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Expansion of T-cells from the cord blood graft as a predictive tool for complications and outcome of cord blood transplantation

Jens Gertow [a,*], Sofia Berglund [a], Mantas Okas [a], Klas Kärre [b], Mats Remberger [a], Jonas Mattsson [a], Michael Uhlin [a,*]

[a] *Centre for Allogeneic Stem Cell Transplantation and Division of Clinical Immunology, Karolinska University Hospital Huddinge, SE-17177 Stockholm, Sweden*
[b] *Strategic Research Center IRIS, Dept of Microbiology, Tumor and Cell Biology, Karolinska Institutet, SE-17177 Stockholm, Sweden*

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Abstract We have previously successfully expanded functional T-cells *in vitro* from cord blood grafts used for clinical transplantation, with the aim of creating donor lymphocyte infusions to treat e.g. malignant relapse. Here we show that the T-cell expansion in addition might work as a prognostic tool for complications after transplantation. We used multi-color flow cytometry to correlate *in vitro* phenotypical and functional data from 33 expansions to clinical outcome post-transplantation. Higher levels of CD69+ activated T-cells in the expansion were associated with prolonged survival of the patient. In addition, we found a correlation between T-cell expansions containing relatively high levels of effector memory T-cells and graft vs. host disease and relapse. Our data suggest that expansions of cord blood T-cells from the graft might not only be used as donor lymphocyte infusions, but also as *in vitro* indicators that could give essential information on how to manage cord blood transplanted patients.

1. Introduction

Umbilical cord blood (CB) is an alternative source of hematopoietic stem cells for allogeneic stem cell transplantation (ASCT) when no HLA-identical adult donor is available [1,2]. The rapid availability of cryopreserved CB units in combination with a higher permissiveness to HLA mismatch has increased the interest in CB as stem cell source even further [3,4]. However, there is still hesitation in using CB partly because this source of stem cells offers no possibility of

* Corresponding authors at: Centre for Allogeneic Stem Cell Transplantation and Division of Clinical Immunology, B87, Karolinska University Hospital Huddinge, SE-141 52 Stockholm, Sweden. Fax: +46 8 746 6699.
E-mail addresses: Jens.Gertow@ki.se (J. Gertow), Michael.uhlin@ki.se (M. Uhlin).

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additional donor lymphocyte infusion (DLI) after transplantation. DLI is routinely administered after bone marrow transplantation and peripheral blood stem cell transplantation as an approach to increase the graft-vs.-leukemia effect and/or to treat a threatening relapse or rejection [5,6]. In order to make DLI available also for CB transplanted patients, we have previously *in vitro* expanded and thoroughly characterized T-cells from given cord blood grafts [7].

In our previous paper, we showed that expansion of T-cells from the given graft was feasible after CD3/CD28 crosslinking and culture in clinical-grade interleukin-2 (IL-2). Sufficient numbers of cells were obtained within 8 days of culture in 13 of 13 expansions. We also compared expanded CB T-cells in terms of phenotype and function to adult peripheral blood T-cells, which form the basis of the current modality of immunotherapy after allogeneic stem cell transplantation, as well as to CB cells acquired *ex vivo*. The expanded T-cells acquired an activated phenotype and could be induced to produce cytokines by both allo- and mitogenic stimuli. When exposed to allogeneic targets, expanded CB T-cells proliferated at levels comparable to those of their e*x vivo* and adult blood counterparts.

To date, we have expanded T-cells from a total of 33 cord blood grafts. As the grafts show diverse *in vitro* proliferation potential and phenotypic markers, we wanted to determine whether the *in vitro* features might reflect *in vivo* characteristics after transplantation. In the present study, we correlate the proliferative expansion rate, phenotypic features and the capacity to produce cytokines of the T-cells from the 33 expansions by multi-color flow cytometry and Luminex assays to clinical features of the patients receiving the corresponding cord blood grafts. It should be noted that the expanded T-cells have not been given to the patients and that future studies will reveal whether these T-cell expansions will be functional as DLI. Regardless of their DLI potential, however, the present study aims to use the T-cell expansions as predictive tools. Furthermore, the expansions are not intended to represent the whole unmanipulated graft, but rather as indicators for the quality of the CB units.

Our findings show that higher levels of activated CD69+ T-cells in the expansion are associated with prolonged patient survival. Additionally, a T-cell expansion containing relatively high levels of effector memory cells is a sign of increased risk of transplant-related complications such as graft vs. host disease (GVHD), bacteremia and post-transplant lymphoproliferative disorder (PTLD). In essence, the present study suggests that T-cell expansion of cord blood grafts may work as a prognostic tool for post-transplant-related issues.

2. Material and methods

2.1. Patient characteristics

Between March 2007 and March 2010, we expanded T-cells from 33 CB units used for 26 hematopoietic stem cell transplantations in 24 patients at our ward. 16 patients were male and 8 were female, and median age was 17 years (range 0.5–65 years, n=24). Two of the patients were retransplanted with another CB unit after rejection of the first. Of the 33 CB units, 14 were used in 7 double CB transplantations (DCBTs) and 19 as single units. Median follow-up time was 191 days (range 5–1364). This study was approved by the Ethical Board at Karolinska Institutet, Sweden. Transplantation characteristics are described in more detail in Table 1.

2.2. Graft vs. host disease-prophylaxis and supportive care

The graft vs. host disease (GVHD)-prophylaxis used was mainly Cyclosporine A (CsA) combined with prednisolone (n=24). The GVHD prophylaxis for the two remaining transplantations was CsA alone, or Tacrolimus and Sirolimus in combination. ATG (Thymoglobulin; Genzyme, Cambridge, MN) was given to all patients except one (CB1-2) at a median total dose of 6.0 (range 5.3–8.3) mg/kg. Higher doses were given to patients with non-malignant diseases. Supportive care has been described in detail previously [8].

2.3. Antibodies and reagents

Fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, and allophycocyanin (APC)-labeled anti-CD8 (RPAT8); APC-Cy7-anti-CD8 (SK1); FITC-, PE-, PE-Cy5-, V450-, and APC-labeled anti-CD3 (UCHT1); peridinin–chlorophyll–protein complex (PerCP)- and PE-Cy7-labeled anti-CD3 (SK7); APC-labeled anti-CD4 (RPA-T4); FITC- and APC-labeled anti-CD45RO (UCHL1); PE-labeled anti-CD45RA (HI100); FITC-, PE-, and PE-Cy5-labeled anti-CD28 (CD28.2); FITC- and PE-labeled anti-CD25 (M-A251); FITC-labeled anti-CD56 (MCAM16.2); FITC-labeled anti-CD94 (HP-3D9); FITC-labeled anti-T-cell receptor (TCR) (WT31); FITC-labeled anti-CD69 (FN50); PE-Cy7-labeled anti-CCR7 (3D12); FITC-labeled anti-TCRVg9 (B3); PE-Cy7-labeled anti interferon-gamma (IFN-γ) (B27); APC-labeled anti- tumor necrosis factor α (TNF-α) (Mab11); and PerCP Cy5.5-labeled anti-CD4 (L200) were purchased from BD Biosciences (Franklin Lakes, NJ). FITC-labeled anti-TCR-gamma-delta pan (IMMU510) was purchased from Beckman Coulter (Fullerton, CA), and PE-labeled anti-CCR7 (150503) and FITC-labeled anti- interleukin-4 (IL-4) (3007) were from R&D Systems (Minneapolis, MN). FITC-labeled anti-forkhead box P3 (FoxP3) (236A/E7) and PE-labeled anti-interleukin-17 (IL-17) (eBio64DEC17) were obtained from eBioscience (San Diego, CA), and PE-Texas Red-labeled anti-CD4 (RFT-4 g) was ordered from Abcam (Cambridge, UK).

2.4. Clinical expansion of cord blood-derived T-cells

The procedure of expanding CB-derived T-cells has been described in detail previously [7]. Briefly, on the day of transplantation, termed day 0, five percent of the mononuclear cells from the thawed CB unit were processed and cultured under GMP-like conditions at our institution. T-cells were positively selected using anti-CD3/CD28 beads (Dynabeads ClinExVivo; Invitrogen Dynal AS, Oslo, Norway), then counted and resuspended at a concentration of 3×10^5 cells/mL in complete RPMI-1640 medium supplemented with recombinant IL-2 at 200 IU/mL (Proleukin; Chiron, Amsterdam, the Netherlands). The cells were maintained at concentrations between 3×10^5 and 5×10^5 during the expansion phase (8–10 days). Expanded