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Correlation of Infused $CD3^+CD8^+$ Cells with Single-Donor Dominance after Double-Unit Cord Blood Transplantation

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Single-donor dominance is observed in the majority of patients following double-unit cord blood transplantation (dCBT); however, the biological basis for this outcome is poorly understood. To investigate the possible influence of specific cell lineages on dominance in dCBT, flow cytometry assessment for $CD34^+$, $CD14^+$, $CD20^+$, $CD3^-CD56^+$, $CD3^+CD56^+$ (natural killer), and T cell subsets ($CD4^+$, $CD8^+$, memory, naïve, and regulatory) was performed on individual units. Subsets were calculated as infused viable cells per kilogram of recipient actual weight. Sixty patients who underwent dCBT were included in the final analysis. Higher $CD3^+$ cell dose was statistically concordant with the dominant unit in 72% of cases ($P = .0006$). Further T cell subset analyses showed that dominance was correlated more with the naive $CD8^+$ cell subset (71% concordance; $P = .009$) than with the naive $CD4^+$ cell subset (61% concordance; $P = .19$). These data indicate that a greater total $CD3^+$ cell dose, particularly of naïve $CD3^+CD8^+$ T cells, may play an important role in determining single-donor dominance after dCBT.

INTRODUCTION

Cord blood (CB) transplantation (CBT) is an accepted treatment for adults and children with hematologic malignancies [1,2]. To overcome the limited cell doses that can be provided by a single CB graft and more reliably achieve sustained donor engraftment, double-unit CBT (dCBT) is commonly performed in adult and larger adolescent patients [3,4]. Interestingly, in the vast majority of dCBT recipients, only one unit emerges as the source of long-term hematopoiesis [1,2,5]; however, the factors that determine which of the 2 units will achieve single-donor dominance after dCBT remain poorly understood.

Unit parameters, such as total nucleated cell (TNC) and $CD34^+$ cell doses, viability, degree of HLA matching, and order of infusion, have not been uniformly associated with donor dominance [6-8]. Conversely, $CD3^+$ cell dose is emerging as a reliable predictor of single-donor dominance [9-11]. Indeed, a report from our institution presented the first direct evidence that only effector T cells derived from the dominant unit produce IFN-γ in response to cells derived from the nonengrafting unit [12]. More recently, we also provided strong evidence indicating that the unit with greater CD3 chimerism at day 7 is more likely to be the dominant unit [13].

In the present study, we further investigated the association between infused CD3 cell dose, along with possible correlations of specific T cell subsets ($CD3^+/CD8^+$, $CD3^+/CD4^+$, naïve/memory, and regulatory), and the emergence of single-unit donor dominance. An extended immunophenotyping flow cytometry panel was performed on a sample obtained from each CB unit just before infusion in 72 consecutive patients undergoing dCBT for hematologic malignancies. Infused cell doses were calculated and used to investigate whether any specific T cell subsets were also highly correlated with single-donor dominance.

METHODS

Patient/Donor Characteristics

Between January 2008 and May 2011, 72 patients received a dCBT at our institution. Twelve patients (17%) were excluded because they either died before obtaining informative chimerism data or had primary/secondary graft failure. In accordance with institutional priority, patients received a CBT if they lacked an available HLA-compatible related or matched unrelated donor. All patients received unrelated donor CB grafts, which were 4/6- to 6/6-matched at HLA-A, -B, and -DRB1 antigens. HLA typing was performed at the antigen level for HLA-A and -B, and high-resolution HLA typing was performed for HLA-DRB1 alleles. The individual CB units were at least 3/6 HLA-A, -B, and -DRB1 matched to one another, and each contained a minimum of 1.5×10^7 TNCs/kg. All study activities were approved by the Fred Hutchinson Cancer Research Center Institutional Review Board, and all participants provided written informed consent in accordance with the principles of the Declaration of Helsinki.

Conditioning Regimens and GVHD Prophylaxis

Myeloablative conditioning consisted of cyclophosphamide 60 mg/kg i.v. daily for 2 days, total body irradiation (TBI) 1320 or 1200 cGy, and fludarabine 40 mg/m^2 i.v. daily for 3 days. Eleven patients received fludarabine 30 mg/m^2 i.v. daily for 5 days, treosulfan at 14 g/m^2 i.v. daily for 3 days, and a single fraction of TBI 200 cGy. Reduced-intensity conditioning consisted of fludarabine 40 mg/m^2 i.v. daily for 5 days, a single dose of cyclophosphamide 50 mg/kg i.v., and a single fraction of TBI 200 or 300 cGy. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine A along with mycophenolate mofetil. Emergent GVHD was treated at the physician's discretion.

Cell Dose Analysis

All CB units were thawed and washed by centrifugation before being resuspended in preparation for infusion. Units were infused sequentially with an interval of <45 minutes between each infusion. A small aliquot was removed from the final product just before infusion for analysis. This sample was then processed for measurement of TNCs and flow cytometry

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assessment of graft composition. In particular, the following viable (7-AAD negative) cell subsets were measured by multicolor fluorescence-activated cell sorting analysis and then expressed as infused cell subsets per kilogram of actual recipient weight: stem/progenitors ($CD34^+$), monocytes ($CD14^+$), B ($CD20^+$), natural killer (NK; $CD3^-CD56^+$), NKT ($CD3^+CD56^+$), and T cell subsets $CD3^+CD4^+$, $CD3^+CD8^+$, $CD45RA^-/CD45RO^+$ (memory) $CD45RA^+/CD45RO^-$ (naïve), and $CD4^+CD25^+CD127^{lo}$ for regulatory T cells. Data were used to calculate total cell subsets per kilogram of recipient weight for each CB unit.

Chimerism Analysis

Analysis of host and CB unit chimerism was performed on flow cytometry–sorted $CD3^+$, $CD56^+$, and $CD33^+$ fractions of peripheral blood on days 7, 14, 21, 28, 56, and 80 after transplantation. Whole bone marrow chimerism analysis was performed on days 28, 56, and 80 after transplantation. DNA chimerism analyses were performed by amplified fragment length polymorphism (detection sensitivity, 1%-5%; range of accuracy, ±5%) [14]. Single-donor dominance was generally defined as >95% single-unit-derived cells in all fractions. In the rare case where the 2 CB units engrafted long term, the unit contributing to >60% hematopoiesis was considered the dominant one. Similarly, in patients who died before day 100, the dominant unit was considered the unit contributing to >60% hematopoiesis at the time of the last chimerism analysis.

Statistical Analysis

If the infused dose of a cell subset was not associated with subsequent development of single-donor dominance, then the expected concordance between the dominant unit and a higher or lower dose of that subset should be the random chance of 50% (eg, coin flip). Thus, a one-sample test of the null hypothesis that a binomial proportion is equal to 0.5 was used to test the pairwise association of concordance of the relatively higher cell subset dose with unit dominance. Similarly, in a separate analysis, if there was no association between cell dose and unit dominance, then the expected mean difference in dose between winning and losing units should be zero. Accordingly, a one-sample *t* test was used to test the null hypothesis that the mean difference in cell dose between winning and losing units was zero. The Wilcoxon rank sum test was used to compare graft characteristics in dominant and nondominant units.

Table 1
Patient Characteristics (n = 60)

Characteristic	Value
Age, years, median (IQR)	38.3 (18.6-51.4)
Female sex, n (%)	30 (50)
Weight, kg, median (IQR)	72.0 (59.9-79.8)
Cytomegalovirus serostatus, n (%)	
Positive	36 (60)
Negative	24 (40)
Transplant type, n (%)	
Myeloablative	46 (77)
Nonmyeloablative	14 (23)
GVHD prophylaxis with cyclosporine/mycophenolate, n (%)	60 (100)
Disease, n (%)	
Acute lymphoblastic leukemia	18 (30)
Acute myelogenous leukemia	26 (44)
Myelodysplastic/myeloproliferative diseases	5 (8)
Chronic lymphocytic leukemia	4 (7)
Other	7 (11)
Donor–recipient sex match, n (%)	
Match–match	22 (37)
Match–mismatch	28 (47)
Mismatch–mismatch	10 (16)
HLA disparity, n (%)*	
4/6 + 4/6	26 (43)
4/6 + 5/6	9 (15)
4/6 + 6/6	—
5/6 + 5/6	13 (22)
5/6 + 5/6	8 (14)
6/6 + 6/6	4 (6)
Days to engraftment, median (IQR)†	
Myeloablative	25 (19-31)
Nonmyeloablative	13 (7-18)

* HLA matching reflects the lowest HLA match of the 2 units.
† The day of neutrophil engraftment was defined as the first of 3 consecutive days of an absolute neutrophil count of ≥500/μL.

RESULTS

Patient and Graft Characteristics

Sixty patients were included in the final analysis. Patient, transplantation, and graft characteristics are summarized in Table 1. Conditioning regimens were myeloablative (MC) in 46 patients (77%) and nonmyeloablative (NMC) in 14 patients (23%). The median time to neutrophil engraftment was 13 days (interquartile range [IQR], 7-18 days) after NMC conditioning and 25 days (IQR, 19-31 days) after MC conditioning. At day +28 posttransplantation, a dominant unit (as defined ≥60% chimerism) was identified in all recipients of MC conditioning. In particular, in all but 1 patient complete contribution from a single unit (as defined ≥95% chimerism) was seen, at a median time of 14 days (IQR, 14-21 days). The only patient with persistent contribution from both units, still present at 1 year posttransplantation, received a treosulfan-based conditioning regimen and 2 6/6 HLA-matched units. In contrast, NMC recipients initially had mixed donor–host chimerism, and single-donor dominance (defined as ≥60% chimerism) was observed in only 4 patients at day +28. The remaining 10 patients converted to single-donor dominance at a median time of 56 days (IQR, 56-80 days) posttransplantation; however, 3 patients still demonstrated significant contributions from both units at day +80.

Among the 120 CB units given to these 60 patients, the degree of matching was as follows: HLA-A, -B, and -DRB1 matched in 15 units; mismatched at 1 antigen in 44 units; and mismatched at 2 antigens in 61 units. HLA mismatch was not a critical factor affecting dominance. Indeed, in 6 cases (10%), the CB unit with the better HLA match to the recipient became the dominant unit, whereas in 9 (15%) cases, the unit with the worse HLA match became dominant. In the remaining cases, both CB units had the same degree of HLA mismatch.

Influence of CD3 + Cells on Determining the Dominant Unit

No significant differences were seen in the median pre-thaw and postwash TNC and CD34+ cell doses and postwash viability between dominant and nondominant CB units (Table 2). For some patients, a sample was not obtained or insufficient material was available from each double CB unit to enable a complete cell subset analysis on both CB units (Table 3). Of the 60 patients in our cohort, 58 had a sufficient sample for analysis of $CD3^+$, $CD4^+$, and $CD8^+$ cell doses. The dominant engrafting unit was the unit with the higher $CD3^+$ dose in 42 patients (72%; $P = .0006$), the unit with the higher $CD4^+$ dose in 41 patients (71%; $P = .002$), and the unit with the higher $CD8^+$ dose in 45 patients (78%; $P < .0001$). In the subset of 14 patients who received NMC, the proportion of positive correlation was identical to that in the whole cohort ($CD3^+$, 10 of 14 [71%]; $CD4^+$, 10 of 14 [71%]; $CD8^+$, 11 of 14 [78%]).

In a smaller cohort of 38 patients (28 with MC and 10 with NMC), further analysis of various $CD4^+$ and $CD8^+$ subsets showed that only the naïve $CD8^+$ cell dose was highly associated with donor dominance (71%; $P = .009$), whereas memory $CD8^+$, memory $CD4^+$, and naïve $CD4^+$ cell doses were not significantly correlated with the dominant unit (Table 3). This association between a higher ratio of infused cell dose and dominance was defined as positive concordance (Figure 1). As expected in CB products, the total $CD8^+$ cell dose was strongly correlated with the majority naïve $CD8^+$ fraction ($R = 0.90$; Figure 2), supporting the concept