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Immunologic Recovery in Children after Alternative Donor Allogeneic Transplantation for Hematologic Malignancies: Comparison of Recipients of Partially T Cell–Depleted Peripheral Blood Stem Cells and Umbilical Cord Blood

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A B S T R A C T

Impaired immunologic recovery (IR) after hematopoietic stem cell transplantation (HSCT) is associated with increased risk for infections and relapse. Stem cell source and graft manipulation influence the kinetics of IR. Partial T cell depletion of peripheral blood stem cell (PBSC) grafts is a novel alternative method of graft manipulation for children. We compared IR in children undergoing HSCT for hematologic malignancies receiving either T cell–depleted (TCD)-PBSCs (n = 55) or umbilical cord blood (UCB) (n = 21) over a 7-year period at a single institution. PBSC grafts underwent ex vivo negative selection for $CD3^+$ cells using the CliniMACS system with partial T cell add-back. Recovery of $CD4^+$ T cells was significantly delayed in TCD-PBSC recipients compared with UCB recipients, owing to impaired $CD4^+/CD45RA^+$ (naïve) T cell lymphopoiesis. Recovery of total $CD3^+$ cells and $CD3^+/CD8^+$ cells was similar in the 2 groups. The TCD-PBSC recipients had a marked deficit in $CD19^+$ and, to a lesser extent, IgA/IgM, owing to the need for B cell depletion of these grafts to attenuate the risk of lymphoproliferative disease after TCD HSCT. There were no significant between-group differences in response to mitogen stimulation, time to independence from intravenous immunoglobulin supplementation, or incidence of viral reactivation. Transplantation outcomes of relapse, transplantation-related mortality, event-free survival, and overall survival were similar in the 2 groups. Efforts to enhance IR after partial TCD-PBSC transplantation, such as selective αβ T cell depletion, hold promise for further improvement of this transplantation approach.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the sole curative option for many children with high-risk hematologic malignancies, but only approximately 30% of these children will have a matched related donor (MRD). To broaden access to this treatment modality, the use of alternative donors is increasing, including bone marrow (BM) or mobilized peripheral blood stem cells (PBSCs) from matched unrelated or partially matched related donors, as well as unrelated umbilical cord blood (UCB). Immunologic recovery (IR) after alternative donor allogeneic HSCT in children is complex and dynamic, influenced by various patient and transplantation-related factors, including age of recipient and donor, indication for transplantation, conditioning regimen, donor type, stem cell source, graft manipulation, infection, and graft-versus-host disease (GVHD) chemoprophylaxis, type, and treatment [1,2]. Impaired IR increases the risk of serious infection [3] and relapse [4], and is associated with decreased survival [5].

Donor type and stem cell source affect IR [2]. The type and quantity of passenger lymphocytes infused as a component of the graft differ by stem cell source and degree of manipulation. Passenger lymphocytes provide initial lymphoid immunity after undergoing thymus-independent homeostatic peripheral expansion [6]. BM and PBSC grafts contain predominately memory T cells, whereas UCB grafts have a higher proportion of naive T cells with differing immunobiology [7]. Graft manipulation with either positive or negative cell selection influences this early thymus-independent lymphoid recovery by altering the cellular composition of the graft [8-10].

In the second wave of IR that occurs several months after transplantation, phenotypically naïve T cells that have undergone maturation in the thymus emerge [11]. This thymic-dependent process is heavily influenced by various clinical factors known to affect thymic function, including age [2], conditioning regimen (particularly irradiation), and presence of GVHD [12]. Because these factors are inextricably linked to donor type and cell source, the kinetics of IR continue to be influenced by graft characteristics even late after transplantation.

At our institution, all types of alternative donors are considered, and the ultimate decision depends on HLA matching, urgency, patient size, and other factors (eg, cytomegalovirus [CMV] status). In an effort to maintain the benefits of mobilized PBSCs—including rapid neutrophil and platelet engraftment [13]—while mitigating the increased risk of cGVHD owing to greater numbers of T cells in the graft [14], we have used partial T cell depletion. Our current method involves ex vivo negative selection for $CD3^+$ cells using the CliniMACS system (Miltenyi Biotec, Bergish-Gladbach, Germany) with partial T cell add-back. To evaluate IR in recipients of alternative donor grafts, we compared IR after HSCT using this graft type with a concurrent cohort of UCB recipients—the other major alternative donor source at

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our hospital. Institutional preference for PBSCs with $CD3^+$ depletion has resulted in relatively few unrelated donor BM transplantations, so this group was not included because meaningful statistical comparison was precluded by small numbers. In addition, we focused our analysis on alternative donor HSCT, given that IR after MRD HSCT has been extensively characterized and is the preferred approach when available. Comparison of IR between alternative graft types may inform decisions regarding donor selection when an MRD is unavailable.

MATERIALS AND METHODS

Patients and Transplantation Regimens

We retrospectively reviewed the charts of 76 consecutive children undergoing first allogeneic HSCT for a hematologic malignancy at our hospital between March 2005 and December 2011. During this period, 55 children received TCD-PBSC grafts that had been $CD3^+$-depleted using the CliniMACS system (clinicaltrials.gov identifier NCT00579124), and 21 patients received UCB grafts. The protocol was approved by our hospital's Institutional Review Board, and written informed consent was obtained from all patients and/or parents, as appropriate.

All patients with an acute hematologic malignancy were in morphological remission (complete remission [CR]) at the time of transplantation. Myeloablative conditioning was provided either with cyclophosphamide 60 mg/kg/day for 2 days and total body irradiation (TBI) 200 cGy twice daily for 3 days (80.3%) or with cyclophosphamide and busulfan 0.8 to 1 mg/kg every 6 hours for 16 doses, adjusted to achieve a target steady-state concentration of 750 to 1100 ng/mL (19.7%), both either with (81.6%) or without thiotepa 5 mg/kg/day for 2 days.

A calcineurin inhibitor was administered as primary GVHD chemoprophylaxis in all patients. UCB recipients also received methylprednisolone 1 mg/kg/day starting on day +7 and tapered starting at day +21, as well as granulocyte-colony stimulating factor (G-CSF; filgrastim) until the absolute neutrophil count exceeded 2000 cells/μL. In the absence of GVHD, immunosuppression was tapered starting on day +100 (or earlier if there was concern for declining chimerism). All patients received standard infectious prophylaxis that was individually tailored to risk. Specifically, for CMV-positive UCB recipients and CMV-positive TCD-PBSC recipients with CMV-negative donors, foscarnet was used until engraftment occurred, at which point it was replaced with valganciclovir, along with IVIG supplementation. Otherwise, CMV prophylaxis was provided with IVIG supplementation alone. Weekly plasma polymerase chain reaction testing for adenovirus, CMV, and Epstein-Barr virus (EBV) was performed on all patients from day +7 up to day +100 and as indicated thereafter; testing for additional viruses was based on clinical indications.

Graft Manipulation

TCD-PBSC grafts were obtained by leukapheresis of peripheral blood mononuclear cells after G-CSF stimulation. $CD3^+$ depletion was performed by negative selection using the automated CliniMACS device, as described previously [15,16]. To mitigate the risk of post-transplantation lymphoproliferative disease, all PBSC grafts underwent some form of B cell depletion, either ex vivo or in vivo. Before the availability of beads conjugated to anti-CD19 antibodies, 38 patients received rituximab 375 mg/m^2/dose on days −1 and +7; thereafter, grafts were depleted of B cells during ex vivo manipulation (n = 17). The number of $CD3^+$ cells added back at the time of the stem cell product infusion was determined by the degree and nature of HLA mismatching and disease status, ranging from 0.2 to 8×10^5 cells/kg of recipient weight (median, 1×10^5 cells/kg). The majority of patients (67%) received between 1×10^5 and 3×10^5 cells/kg. In general, patients with high-risk leukemia, less HLA disparity, and malignancies associated with greater graft-versus-leukemia effects (eg, chronic myelogenous leukemia [CML]) received higher $CD3^+$ cell doses. Of the 7 patients who received $>3 \times 10^5$ cells/kg, 4 were 10/10 HLA matches, and all had CML, juvenile myelomonocytic leukemia, or acute leukemia with evidence of minimal residual disease at the time of transplantation. Recipients of low $CD3^+$ cell doses generally had ≥2 HLA antigen mismatches and were in durable CR.

Measurement of Immunologic Recovery

Formal assessments of immunologic recovery were made at 4, 8, 12, and 24 months after HSCT as the standard of care. Monoclonal antibodies to surface antigens were used in flow cytometry analysis to define the following immunophenotypes: $CD3^-/CD16^+$ and/or $CD56^+$ (natural killer [NK] cells), $CD3^+$ (T cells), $CD3^+/CD4^+$ ($CD4^+$ T cells), $CD3^+/CD8^+$ (cytotoxic T cells), $CD4^+/CD45RA^+$ (naïve $CD4^+$ T cells), $CD4^+/CD45RO^+$ (memory $CD4^+$ T cells), $CD19^+$ (B cells), and $CD20^+$ (B cells). From these data, the ratios of $CD4^+$ cells to $CD8^+$ cells (4:8) and that of naïve cells to memory cells (RA:RO) were calculated. Immunoglobulins (IgG, IgA, and IgM) were measured at the same intervals by standard nephelometry. The time to independence from intravenous gamma globulin (IVIG) supplementation was based on the interval between day 0 and the last dose of IVIG, in days. Patients received supplemental IVIG until the IgG level was maintained at >500 mg/dL without support.

The response of peripheral blood mononuclear cells to stimulation with the mitogens phytohemagglutinin (PHA), pokeweed (PWM), and concanavalin A (ConA) was measured at 8, 12, and 24 months post-transplantation. Results are reported as ratios of patient to control counts per minute (CPM_{rel}); a normal response was considered to be ≥50% of normal ($CPM_{rel} \geq 0.5$).

Among the subjects alive at 1 year, published age-based ranges in healthy children for number of total lymphocytes, $CD3^+$ cells, $CD16^+/CD56^+$ cells, $CD3^+/CD4^+$ cells, $CD3^+/CD8^+$ cells, and $CD19^+$ cells were used to define patients who were in at least the 5th percentile as "normal" [17].

Definitions of Transplantation Outcomes

Neutrophil engraftment was defined as the first of 3 consecutive days on which the peripheral blood absolute neutrophil count was ≥500 cell/uL, and platelet engraftment was defined as the first of 7 consecutive days with an unsupported platelet count ≥20,000 cells/uL. Pre-engraftment bacteremia was defined as any positive blood culture obtained between the time of initiation of the conditioning regimen and neutrophil engraftment. Viral reactivations were included only if they were considered clinically significant, as defined by either the need for antiviral therapy or the presence of associated clinical manifestations. Untreated reactivations or infections without clinical disease (eg, human herpesvirus-6 [HHV6] viremia without symptomatic disease) were not counted.

Staging and grading of acute GVHD (aGVHD) and chronic GVHD (cGVHD) was based on Center for International Blood and Marrow Transplant Research guidelines. Patients who developed GVHD after donor lymphocyte infusion (DLI) were considered to have GVHD. The time to cessation of immunosuppressive therapy (IST) was defined as the first time after HSCT or diagnosis of aGVHD or cGVHD at which the patient achieved freedom from immunosuppressive medications for at least 1 month.

Relapse was defined as morphological evidence of recurrent disease in the peripheral blood or BM. Patients with mixed chimerism who responded to DLI were not considered to have relapsed. Transplantation-related mortality (TRM) was defined as all nonrelapse deaths. Event-free survival was calculated based on the following events: relapse, TRM, or DLI.

Statistical Analysis

Baseline demographic and transplant characteristics were compared using Fisher exact tests for categorical data and Wilcoxon rank-sum for continuous variables. The outcome variables of absolute lymphocyte numbers, 4:8 and RA:RO ratios, immunoglobulin levels, and mitogen responses were logarithmically (base 10) transformed to obtain data showing symmetric distribution. All analyses of the primary outcome measures of these variables were performed on these transformed values. A linear mixed effects model was fitted to each outcome variable using Proc Mixed in SAS 9.2 (SAS Institute, Cary, NC). The model included group, time, and group-by-time interaction as fixed effects, along with a random intercept and a random slope for each patient. This approach accounts for potential correlations among repeated measurements, and was used to test differences between TCD-PBSC and UCB recipients and differences between patients with and without cGVHD.

Two multivariate models were constructed to control for potential confounders. The first model incorporated pretransplantation variables with a known association with IR after HSCT: age, sex, disease category (acute lymphoblastic leukemia, acute myelogenous leukemia, other), conditioning regimen (TBI, no TBI), HLA disparity (any mismatch, fully matched), and receipt of antithymocyte globulin. A second model incorporated all of these variables with the addition of cGVHD, the sole post-transplantation variable included in multivariate analysis.

Differences in the proportion of patients reaching age-based normal lymphocyte levels and mitogen response (as defined above) were assessed using the Fisher exact test. The following separate exposures were used: UCB and TCD-PBSC; aGVHD grade II-IV and no aGVHD/grade I; cGVHD and no cGVHD; and use of IST and no IST at 1 year.

The Kaplan-Meier method was used to estimate overall survival (OS) and event-free survival (EFS), and differences between groups were tested using the log-rank statistic. Patients who were event-free were censored at the time of last follow-up. Cumulative incidence curves were generated for relapse and TRM, adjusting for the other outcome as a competing risk. Differences between the groups were tested using Gray's test [18].

All statistical tests were 2-sided, with a significance level of $P < .05$. All analyses were performed using Stata 12.1 (StataCorp, College Station, TX) or SAS 9.2.