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Clinical Research: Alternative Donors

General and Virus-Specific Immune Cell Reconstitution after Double Cord Blood Transplantation

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A B S T R A C T

Cord blood transplantation (CBT) is curative for many patients with hematologic malignancies but is associated with delayed immune recovery and an increased risk of viral infections compared with HLA-matched bone marrow or peripheral blood progenitor cell transplantation. In this study we evaluated the significance of lymphocyte recovery in 125 consecutive patients with hematologic malignancies who underwent double-unit CBT (DUCBT) with an antithymocyte globulin–containing regimen at our institution. A subset of 65 patients was prospectively evaluated for recovery of T, natural killer (NK), and B cells, and in 46 patients we also examined viral-specific T cell recovery against adenovirus, Epstein-Barr virus, cytomegalovirus, BK virus, respiratory syncytial virus, and influenza antigen. Our results indicate that in recipients of DUCBT, the day 30 absolute lymphocyte count is highly predictive of nonrelapse mortality and overall survival. Immune recovery post-DUCBT was characterized by prolonged $CD8^+$ and $CD4^+$ T lymphopenia associated with preferential expansion of B and NK cells. We also observed profound delays in quantitative and functional recovery of viral-specific $CD4^+$ and $CD8^+$ T cell responses for the first year post-CBT. Taken together, our data support efforts aimed at optimizing viral-specific T cell recovery to improve outcomes post-CBT.

INTRODUCTION

Umbilical cord blood (CB) is being increasingly used as a source of hematopoietic stem and progenitor cells for allogeneic stem cell transplant candidates lacking suitable matched donors. Although CB transplantation (CBT) is successful in many patients, its efficacy has been restricted by slow hematopoietic and immunologic reconstitution because of the quantitative and qualitative differences in the composition of CB grafts [1-5]. Although the frequency of hematopoietic stem and progenitor cells is greater in CB units, CB grafts contain an average of 1 to 2 logs fewer total cells compared with peripheral blood (PB) or bone marrow allografts. Moreover, the vast majority of T, B, and dendritic cells in CB grafts are immature [6,7], which likely explains the low rates of graft-versus-host disease (GVHD) seen after CBT given the degree of HLA mismatches typically used [8,9]. The use of dual CB grafts represents a potentially important approach to reducing nonrelapse mortality (NRM) among patients undergoing double unit CBT (DUCBT), particularly in adult patients. In this setting, although 2 CB units are initially transplanted, only 1 provides prolonged engraftment and becomes the "dominant" engrafted unit. Yet, even after DUCBT, severe complications related to infections remain a major cause of morbidity and mortality [10-15]. Although this may be a

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Table 1
Patient Characteristics (N = 125)

Characteristic	Value
Age (years), median, range	49 (18-73)
Gender	
Female	61 (49%)
Male	64 (51%)
Diagnosis	
AML	64 (51%)
ALL	20 (16%)
CML/CLL	22 (18%)
NHL/HL/MM	19 (15%)
Disease status	
CR1/CP1	26 (21%)
CR2/CP2	30 (24%)
Advanced disease	69 (55%)
Recipient CMV serostatus	
Seropositive	109 (87%)
Seronegative	16 (13%)
Conditioning	
Bu/Clo/thiotepa	62 (50%)
Flu/Bu/Clo/TBI 200 cGy	46 (37%)
FM	10 (8%)
Flu/Bu	7 (6%)
GVHD prophylaxis	
Tacrolimus/MMF	125 (100%)

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; MM, multiple myeloma; CR, complete remission; CP, chronic phase; Bu, busulfan; Clo, clofarabine; Flu, fludarabine; TBI, total body irradiation; FM, fludarabine and melphalan; MMF, mycophenolate mofetil.

consequence of the lower cell dose in CB grafts, it also reflects the relative immaturity of CB immune subsets.

A number of studies have reported on immune reconstitution after single CBT [16-20], but few have studied immune recovery after DUCBT [21-23]. Here we report the results of a prospective longitudinal study of immune recovery and viral-specific T cell reconstitution in recipients of double CB grafts. Our results indicate that the day 30 absolute lymphocyte count (ALC30) is highly predictive of NRM and overall survival (OS) in recipients of DUCBT who receive serotherapy for GVHD prophylaxis and that recovery of quantitative T cells as well as recovery of functional (cytokine-producing) viral-specific T cells is delayed.

METHODS

Patient Selection and Management

One hundred twenty-five consecutive adult patients undergoing DUCBT at our institution from January 2006 to November 2011 were studied (Table 1). Less than half of the patients (45%) were in first or second complete remission or first or second chronic phase disease, whereas the rest had advanced disease at the time of transplant. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki for protocols approved by the MD Anderson Cancer Center Institutional Review Board.

All patients received serotherapy with rabbit thymoglobulin 1.25 mg/kg on day −4 and 1.75 mg/kg on day −3. GVHD prophylaxis consisted of tacrolimus and mycophenolate mofetil (1 g p.o. twice daily), with a taper of mycophenolate mofetil at day 100 and tacrolimus at 6 months if no GVHD was present. In the event of confirmed or suspected GVHD, initial therapy consisted of methylprednisolone (2 mg/kg/day), with a taper based on clinical response.

The surveillance for cytomegalovirus (CMV) was performed by antigenemia assay in patients with absolute neutrophil counts > 1000/μL or with quantitative PCR if the absolute neutrophil count was lower. This was done twice weekly for the first 100 days after CBT or longer if any complications were present. Other viruses including adenovirus (AdV), Epstein-Barr virus (EBV), BK virus (BKV), respiratory syncytial virus (RSV), human herpesvirus 6, influenza, and parainfluenza were tested as clinically indicated. Donor engraftment was assessed using PCR with primer sets flanking microsatellite repeats.

Immunophenotyping

Immunophenotyping was performed by the flow laboratory at MD Anderson Cancer Center on PB samples collected at days +30, +100, and +180 and 1 year post-CBT. PB mononuclear cells were surface stained with monoclonal antibodies against CD3, CD4, CD8, CD19, and CD56 (all from BD Biosciences, San Jose, CA). Cells were acquired on a Cyan flow cytometer (Dako, Fort Collins, CO) and data analyzed with FlowJo software (Tree Star, Ashland, OR).

Enzyme-Linked Immunospot Assay

In a subset of 46 patients, IFN-γ enzyme-linked immunospot analysis was used to quantitate the frequency of T cells that secreted IFN-γ in response to hexon and penton (Adv); IE1 and pp65 (CMV); EBNA1, EBNA3a-c, LMP1, LMP2, and BZLF1 (EBV); VP1 and large T (BKV); N and F (RSV); and MP1 and NP1 (influenza) pepmixes (JPT Peptide Technologies GmbH, Berlin, Germany), all diluted to 1 μg/mL per peptide. Staphylococcal enterotoxin B (1 μg/mL; Sigma-Aldrich, Spring, TX) was used as positive control. PB mononuclear cells collected before and after transplant were resuspended at 2×10^6/mL in T cell media (Advanced RPMI 1640 [Life Technologies, Grand Island, NY] supplemented with 45% Click's medium [Irvine Scientific, Santa Ana, CA], 2 mM GlutaMAX [Life Technologies], and 10% FBS [Hyclone, Logan, UT]). Each condition was run in duplicate. After 20 hours of incubation, plates were developed, dried overnight at room temperature in the dark, and then sent to Zellnet Consulting for quantification. Spot-forming cells (SFCs) and input cell numbers were plotted, and the frequency of T cells specific to each antigen was expressed as specific SFC per input cell numbers.

Statistical Analyses

Actuarial OS was estimated using the Kaplan-Meier method. The cumulative incidence of NRM was estimated considering disease progression or death attributable to malignancy as competing risks. Predictors of NRM were evaluated in landmark analysis using Cox proportional hazards regression analysis. Engrafted patients who were alive and progression-free on the date ALC30 was measured were eligible for the risk factor analysis. ALC30 was evaluated in quartiles. All analyses were performed using STATA 11 (StataCorp LP, College Station, TX), and statistical significance was defined at the .05 level.

RESULTS

Clinical Characteristics and Clinical Outcome

One hundred twenty-five adult patients with high-risk hematologic malignancy who underwent DUCBT during the study period were assessed, with a median follow-up of 979 days (range, 56 to 1907) in surviving patients. Details of the conditioning regimens are included in Table 1. Thirty percent of patients received 2 unmanipulated CB units, and 70% received 1 unmanipulated and 1 CB unit that was expanded before infusion as previously described [24]. Successful neutrophil engraftment, as defined by the first date of 3 consecutive days of absolute neutrophil count $\geq .5 \times 10^9$/L, was achieved in 110 patients, whereas 9 patients

Table 2
Donor Engraftment at Days +30 and +100 Post-DUCBT

	Day +30			Day +100		
	Total	T Cell	Myeloid Cell	Total	T Cell	Myeloid Cell
Overall	100% (97-100)	100% (100)	100% (99-100)	100% (100)	100% (100)	100% (100)
CB1	82% (39-100)	99% (75-100)	91% (42-100)	99% (60-100)	100% (69-100)	100% (55-100)
CB2	0% (0-31)	0% (0-16)	0% (0-28)	0% (0-16)	0% (0-4)	0% (0-15)

Donor engraftment for total, T cell, and myeloid cell fractions are presented as median percentages, with interquartile range in parentheses.