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Impact of Viable CD45 Cells Infused on Lymphocyte Subset Recovery after Unrelated Cord Blood Transplantation in Children

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We studied lymphocyte recovery in 88 children who consecutively underwent unrelated cord blood transplantation for malignant (n = 64) or nonmalignant (n = 24) diseases. All children but 3 received myeloablative conditioning regimens with pretransplant antithymocyte globulin. Median age was 5.6 years (0.1-18 years) and median follow-up was 40 months (10-136 months). The median dose of infused viable $CD45^+$ cells (vCD45) was 3.35×10^7/kg with a ratio infused vCD45/collected total nucleated cell at 0.46. Immunologic endpoints were: time to achieve $CD3^+$ >500 and 1500/mm^3, $CD4^+$ >500/mm^3, $CD8^+$ >250/mm^3, $CD19^+$ >200/mm^3, natural killer >100/mm^3. These endpoints were analyzed through the use of cumulative curves for estimating incidence over time in the context of competing risks, and through Fine and Gray models to assess prognostic factors. The median time to reach these endpoints was 33, 97, 214, and 340 days for natural killer, B, CD8, and CD4 cells, respectively. In multivariate analysis, a high infused vCD45 cell dose improved CD3 ($P = .014$) and CD4 ($P = .032$) reconstitutions. A young recipient age also favored CD3 recovery ($P = .013$). With patients grouped according to vCD45 cell dose quartiles, the threshold for a better recovery was 3.35×10^7/kg. Considering the ratio vCD45/TNC, this "immune recovery based" threshold corresponds to a higher cell dose than the minimum usually recommended dose for myelogenous engraftment. This may have important implication for UCB selection.

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INTRODUCTION

Since initial studies reported by Kurtzberg et al. [1], Wagner et al. [2], and Gluckman et al. [3], hematopoietic stem cell transplantation (HSCT) from an unrelated cord blood unit (UCBT) has become an accepted therapeutic strategy for children without an HLA identical donor who need an allogeneic HSCT [4]. The increasing use of CB, relative to bone marrow transplantation (BMT), in the unrelated donor setting is explained by such reasons as noninvasive hematopoietic collection, prompt availability of UCB units, less stringent criteria for HLA matching, lower incidence and severity of graft-versus-host disease (GVHD), and overall similar outcomes with both types of grafts in children [5-7] and adults [8,9]. Despite the low incidence of GVHD, relapse rate of leukemia appears to be similar after UCBT or unrelated BMT [10,11]. Nevertheless, UCBT remains associated with a high incidence of life-threatening infections, especially during the early posttransplant period [10,12,13]. Infection-related mortality is the major cause of nonrelapse mortality following UCBT, and is often directly related to delayed immune recovery [7,14,15]. Several reports have described lymphocyte subset reconstitution and factors affecting their speed of recovery after UCBT [16-20]. Although these studies constitute major contributions, their conclusions may

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be viewed as limited, owing to some of their features such as relatively low number of transplanted patients, patient selection leading to potential bias, and multicentric data resulting in heterogeneity of transplant procedures and evaluation of posttransplant immune recovery. Here, we report a detailed analysis of lymphocyte subset recovery homogeneously conducted in 88 children who consecutively received UCBT in a single pediatric transplant center.

MATERIALS AND METHODS

Patients

Patients treated in our pediatric transplant program were eligible for the study if they met all the following criteria: (1) having received HSCT with a single unrelated cord blood unit, (2) being 18 years old or less at time of transplant, (3) being in remission at the time of transplant for children with malignant diseases. Eighty-eight children consecutively transplanted between February 1997 and December 2007 fulfilled these criteria and were included in the study.

Evaluation of HLA Compatibility and Prefreezing Transplant Dose

Donor-recipient HLA matching was assessed at generic level for HLA-A and -B (ie, low/intermediate resolution molecular typing). High-resolution genotyping of HLA DRB1 of recipients and UCB was performed by polymerase chain reaction-sequence specific primer (PCR-SSP). HLA compatibility was expressed as the number of identical loci among 6.

CB units were obtained from CB banks located in France (n = 45), the United states (n = 18), Germany (n = 7), the United Kingdom (n = 5), Italy (n = 5), Spain (n = 4), Belgium (n = 1), and Denmark (n = 1), which provided the prefreezing total nucleated cell (TNC) dose for each unit.

Evaluation of Transplant Cell Dose and Quality Controls on Thawed CB

The infused transplanted cell doses were evaluated by the TNC count as well as by absolute $CD34^+$ and $CD45^+$ cells counts. Absolute counts of $CD34^+$ and $CD45^+$ cells were determined using the Stem-Kit numeration kit (Beckman-Coulter Company, Fullerton, CA), as previously described [21]. Briefly this commercially available diagnostic kit includes the viability dye 7-amino actinomycin D (7-AAD). Thus, the viable CD45 (vCD45) count corresponds to viable infused cells expressing the CD45 common leukocyte antigen. Including fluorescent microspheres, this single platform assay allows for a quantification of viable $CD34^+$ and $CD45^+$ cells (v$CD34^+$ and v$CD45^+$, respectively) [22,23]. Data acquisitions and analyses were performed on a FACSCalibur 4-color flow cytometer using the Cellquest software (Becton Dickinson Immunocytometry System [BDIS], San Jose, CA). The gating strategy is adapted from ISHAGE guidelines following recommendation for cord blood CD34 cell enumeration [22].

Evaluation of Lymphocyte Subsets Recovery and Blood Chimerism

All recipients underwent blood samples at days +30, +60, +90, months +6, +9, +12, +18, +24, and then every year. Phenotypic analysis of blood lymphocytes was performed using 4-color immunofluorescence flow cytometry to characterize the following lymphocyte subsets: $CD3^+$ T lymphocytes, $CD3^+CD4^+$ and $CD3^+CD8^+$ T lymphocytes, $CD19^+$ B lymphocytes, and $CD3^-/CD16^+56^+$ natural killer (NK) lymphocytes.

Chimerism was analyzed in peripheral blood cells of all recipients at days +30, and +90, and months +6, +9, +12, +18, and +24 after transplantation. Chimerism was determined by real-time PCR amplification of single nucleotide polymorphisms. Total chimerism was defined as >99% donor-derived cells, mixed chimerism as the presence of 20% to 99% donor-derived cells, and autologous recovery as hematopoietic recovery (absolute neutrophil count [ANC] >0.5 G/L and platelets >20 G/L) with >80% blood host chimerism.

Endpoints

Standard definitions were used for common posttransplant endpoints, namely, overall survival (OS), relapse incidence, treatment-related mortality (TRM), acute GVHD (aGVHD), and chronic GVHD (cGVHD). Several immunologic endpoints were chosen for their potential clinical significance: time to achieve a $CD3^+$ cell count $>500/mm^3$, CD3 $>1500/mm^3$, CD4 $>500/mm^3$, CD8 $>250/mm^3$, CD19 $>200/mm^3$, and $CD3^-CD56^+$ $>100/mm^3$.

Statistical Methods

November 1, 2008, was used as the reference date, that is, the day when we locked data on patient outcomes. As for OS, Kaplan-Meier estimates provided estimated incidence over time. However, the other endpoints shared a competing risk setting, meaning patients could develop events that avoid the occurrence of the event of interest. As an example, after death or relapse before achievement of an immunologic endpoint, no further immunologic recovery could occur. Therefore, these endpoints (TRM, relapse, and all endpoints used for assessment of immunologic recovery) were analyzed through the use of cumulative incidence curves for estimating incidence over time [24] and through Fine and Gray models [25] to assess prognostic factors. In this study, TRM,

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