 than used in previous clinical trials may be sufficient to sustain the viability of infused CB T cells and, thus, help to accelerate naïve T cell reconstitution without potentiating their alloreactivity.

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INTRODUCTION

Slow reconstitution of T cell adaptive immunity and graft-versus-host disease (GVHD) remain critical issues after allogeneic hematopoietic stem cell transplantation. Compared with conventional allografts from matched unrelated donors, umbilical cord blood (CB) transplantation may allow a greater degree of HLA disparities, but it often leads to a more gradual immune reconstitution [1,2]. T cells brought by CB grafts are almost exclusively naïve, include a high proportion of functionally immature recent thymic emigrants (RTEs), and are particularly prone to apoptosis [3,4]. Together with a relatively low graft nucleated cell dose, infusion of apoptosis-prone T cells would likely lead to their shortened survival in the host, which suggests that the long-lasting immunodeficiency after CB transplantation may be due, in part, to the rapid disappearance of most of the infused T cells.

Interleukin-7 (IL-7) is a major homeostatic cytokine essential to naïve T cell survival as well as homeostatic expansion of T cells [5], which is the primary process involved in the first months of immune reconstitution and remains the main contributor to reconstitution in adult recipients, particularly the older recipients. Peak circulating

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levels of IL-7 in the nanogram per milliliter range are obtained after administration of recombinant human IL-7 to improve T cell recovery in lymphopenic patients [6,7]. Such a high concentration is reportedly effective at preventing resting CB T cells from death in culture [8,9] and may induce a variable capacity to produce interferon-γ and other cytokines upon in vitro stimulation [10]. High IL-7 concentrations may expand alloreactive and autoreactive T cells [11], which may induce and/or potentiate GVHD, as indicated both by preclinical models [12] and by the association found in some clinical studies between high systemic IL-7 levels and acute GVHD rates [13,14]. With this in mind, we sought to investigate whether lower IL-7 concentrations could suffice to maintain CB T cell viability without exaggerating their activation and alloreactivity. We observed a broad individual variation in survival of CB T cells from term placentas and demonstrated that culture of cryopreserved/thawed CB T cells in the presence of .1 ng/mL of recombinant human IL-7 could be sufficient to sustain T cell viability without boosting the allo-immune response.

MATERIALS AND METHODS

CB and Adult Peripheral Blood Cell Isolation

The CB and adult peripheral blood samples were collected under the ethical approval of the Lille University Hospital Human Research committee (DC 2011-1288). Umbilical CB (50 to 100 mL) was obtained from 34 healthy full-term neonates (38 to 42 weeks of gestation) immediately after uncomplicated vaginal delivery of a low-risk pregnancy and single gestation, using methods that met the standards for the collection for CB banking and after signed informed consent from the mothers. Birth characteristics were collected (Table 1). CB plasma samples were analyzed using a high-sensitivity ELISA (IL-7 highly-sensitive Quantikine ELISA kits, R&D systems, Minneapolis, MN). Mononuclear cells were isolated by standard Ficoll-paque (GE, Uppsala, Sweden) density-gradient centrifugation within 3 hours after CB collection, washed in phosphate-buffered saline, and then either used directly or suspended in freezing medium (Recovery Cell Culture Freezing Medium, Gibco, Invitrogen, Oslo, Norway) containing 10% DMSO and cryopreserved in liquid nitrogen. Cryopreserved cells were kept frozen for 4 months on average (range, 15 days to 1 year) and then thawed quickly in a 37°C water bath and washed immediately before use in RPMI medium containing 1 mM of ethylenediamine tetraacetic acid. T cells were isolated from fresh or cryopreserved/thawed CB mononuclear cells by negative immunomagnetic selection (EasySep Human T cell Enrichment kit, StemCell Technologies, Vancouver, Canada), yielding >95% $CD3^+$ cells by flow cytometry. Mononuclear cells and T cells were similarly purified from peripheral blood from consenting healthy adults and were used as controls and to isolate allogeneic stimulators by negative immunomagnetic selection (EasySep Monocyte Enrichment kit, StemCell Technologies).

Immunophenotypic Analysis of CB T Cells

CB T cell populations were analyzed before and after culture. All directly conjugated antibodies and isotype controls were from Beckman Coulter (Fullerton, CA), with the exception of the monoclonal antibody to CD127 (IL-7 receptor alpha chain) from eBioscience (Paris, France) and the monoclonal antibody to protein tyrosine kinase-7 (PTK7) from Miltenyi Biotech (Bergisch Gladbach, Germany). Live-gated lymphocytes were simultaneously acquired with internal bead standards (Flow-Count fluorospheres, Beckman Coulter) by multiparameter flow cytometry (Navios, Beckman Coulter) to obtain absolute cell counts. Expression of CD127 and CD31 was evaluated by determining the proportion of cells with positive staining and their mean fluorescence intensity. Cell viability was assessed by flow cytometric scatter analysis and colabeling with 50 nM 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6(3)$; Invitrogen, Carlsbad, CA) and propidium iodide (Sigma-Aldrich, St. Louis, MO) as instructed by the manufacturers. To determine whether cultured cells had undergone cellular proliferation, CB T cells (1×10^6/mL) were first pulsed with carboxyfluorescein diacetate succinimidyl ester (CFSE) (5 μM; Invitrogen), then quenched with 50% fetal calf serum, and washed 2 times in phosphate-buffered saline before initiation of cultures. The population of cells that have undergone successive cellular divisions was characterized by serial halving of the fluorescence intensity.

Unstimulated CB T Cell Cultures

Unstimulated CB T cell cultures were established at 1×10^5/well in round bottom 96-well plates in complete RPMI medium containing 2 mM L-glutamate, 2 mM pyruvate, 10% heat-inactivated human AB serum (J. Boy Institute, Reims, France) and antibiotics. Depending on the experiments, cultures of CB T cells were either matched with cultures of adult T cells or run in parallel in the presence or absence of recombinant human IL-7 (R&D Systems) at an initial concentration of 25 to 1000 pg/mL, with additional IL-7 added daily thereafter at the same concentration. After different times of culture at 37°C and 5% CO_2, the number of viable cells and cellular proliferation were determined as described above.

One-way Mixed Lymphocyte Reactions

Mixed lymphocyte reactions (MLR) were established in triplicate using unlabeled or CFSE-labeled CB T cells (1×10^5 cells/well) as responders. Allogeneic monocytes from adults with 4 to 6/6 HLA disparities with the responders, including at least 1 HLA-DR disparity, were chosen as stimulators (1×10^4 cells/well). HLA typing for A, B, and DR loci was done in our tissue typing laboratory. Proliferation was assessed after 5 days by CFSE dye dilution or by pulsing with methyl ^{3}H-thymidine (18.5 kBq/well; PerkinElmer, Waltham, MA) for the final 18 hours of the culture and microbeta scintillation counting (TopCount Microplate Scintillation Counter, PerkinElmer). Interferon-γ enzyme-linked immunosorbent spot assays (IFN-γ ELISPOT) were performed with 2×10^5 viable cells harvested after 7 days from the triplicate MLR cultures and recultured for an additional 20 hours in immunospot plates coated with a monoclonal antibody to IFN-γ (TB-SPOT TB, Oxford Immunotech, Oxford, UK). IFN-γ spots were enumerated by using TB Scan software (Immunoscan, CTL-Europe, Bonn, Germany).

Statistical Analysis

Where appropriate, comparisons were made using the Mann-Whitney U-test or the Kruskal-Wallis test with Dunn post-test evaluation and the Wilcoxon signed rank test for pairwise comparisons. Correlations were based on Spearman's ρ. Multivariate analysis (general linear model analysis) was used for association between multiple variables.

Table 1
Analysis of the Association between Clinical Characteristics of CB Donors and the Viability of their CB T cells after Four Days in Complete Culture Medium without Exogenous Cytokine Supplementation

Characteristic	Median (range)	Correlation with CB T Cell Viability*			
		Spearman's ρ	*P* Value	β†	*P* Value
Gestational age at partition, wk	40 (37.8-42)	.022	NS		NS
Infant birth weight, kg	3.49 (1.98-4.43)	−.376	.031	−.471	.023
Infant gender (M/F)‡	12/22		NS		NS
Apgar score	10 (2-10)	.054	NS		NS
Venous CB pH	7.37 (7.14-7.48)	.397	.027	.609	.005
CB plasma IL-7 level (pg/mL)§	0 (0-6.67)	.335	NS		NS
RTE: $CD4^+$ $CD31^+$ $PTK7^+$ cells (%)	76.6 (61-85)	−.59	NS		NS

NS indicates not significant; M, male; F, female.

* Cryopreserved CB T cells were thawed and maintained in complete culture medium without addition of exogenous cytokines, and viable cells ($DiOC_6$(3)-positive and propidium iodide−negative) were enumerated after four days.

† Multivariate analysis by using the general linear model. Only values achieving a significance of $P < .05$ are shown.

‡ Mann-Whitney U test was used to test for differences by gender.

§ Normal values in healthy adults: median 2 pg/mL (range, .6 to 7.7 pg/mL) [15].

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