Ex Vivo Expansion of Suboptimal (Low Dose) Cord Blood Products Provides Sufficient Cells for Transplantation of Adult Patients

Abstract

Background: Recent trials have demonstrated the potential to combine two cord blood (CB) products to increase the cell dose delivered, however often CB products with sufficient cell dose, have a 2 antigen mismatch to each other or the recipient. Better matched products are routinely available however, their use is limited due to low cell content. We have developed ex vivo expansion techniques that can provide a 10 to 20 fold increase in cell numbers and evaluated the potential of ex vivo expansion to provide sufficient doses for these suboptimal products.

Methods: CB products which were frozen for banking and rejected due to low cell doses (median cells dose 0.7x10^7 total nucleated cells (TNC)/kg for a 50kg individual) were expanded using co culture on allogeneic mesenchymal stem cells (MSC). The CB products were thawed and the mononuclear cell (MNC) fraction isolated by density gradient centrifugation. The CB MNCs were cultured in T162 cm^2 tissue culture flasks pre established with MSC in expansion media plus SCF, G-CSF and Tpo. After culture for 2 weeks the TNC/kg were determined.

Results: A median fold expansion of 9 fold was obtained (N=5) resulting in a median cell dose post expansion of 4.3 x10^7 TNC/kg (for a 50 kg individual). In addition, fractionation of the CD34+ve and CD34-ve subsets from the CB MNC, demonstrated that the expansion was almost exclusively from the CD34+ cells.

Discussion: These data demonstrate that co culture of CB MNCs on MSC result in significant expansion of suboptimal CB products. The expanded CB products would meet the minimal cell requirements for use of CB in adults and therefore provide the potential use of better matched CB products. This could result in enhanced engraftment and less toxicities such as graft versus host disease that occurs with lower level HLA matched CB products.

Keywords: Cord Blood; Ex vivo expansion; CD34+ cells

Introduction

The use of umbilical cord blood (CB), as a source of hematopoietic cells for patients undergoing high dose chemotherapy, has become a standard therapeutic option for pediatric patients with hematologic malignancies [1-5]. However, the low cell doses in CB products has limited the use in adult patients due to delayed engraftment and survival [6,7]. This has led to evaluation of approaches to increase the cell dose by ex vivo expansion [8-10]. Several groups have evaluated the potential of ex vivo expanded CB units to enhance engraftment [8-10], however, the results to date have failed to demonstrate significant improvement in the time to neutrophil engraftment. We have proposed that current methodologies employed in these trials, which include selection of CD34+ or CD133+ cells from frozen CB units, are problematic due to significant cell losses during selection and low purities in a number of products [10,11]. Based upon the issues associated with selection of frozen CB units, we have developed culture methods for co culture of CB mono nuclear cells (CB-MNC) on mesenchymal stem cells (MSC) [11]. We have demonstrated an expansion of total nucleated cells (TNC) of 10 to 20 fold and clinical trials have demonstrated decreased time to neutrophil and platelet engraftment [12].

Another approach that has been explored for delivering higher cell doses with CB products is the use of two CB products [13]. Wagner and colleagues have demonstrated the feasibility of this approach for use in adults; however the majority of recipients receive 4 of 6 HLA CB products. A number of patients have better matched CB products available (5 of 6 or 6 of 6), however these products have low cell doses (<2x10^7 TNC/kg) and are not suitable for use. We hypothesized that the co culture expansion conditions we developed could be suitable to expand suboptimal CB products, ie products with cell doses below 2x10^7 TNC/kg, to provide a sufficient cell dose for adult patient and the use of better matched products would result in decreased immunological complications including graft versus host disease (GVHD).

Methods

Cord blood products

Frozen CB products: Frozen CB products were supplied by Joanne Kurtzberg (Duke University, Durham, NC). The products had been frozen for banking however they were deemed unsuitable due to low cell numbers. All products were collected under a Duke University IRB approved protocol with signed consent. The products were shipped in a dry shipper and stored in liquid nitrogen until used.
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**Fresh CB products:** Fresh CB products were supplied by Dr. Pablo Rubenstein (New York Cord Blood Bank, New York, NY) and were shipped overnight at room temperature. These products were collected for banking under appropriate IRB approval and signed consent but were deemed unsuitable for banking due to low cell numbers or other issues. CB-MNC were isolated from fresh CB products by density centrifugation over ficoll and the low density cells recovered. CD34+ cells were isolated from the CB-MNC using the Vario MACS selection device (Miltenyi Biotech, Cologne, Germany). Expansion was performed on MSC as described below in Stemline II media plus growth factors.

**Isolation of MSC**

Bone marrow cells were purchased from Allcells Inc (Emeryville, CA) who obtained the BM aspirates from normal donors under appropriate IRB approvals. The MNC fraction was isolated by ficoll separation and MSC were grown to confluency in T162 cm² tissue culture flasks (Corning, Acton, MA) in alpha MEM plus 20% FCS.

**Ex Vivo expansion of CB-MNC on MSC**

CB products were thawed and washed, resulting in a median of 29 fold expansion of CD34+ cells. Co-culture of CB-MNC on MSC resulted in a median of 15 fold expansion of TNC with a 3 fold expansion of TNC and a 4 fold expansion of CD34+ cells. The levels of TNC and CD34+ cells harvested from the CB MNC fractions. CB MNC, CB CD34+ and CB CD34- cell populations were collected for banking under appropriate IRB approval and signed consent but were deemed unsuitable for banking due to low cell numbers or other issues. CB-MNC were isolated from fresh CB products by density centrifugation over ficoll and the low density cells recovered. CD34+ cells were isolated from the CB-MNC using the Vario MACS selection device (Miltenyi Biotech, Cologne, Germany). Expansion was performed on MSC as described below in Stemline II media plus growth factors.

**Flow cytometric analysis**

Cells were analyzed for phenotypic expression of surface proteins specific for subpopulations of hematopoietic cells. Approximately 1 million cells were stained with anti-CD34-PE and anti-CD45-FITC (Becton-Dickinson, San Jose, CA) and a minimum of 50,000 events collected in a list mode file format by flow cytometry (FACS Vantage, Becton-Dickinson). Aliquots of cells were also stained with isotype control antibodies.

**Results**

**Expansion of CB mononuclear cells on MSC**

CB products were thawed and washed, resulting in a median of 3.3x10^7 TNC/kg (range 2.2 to 16x10^7 TNC/kg) (Table 1). For a 50 kg recipient, the expanded CB product would be equivalent to 4.3x10^7 TNC/kg (range 2.2 to 16x10^7), with all expanded products reaching the minimal target dose of 1x10^7 TNC/kg (Table 2). In fact all expanded products contained more than 1x10^7 TNC/kg based upon a 100kg recipient.

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**Table 1:** Ex Vivo Expansion of TNC from Frozen CB Products.

**Expansion of CB CD34+ Cells on MSC**

We hypothesized that the major cell population in the CB units responsible for expansion would be CD34+ cells. To test this hypothesis we fractionated CB MNC into CD34+ and CD34- fractions. Expansion of CB CD34+ cells populations were then cultured on preformed layers of MSC. Ten (10) T162 cm² tissue culture flasks (Corning, Acton, MA) in alpha MEM plus 20% FCS.

<table>
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**Table 2:** Cell Doses Based Upon Recipient Weight of 50kg (A) and 100kg (B).

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Discussion

We would propose two potential advantages to the use of co-culture for expansion based upon the above results. Firstly the possible enhanced engraftment and secondly the ability to use better matched CB products that may have a low cell dose. Wagner and colleagues have described the use of two CB products to provide an increased cell dose; however, the majority of patients receive a 2 antigen mismatched CB unit [13]. Better matched CB units are routinely identified but are not suitable due to low cell doses. The expansion of the better matched CB units could potentially result in decreased toxicities relating to graft versus host disease (GVHD).

The culture conditions used in this study have been used in previous clinical trials of ex vivo expansion of both CB [10] and peripheral blood progenitor cell products (PBPC) [14]. In particular the expanded PBPC products resulted in faster neutrophil engraftment which correlated with the total nucleated cells (TNC) infused [14]. In addition, we have previously reported that CD34 selection of frozen CB products results in low purities and low levels of expansion [11]. When CB MNC were cultured for 14 days, little if any expansion resulted, however, co-culture on MSC resulted in significant expansion of TNC and progenitor cells (CD34+ cells and CFU) [11]. The expanded CB products contain high numbers of neutrophils and promyelocytes and less than 1% T and B lymphocytes [10,11]. Although the focus of this study was expansion of TNC, our previous studies have demonstrated that long term repopulating stem cells are decreased following expansion. When ex vivo expanded CB cells were injected into fetal sheep the levels of human engraftment were decreased compared to sheep transplanted with non expanded CB cells [15]. Based upon these data, the clinical trial design currently being undertaken with co-culture of CB MNC on MSC [12], utilizes two CB products with the expanded CB product providing enhanced short term engraftment while the un expanded CB product provides the long term engraftment. This study reported the median time to neutrophil and platelet engraftment of 15 days and 42 days respectively [12]. The median fold expansion of TNC was 12 fold. This study has led to a phase III trial for evaluation of CB expansion on MSCs.

A second clinical trial of expanded CB cells is being conducted at the Fred Hutchinson Cancer Center by Dr. Bernstein and colleagues [16]. This study targets expansion of CD34+ cells using an engineered form of the Notch ligand, Delta 1. Preliminary data was reported at meeting of the American Society of Hematology on the first 6 patients enrolled in this study and reported rapid engraftment with a median time to neutrophil recovery of 14 days (range 7-34 days).

Taken together these studies demonstrate the potential of expanded CB cells to provide more rapid engraftment. The data we have presented in this report expands the range of CB products that may be used for therapy. The clinical trials described above utilize CB products with higher cell numbers and typically CB products with smaller cell doses would not meet minimum cell dose requirements. Testing of this approach will require a clinical trial to evaluate the engraftment potential of these expanded products.

References


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