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Low-Dose Serotherapy Improves Early Immune Reconstitution after Cord Blood Transplantation for Primary Immunodeficiencies

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Cord blood transplantation (CBT) is curative for many primary immunodeficiencies (PIDs) but is associated with risks of viral infection and graft-versus-host disease (GvHD). Serotherapy reduces GvHD but potentially increases the risk of viral infection by delaying immune reconstitution. Because many PID patients have pre-existing viral infections, the optimal dose of serotherapy is unclear. We performed a retrospective analysis in 34 consecutive PID patients undergoing CBT and compared immune reconstitution, viral infection, GvHD, mortality, and long-term immune function between high-dose (n = 11) and low-dose (n = 9) serotherapy. Serotherapy dose had no effect on neutrophil engraftment. Median $CD3^+$ engraftment occurred at 92.5 and 97 days for high- and low-dose serotherapy, respectively. The low-dose serotherapy group had higher $CD3^+$, $CD4^+$, and early thymic emigrant counts at 4 months compared with the high-dose group. GvHD severity and number of viral infections did not differ between serotherapy doses. Survival from the transplantation process was 90.9% for high-dose and 100% for low-dose groups. In conclusion, low-dose serotherapy enhanced T cell reconstitution and thymopoiesis during the first year after CBT with no increase in GvHD.

INTRODUCTION

Primary immunodeficiencies (PIDs) are inherited conditions characterized by recurrent infections, inflammatory disorders, and autoimmunity. Severe combined immunodeficiency (SCID), the most severe form of PID, is usually fatal within the first year of life unless corrected [1]. Other T cell and innate immune defects have excessive morbidity and mortality through childhood and early adulthood. Hematopoietic stem cell transplantation (HSCT) is curative for many patients with a 10-year survival of 84% in HLA-matched sibling HSCT for SCID [2,3]. Ten-year survival for non-SCID PID is 71% for HLA-matched sibling HSCT, with survival rates improving over time [2,3].

Umbilical cord blood offers an alternative source of stem cells for transplantation (cord blood transplantation, CBT) when a matched sibling donor is unavailable. Advantages and disadvantages of CBT over bone marrow transplantation (BMT) and peripheral blood stem cells (PBSC) are reviewed in detail elsewhere [4,5]. Advantages include (1) easy access to the cord blood unit and, therefore, earlier transplantation; (2) absence of risk to donor; (3) lower risk of latent viral transmission and graft-versus-host disease (GvHD); and (4) higher chance of matching rare HLA haplotypes. However, the stem cell dose is often low and unrelated donors are not available for boost HSCT. Cord blood units are virologically naïve and often show slower engraftment due to a lower $CD34^+$ dose compared to BMT and PBSCs. Both of these factors increase the risks from pre-existing infections in CBT for PID patients.

Evidence for the effectiveness of CBT in PID comes from a few single center and multicenter studies [6-10]. A recent study demonstrated a lower rate of grades II to IV GvHD and improved survival in unrelated CBT when the authors compared their results with similar studies of BMT for PID and that mortality was associated with pre-existing infection, no conditioning, ≥2 HLA mismatch and underlying disease [10]. However, this study does not provide data on lymphocyte reconstitution or long-term graft function. Other studies with limited data on lymphocyte reconstitution show that absolute lymphocyte counts increase from 2 months with a proportional increase in $CD4^+$ and $CD8^+$ T cells from 3 months post CBT and that age-related normal values for $CD19^+$, $CD3^+$, and $CD4^+$ are reached by 24 months in all patients studied [7,11]. All surviving patients in 1 series were independent of intravenous immunoglobulin and responded to T cell stimulation, tetanus, and hepatitis B vaccination [7].

Serotherapy in the form alemtuzumab (T and B cell depleting anti-CD52 humanized monoclonal antibody) is added to conditioning regimens to reduce the incidence of GvHD [12-16]. Immune reconstitution is delayed by alemtuzumab in patients with malignant and nonmalignant hematological conditions [13,16,17] with some studies suggesting slower immune reconstitution with higher doses used [12,18]. This slower immune reconstitution is, in turn, associated with an increased incidence of viral reactivation, notably cytomegalovirus [17, 20-22], adenovirus [14,23,24] and respiratory viruses [25]. This is particularly pertinent when considering CBT for PID because many patients have

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active or latent viral infections at the time of HSCT and CBT is considered to have slower immune reconstitution and be virologically naïve [4,5]. The role of serotherapy in CBT for PID has not been explored to date.

We report the results of a retrospective study of the effects of 2 alemtuzumab-based serotherapy dose regimens on lymphocyte reconstitution, GvHD, viral infection, and mortality in PID patients who underwent CBT at an international center.

MATERIALS AND METHODS

Patients and Exclusion Criteria

Thirty-four consecutive patients who underwent CBT for PID between May 1999 and December 2010 at the Children's BMT Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, United Kingdom were identified from the unit's database. A retrospective study of these patients was performed, of which 14 have been previously reported [6]. There was a systematic change in serotherapy policy at the Children's BMT Unit in 2006 to 2007, when the alemtuzumab dose was reduced to determine if it had the same protective effects on GvHD without an increase in viral infection. Two patients were excluded from further analysis because they received alemtuzumab before conditioning at 3 and 4 weeks before CBT, and another was excluded because the patient received rabbit antithymocyte globulin (ATG) instead of alemtuzumb. Those who received no serotherapy (n = 11) were also excluded because 6 did not receive chemotherapeutic conditioning and others were selected to this group based on clinical presentation. The remaining patients were retrospectively nonrandomly assigned to high-dose (alemtuzumab ≥ .9 mg/kg given in 5 divided doses before day -10 or between day -8 and day -4; n = 11) or low-dose (alemtuzumab .3 to .6 mg/kg given in 3 divided doses on consecutive days between day -11 and day -6; n = 9) serotherapy groups for the purpose of analysis. No alemtuzumab pharmacokinetic studies were performed. Underlying diagnoses by serotheraphy group are shown in Supplementary Table S1.

Umbilical Cord Transplantation Procedure

Search for a suitable umbilical cord donor unit was initiated if a matched sibling cord was stored or if a matched sibling donor was unavailable. Transplantation characteristics are summarized in Table 1. HLA matching was based on low-resolution molecular class I and high-resolution molecular class II typing at 10 HLA loci as previously described [6]. Patients were conditioned according to the European Blood and Bone Marrow Transplant Group (EBMT) guidelines, EBMT Working Party for Inborn Errors, and clinical condition, and they received either myeloablative conditioning (busulphan 16 mg/kg + cyclophosphamide 200 mg/kg or treosulphan 36 g/m^2 + cyclophosphamide 200 mg/kg; n = 9) or reduced-intensity conditioning (treosulphan 36 g/m^2 + fludarabine 150 mg/m^2 or fludarabine 150 mg/m^2 + melphalan 140 mg/m^2; n = 11). Patients also received either high-dose or low-dose serotherapy as part of their conditioning regimen allocated as described above. The cord blood unit was transfused on day 0 with a median nucleated cell count of 1.30 × 10^8 cells/kg (range, .36 to 3.8) and a median CD34$^+$ count of 3.70 × 10^5 cells/kg (range, .67 to 19.0).

Table 1
Patient and Donor Cord Blood Unit Characteristics with Respect to Serotherapy Dose

	Serotherapy Dose		*P* Value
	High	Low	
No. of patients	11	9	
Year of CBT			.001
1999-2005	8	0	
2006-2010	3	9	
Age at CBT, median (range), wk	26 (8-51)	24 (7-52)	.790
Underlying diagnosis*			1.00
SCID	5	5	
Non-SCID	6	4	
Cord unit source			1.00
MUD	5	5	
MMUD	6	4	
HLA match			.496
10/10	5	5	
9/10	6	3	
8/10	0	1	
Nucleated cell dose, median (range), × 10^8/kg	1.1 (.36-3.10)	1.3 (.55-3.8)	.648
CD34$^+$ cell dose, median (range), × 10^6/kg	.29 (.067-1.45)	.63 (0.15-1.90)	.239
Chemotherapy conditioning			.406
Myeloablative	6	3	
RIC	5	6	

CBT indicates cord blood transplant; SCID, severe combined immunodeficiency; MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; RIC, reduced-intensity conditioning.
* Further details of diagnoses are available in Supplementary Table S1.

Supportive Measures and Prophylaxis

All patients were nursed in HEPA-filtered cubicles. Patients received cyclosporine for GvHD prophylaxis, cotrimoxazole for *Pneumocystis jiroveci* prophylaxis, liposomal amphotericin for antifungal prophylaxis, and aciclovir for antiviral prophylaxis. Patients received blood and platelet support and granulocyte colony stimulating factor until engraftment, at the discretion of the managing physician. All parents gave written consent according to our local centre and European Blood and Marrow transplantation guidelines.

Immunological Studies

Time to neutrophil (third consecutive day of absolute neutrophil count (ANC) > .5 × 10^9/L), platelet (platelet count of >50 × 10^9/L independent of transfusion support) and T lymphocyte (first day of a CD3$^+$ count > 200 cells/μL) engraftment was recorded. Lymphocyte subset analysis was measured by 4-color flow cytometry as previously described [26]. Briefly, lymphocyte surface marker studies were performed on fresh whole blood collected in EDTA using appropriate markers (CD45 PerCP, CD3 FITC, CD4 APC, CD8 PE, CD19 APC, CD16/CD56 PE, CD3 PerCP/CD4 APC/CD45RAFITC/CD27 PE, CD19 PerCP/CD27 FITC/IgM APC/IgD PE [Becton Dickinson, UK Ltd, Oxford]), and analyzed on a Becton Dickinson FACS Calibur flow cytometer. The markers CD4$^+$/CD45RA$^+$ and CD4$^+$/CD45RA$^+$/CD27$^+$ were used as surrogates for early thymic emigrant equivalents (ETEEs) [27]. Lymphocyte subsets (CD3$^+$, CD4$^+$, CD8$^+$, CD19$^+$, natural killer [NK] cells and CD4$^+$ ETEEs) at 2, 4, 6, and 12 months follow-up were recorded. Specific antibody responses to pneumococcal (polysaccharide or Prevenar), tetanus toxoid and *Haemophilus influenzae* b vaccine antigens were measured by ELISA and defined as present or absent after vaccination [28].

Chimerism Studies

Whole blood was stained with CD3, CD19, or CD15 micro beads and cell lines were separated using an autoMACS automated bench-top magnetic cell sorter (Miltenyi Biotec Ltd, Surrey, UK). Chimerism was measured in sex-mismatched cases by XY-fluorescent in-situ hybridization (FISH) using standard cytogenetic techniques. Briefly, interphase FISH was performed using a Vysis 2-color CEP X/CEP Y probe set according to the manufacturer's protocol. Where donor and recipient were same sex, chimerism was measured by short tandem repeat marker analysis of genomic DNA, as previously described [29]. Most recent donor chimerism for T cell, B cell, and myeloid lineages was recorded. Chimerism was defined as donor (>95% donor cells), high-level mixed (50% to 95% donor cells), mixed (5% to 49% donor cells) or recipient (<5% donor cells) in specific cell lineages.

Viral Clearance

Patients were considered to have viral infection when a positive virology result by PCR, tissue culture, electron microscopy, or immuno-chromogenic methods was present. Viral PCR for adenovirus, cytomegalovirus, human herpes virus 6, Epstein-Barr virus, enterovirus, parainfluenza virus 3, respiratory syncytial virus, coronavirus, astrovirus, varicella-zoster virus, and norovirus; electron microscopy for small round structured virus, coronavirus, norovirus, astrovirus, and rotavirus; tissue culture for poliovirus vaccine strains; and immuno-chromogenic methods for adenovirus and rotavirus were undertaken at Newcastle Health Protection Agency Laboratories, Newcastle, United Kingdom. Viral infections were considered cleared on the first of 3 consecutively negative virology results. If there was recurrence of the same infection at a later date, this was treated as failure to clear the virus. Viral infection was considered present if the patient died with most recent result being positive or if there was still virological evidence of infection at last follow-up.

GvHD, Mortality and Follow-up

GvHD grade, target organ, and whether it was acute or chronic were recorded. GvHD was defined by modified Glucksberg criteria. Time to death and cause of death were recorded. Transplantation-related mortality (TRM) was defined as cause of death as a direct result of the transplantation procedure. Time to most recent follow-up and independence of replacement immunoglobulin were recorded.