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Early and Long-Term Impaired T Lymphocyte Immune Reconstitution after Cord Blood Transplantation with Antithymocyte Globulin

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A B S T R A C T

Immune reconstitution is crucial to the success of allogeneic hematopoietic stem cell transplantation. Umbilical cord blood transplantation (UCBT) has been associated with delayed immune reconstitution. We characterized the kinetics and investigated the risk variables affecting recovery of the main lymphocyte subsets in 225 consecutive pediatric and adult patients (males, n = 126; median age, 15; range, .3 to 60; interquartile range, 4 to 35) who underwent myeloablative single UCBT between 2005 and 2015 for malignant and nonmalignant disorders. Low $CD4^+$ and $CD8^+$ T cell counts were observed up to 12 months after UCBT. In contrast, B and natural killer cells recovered rapidly early after transplantation. In a multivariate regression model, factors favoring $CD4^+$ T cell recovery ≥ 200 cells/μL were lower dose antithymocyte globulin (ATG) (hazard ratio [HR], 3.93; 95% confidence interval [CI], 2.3 to 5.83; $P = .001$), negative recipient cytomegalovirus (CMV) serostatus (HR, 3.76; 95% CI, 1.9 to 5.74; $P = .001$), and younger age (HR, 2.61; 95% CI, 1.01 to 3.47; $P = .03$). Factors favoring $CD8^+$ T cell recovery ≥ 200 cells/μL were lower dose ATG (HR, 3.03; 95% CI, 1.4 to 5.1; $P = .03$) and negative recipient CMV serostatus (HR, 1.9; 95% CI, 1.63 to 2.15; $P = .01$). Our results demonstrate the significant negative impact of ATG on lymphocyte recovery. A reduction of the dose or omission of ATG could improve immune reconstitution and perhaps reduce opportunistic infections after UCBT.

INTRODUCTION

Umbilical cord blood transplantation (UCBT) is commonly used for patients with hematological and nonhematological malignancies who require allogeneic (allo) hematopoietic stem cell transplantation (HSCT), when there are no HLA-matched donors available.

A major limitation for the use of UCBT is the relatively small number of infused hematopoietic stem cells that results in delayed engraftment [1,2]. Previous studies have shown that T cell recovery is often delayed after UCBT [3-5]. In contrast, B and natural killer (NK) cell appear to recover rapidly after UCBT [6]. Of note, major outcomes after transplantation improve in patients with a rapid T cell recovery [7,8].

Initial cellular immune reconstitution after transplantation largely depends on thymic-independent peripheral expansion of donor-derived memory T cells. After that, thymic-dependent maturation is important for diversification of the T cell repertoire and consolidating host immune reconstitution against pathogens or recurrence of malignancy [9,10].

Previous studies demonstrated that post-transplantation immune recovery is affected by several factors, including thymic involution associated to patient age, the conditioning regimen, HLA disparity between donor and recipient, occurrence of graft-versus-host disease (GVHD), and drugs used to prevent or treat GVHD, such as antithymocyte globulin (ATG) [11-21].

Various groups evaluated the lymphocyte kinetics after UCBT and confirmed a delay in T cell subsets' recovery up to 6 months after transplantation; however, by 12 months,

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immune recovery is often at least on par with that seen after conventional HSCT. However, despite this, there are limited data on factors involved in lymphocyte subsets' recovery after UCBT [6,9].

Consequently, to understand the key factors influencing lymphocyte recovery after UCBT, we retrospectively explored the main lymphocyte subset kinetics profile and analyzed the predictive factors associated with a prompt lymphocyte recovery in a cohort of 225 pediatric and adult patients diagnosed with neoplastic and non-neoplastic hematological diseases who underwent myeloablative single unit UCBT (sUCBT) using a very consistent selection criteria and conditioning protocol.

MATERIALS AND METHODS

Patient Cohort

A total of 271 patients received an UCBT from January 2005 to April 2015. For the purpose of the study, we excluded patients who received a second allo-HSCT (n = 7), a related UCBT (n = 18), coinfusion of bone marrow with UCBT (n = 6), or UCBT with haploidentical third-party $CD34^+$ selected cells (n = 15). We included all 225 (121 [54%] pediatric and 104 adult) patients who received a first sUCBT in the Hospital Vall d'Hebron (Barcelona), Hospital de Sant Pau (Barcelona), and Hospital Germans Trias i Pujol (Barcelona), consisting of 3 adult and 2 pediatric transplantation programs.

Enrollment Criteria

All patients with hematological malignancies and nonhematological diseases were eligible for enrollment if there were a lack of a suitable HLA-matched unrelated donor within a reasonable time after the search through international registries and there was a suitable umbilical cord blood unit (CBU) available, as described below. Patients or their guardians gave written informed consent for their inclusion in each transplantation protocol. For all patients included in the analysis, the sUCBT was the first allo-HSCT received.

Transplantation Procedure and CBU Selection

All patients received myeloablative conditioning. The most commonly used protocol has been previously published [22] and was based on thiotepa (10 mg/kg i.v.), fludarabine (150 mg/m^2 i.v.), busulfan (9.6 mg/m^2 i.v.), and in vivo T cell depletion with ATG, 6 to 10 mg/kg i.v (Thymoglobulin, Sangstat/Genzyme, Lyon, France). ATG was administered in different schedules starting on day –5 or –4 to –2 day depending on the overall dose administered.

GVHD prophylaxis was based on cyclosporine, 1.5 mg mg/kg/12 hours i.v. followed by 3 to 5 mg/kg/12 hours orally when oral intake was possible and slow tapering starting between day +90 and +180 if feasible. Cyclosporine was combined with a short-course of steroids (1 mg/kg/daily) from day +14 to +28 or mycophenolate mofetil (15 mg/kg/day from –1 to +30 day). As supportive care, all patients received post-transplantation granulocyte colony–stimulating factor from day +7 until neutrophil recovery.

For adult patients, the minimum precryopreserved cell counts recommended was total nucleated cells (TNC) > 1.5×10^7/kg and $CD34^+$ cells ≥ $.6 \times 10^5$/kg. A degree of HLA matching between CBU and the recipient greater or equal to 4 of 6 (considering HLA-A and -B at antigen level and -DRB1 at allele level) was required. For pediatric patients with malignant diseases, the minimum precryopreserved cell counts recommended for selection was TNC ≥ 3×10^7/kg and $CD34^+ \geq 1.5 \times 10^5$/kg for 4/6 to 6/6 degree HLA mismatch. For children with nonmalignant diseases, the minimum precryopreserved cell dose recommended was TNC ≥ 5×10^7/kg and $CD34^+ \geq 2 \times 10^5$/kg for 5/6 to 6/6 degree HLA mismatch.

Definitions

Assessment of GVHD, nonrelapse mortality, relapse, disease-free survival, overall survival, and disease status

Recipients were evaluated weekly for development and grading of acute GVHD (aGVHD). Acute and chronic GVHD (cGVHD) were diagnosed and graded according to the standard criteria [23,24]. Patients dying before +100 day were not considered for cGVHD analysis. *Nonrelapse mortality* (NRM) was defined as death from any cause without evidence of relapse. *Disease-free survival* was defined as survival from the time of transplantation without evidence of disease relapse. *Overall survival* was defined as survival from the time of transplantation. Disease status at the time of transplantation was classified as follows: (1) early phase, including acute leukemia, myelodysplastic syndrome (MDS) and lymphoma on the first complete remission, untreated MDS with < 5% blasts and/or chronic myeloid leukemia (CML) in the first chronic phase; (2) intermediate phase, including acute leukemia, lymphoma, or MDS in a second remission and CML in a second or further chronic or accelerated phase; and (3) advanced phase, including acute leukemia and lymphoma not in remission, CML in blast crisis, and untreated refractory anemia with excess blasts [25].

Flow cytometry analysis of peripheral blood

Immunophenotyping was performed on whole-blood samples generally obtained at 3, 6, 12, 18, and 24 months after transplantation. Quantification of the following subsets was performed: absolute number of T cells ($CD3^+$), helper T cells ($CD3^+CD4^+$), cytotoxic T cells ($CD3^+CD8^+$), B cells ($CD19^+$), and NK cells ($CD3^-CD16^+CD56^+$) and were determined using 4-color immunofluorescence and fluorescence-activated cell sorting analysis. Briefly, a volume of 10 μL of CD3-FITC, CD45-PerCP, CD19-APC or CD3-FITC, CD8-PE, CD45-PerCP, CD4-APC reagent (PerfecT count, Cytognos, Salamanca, Spain) was added to a tube containing a known quantity of beads, followed by 25 μL of EDTA-treated whole blood and incubated for 15 minutes at room temperature. Red blood cells were subsequently lysed for 15 minutes with 450 μL of FACS Lysing Solution (Cytognos). Samples were acquired using FACSCalibur and analyzed with Multiset software (Becton-Dickinson, Franklin Lakes, NJ).

Kinetics of lymphocyte recovery and risk factors assessment

Lymphocyte recovery kinetics were studied calculating the median and range of $CD3^+$ T cell, $CD4^+$ T cell, $CD8^+$ T cell, B, and NK cells measured at 3, 6, 12, 18, and 24 months after UCBT by age (<20 and ≥20 years) and in the whole population and we compared the results with our laboratory reference value. We also calculated the median time to reach several lymphocyte endpoints because of the clinical significance based on a previous report [26], as follows: time to reach $CD3^+$ T cell ≥ 500 cells/μL, $CD3^+$ T cell ≥ 1500 cell/μL, $CD4^+$ T cells ≥ 50 cell/μL, $CD4^+$ T cells ≥ 200. To evaluate the potential effect of ATG on T lymphocyte recovery in the post-transplantation period, we calculated the median and range of $CD4^+$ and $CD8^+$ T cell at 3 and 6 months after CBT in the patients who did not receive ATG in the conditioning regimen.

Pretransplantation variables studied for their potential impact on the lymphocyte endpoints were year of transplantation, recipient age, disease type, disease phase, autologous stem cell transplantation before UCBT, pretransplantation recipient cytomegalovirus (CMV) serology, HLA match at antigenic and allelic level, infused TNC dose, infused $CD34^+$ cell dose, infused $CD3^+$ cell dose, infused colony-forming unit, pretransplantation ATG dose, and GVHD prophylaxis.

Definition of infections

Severe infections starting from the day of progenitors infusion (day 0) to 24 months after transplantation were collected from all participating centers, according to predefined criteria [27].

Statistical analysis

Baseline characteristics were described as median, range, and interquartile range (IQR) for quantitative variables and frequency and percentages for categorical variables. Lymphocyte kinetics was described as median and range for each lymphocyte subset ($CD4^+$ T cell, $CD8^+$ T cell, B cell, and NK cell) at different time-points (3, 6, 12, 18, and 24 months) after transplantation by age (<20 and ≥20 years) and in the whole population. Additionally, we calculated the median and range of time to reach the different lymphocyte endpoints mentioned above in those patients at risk at the time of the analysis (those alive patients showing sustained engraftment).

We conducted a univariate analysis to assess the factors influencing lymphocyte recovery. Characteristics selected for inclusion in the multivariate model were those with $P < .10$ in univariate analysis. Cumulative incidence curves were used in a competing risk setting to calculate the cumulative incidence of neutrophil and platelet engraftment, aGVHD, cGVHD, relapse, and NRM for the entire population [28]. Death without engraftment was the competing event for neutrophil and platelet engraftment. Death without relapse was the competing event of relapse. Relapse or death without developing aGVHD or cGVHD were the competing events for aGVHD and cGVHD, respectively. Survival probability was calculated using Kaplan-Meier estimation in whole population [29]. A Cox proportional hazard model or the Fine and Gray method for competing events were used for multivariate analysis [30]. All statistical tests were conducted using SPSS statistical software (SPSS version 20.0, Chicago, IL). Cumulative incidence with competing risks was conducted in R software, version 3.1.1 (The CRAN project).

RESULTS

Patient and CBU Characteristics

A total of 225 patients were included in this study. Clinical characteristics are summarized in Table 1. During the study period, 225 consecutive patients underwent