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

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Abstract

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 90.5% 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

Financial disclosure: See Acknowledgments on page 248.

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Article history:
Received 2 April 2013
Accepted 6 November 2013

Key Words:
Primary immunodeficiency
Cord blood transplantation
Immune reconstitution
Serotherapy
Alemtuzumab

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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 naive [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 alemtuzumab. 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 ≥ 0.9 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 5 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 serotherapy 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. The markers CD4+/CD20−, CD19+ NK cells and CD4+ T cells were recorded. Lymphocyte subset analysis was performed on fresh whole blood 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 (3% 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 method 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 immunochromogenic 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.

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.

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] > 5.0 × 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/CD5RAFITC/ CD27 PE, CD3 PerCP/CD27 FITC/μg APC/μg PE [Becton Dickinson, UK Ltd, Oxford]), and analyzed on a Becton Dickinson FACS Calibur flow cytometer. The markers CD4+, CD8+, CD19+, natural killer (NK) cells and CD4+ and CD8+ T-cells were 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/CD5RAFITC/ CD27 PE, CD3 PerCP/CD27 FITC/μg APC/μg PE [Becton Dickinson, UK Ltd, Oxford]), and analyzed on a Becton Dickinson FACS Calibur flow cytometer. The markers CD4+, CD8+, CD19+, natural killer (NK) cells and CD4+ and CD8+ T-cells were recorded. Lymphocyte subset analysis was measured by 4-color flow cytometry as previously described [26].
RESULTS

Transplantation Characteristics and Serotherapy Dose

Demographics and transplantation characteristics are summarized in Table 1. All patients were 12 months of age or younger at transplantation. Only year of transplantation was significantly different between high-dose and low-dose serotherapy groups (P = .001). Low-dose serotherapy CBT all occurred after 2006, whereas high-dose serotherapy CBT mainly occurred before 2006, consistent with the systematic change in practice.

Engraftment

Neutrophil engraftment was similar for high- and low-dose serotherapy (P = .675), with median engraftment occurring at 21 days (range, 13 to 38) and 20 days (range, 15 to 31), respectively. Platelet engraftment occurred at a median of 38 days (range, 18 to 114) and 31 days (range, 0 to 46) for the high- and low-dose serotherapy groups respectively (P = .095). Platelet engraftment took over 100 days in 1 patient in the high-dose serotherapy group.

Lymphocyte Reconstitution

CD3⁺ lymphocyte engraftment occurred at a median time of 92.5 days (range, 56 to 222) and 97 days (range, 14 to 107) for high- and low-dose serotherapy, respectively (P = .171).

T lymphocyte counts increased throughout the first 12 months post CBT across the different T lymphocyte subsets (Table 2, Figure 1A-D). Reconstitution was similar at 2 months for high- and low-dose serotherapy groups (Table 2, Figure 1A-D). By 6 and 12 months, CD3⁺, CD4⁺, and CD4⁺ ETEE cell reconstitution in the high-dose group was similar to low-dose group. CD8⁺ lymphocyte reconstitution was similar for high- and low-dose serotherapy groups throughout the first 12 months after CBT.

B lymphocyte counts showed a trend of increasing in number over the first 12 months post CBT for both high- and low-dose serotherapy. There was no significant difference in B cell reconstitution between high- and low-dose serotherapy groups (Table 2, Figure 1E). NK cell counts were significantly higher at 2 months (P = .006) for high-dose serotherapy as compared with low-dose serotherapy. At 4, 6, and 12 months, there was no significant difference in NK cell counts between serotherapy groups (Table 2, Figure 1F).

Viral Clearance

Seven patients had at least 1 viral infection at time of CBT and 7 patients contracted at least 1 viral infection post transplantation. Of these, 9 patients achieved clearance of all virus and a further 3 patients had insufficient data to determine achievement of viral clearance. There was no difference in the number of viral infections present at CBT or new viral infection after CBT between high- and low-dose serotherapy groups. All evaluable patients who received serotherapy cleared viral infections (Table 3). There were insufficient results to analyze the time to viral clearance further.

Chimerism and Graft Function

There was no significant difference in donor chimerism between high-dose and low-dose serotherapy in any of the 3 cell lineages (Figure 2). One patient with Chronic Granulomatous Disease who received high-dose serotherapy has undergone a second HSCT for falling myeloid chimerism.

All except 2 patients discontinued replacement immunoglobulin by 24 months and all those who have been vaccinated have responded to Haemophilus influenza b, tetanus toxoid, and pneumococcal vaccinations (Table 3). Two patients who received high-dose serotherapy were dependent on immunoglobulin therapy to 32 and 34 months and have since made a good response to primary vaccinations. One patient in the low-dose serotherapy group has

Table 2

| Lymphocyte Subset | Serotherapy Dose | Lymphocyte Count, Median (range) in Cells/μL |
|-------------------|-----------------|---------------------------------------------|
|                   |                 | 2 Months | 4 Months | 6 Months | 12 Months |
| CD3⁺              | High            | 48 (0-515) | 555 (0-1339) | 1423.5 (82-3774) | 3423.5 (158-7045) |
| Low               | 146 (0-638)    | 1103 (176-1694) | 1871 (203-4201) | 3477 (1587-5952) |
| P value           | .614           | .037     | .675     | .113     |
| CD4⁺              | High            | 20 (0-382) | 248 (0-1123) | 746.5 (48-2711) | 2139 (123-6077) |
| Low               | 0 (0-319)      | 177 (34-1243) | 1515 (176-2232) | 2754 (923-4033) |
| P value           | .685           | .037     | .685     | .288     |
| CD8⁺              | High            | 46 (0-320) | 143 (0-308) | 304.5 (27-2821) | 1071 (29-2813) |
| Low               | 0 (0-141)      | 179 (0-467) | 357 (49-759) | 996 (415-1750) |
| P value           | .403           | .569     | .855     | .965     |
| CD4⁺ ETEE         | High            | Not measured | 31.5 (0-455) | 318.5 (0-1691) | 1614 (0-4862) |
| Low               | Not measured   | 339 (13-677) | 724 (23-1318) | 1786 (556-2619) |
| P value           | NA             | .021     | .286     | .270     |
| CD19⁺             | High            | 415 (0-2323) | 1320 (0-4517) | 1473 (32-3355) | 1475 (577-2155) |
| Low               | 1143 (405-6833) | 1445 (650-2630) | 1286 (278-2294) | 1840 (0-2410) |
| P value           | .119           | .382     | 1.00     | .310     |
| NK cells          | High            | 357 (184-771) | 485 (167-1399) | 434.5 (32-1547) | 288 (163-1277) |
| Low               | 226 (136-363)  | 346 (93-489) | 382 (125-1562) | 417 (202-787) |
| P value           | .006           | .113     | .514     | .627     |

Mann-Whitney U P values stated for difference between high- and low-dose serotherapy and considered significant if P < .05.
discontinued immunoglobulin but has not yet received primary vaccinations.

**GvHD and Mortality**

Eight patients developed acute GvHD (40%); 5 grade II GvHD (25%). No patients experienced grade IV GvHD. There was no statistically significant difference in the severity of GvHD between high- and low-dose serotherapy groups ($P = .464$; Table 3). One patient, who received high-dose serotherapy, developed chronic skin GvHD.

All TRM occurred within 6 months of transplantation. One patient who received high-dose chemotherapy died from TRM (possible veno-occlusive disease and gastrointestinal hemorrhage complicated by chronic lung disease). Another patient who received high-dose serotherapy died from sudden infant death syndrome and was not classified as TRM.

Cumulative survival from TRM in high-dose and low-dose serotherapy groups was 90.9% (95% confidence interval, 50.8% to 98.7%) and 100%, respectively, with no difference in survival between groups ($P = .366$; Figure 3).

**DISCUSSION**

NK and B cell reconstitution has been demonstrated to occur early after CBT, whereas low T cell numbers have been demonstrated to remain for the first 6 months post CBT [11,30], increasing the potential risk of viral infections as compared to BMT or PBSC [4,5]. Giving T lymphocyte-depleting serotherapy as GvHD prophylaxis prolongs T lymphopenia post transplantation [12,18] and giving high doses can increase the risk of viral infection further [14,17,20-25]. In our study, lower dose serotherapy improved T lymphocyte reconstitution with better thymopoiesis at 4 months post transplant than high-dose serotherapy. This is particularly pertinent in the setting of CBT for PID. In our study, the majority of patients had SCID and, therefore, further delay in T cell reconstitution from excessive serotherapy might delay clearance of pre-existing viral infection. The delay in immune reconstitution between high- and low-dose serotherapy could be explained by the prolonged exposure of the graft to lympholytic antibodies when higher doses are given. Lympholytic concentrations of alemtuzumab...
have been shown to be present up to 56 days after HSCT when very high doses are given in vivo [31].

A recent prospective series of 30 pediatric patients who underwent CBT for malignant and nonmalignant disease without serotherapy conditioning showed early restoration of T lymphocytes within the first 2 months post CBT from a thymic independent pathway that produced effective viral specific T cells. Thymopoiesis was evident at 1 year after transplantation [32]. In contrast to this study by Chiesa et al., our study demonstrated a delayed restoration of median T cell counts until 4 months post CBT when low-dose serotherapy was given and a further delay in T lymphocyte reconstitution to 6 months post CBT when using high dose serotherapy. CD3+ reconstitution in our study improved at 4 months when low-dose serotherapy was given as compared with high-dose serotherapy, which is likely caused by corresponding improvements in CD4+ and CD4+ ETEE counts in the low-dose serotherapy group. The evidence of thymopoiesis in the low-dose serotherapy group at 4 months again contrasts with the study by Chiesa et al. The T cell reconstitution in the low-dose serotherapy group seen in our study at 4 months post transplantation is, therefore, likely to be explained by expansion in both a thymic dependant and independent manner.

Patients in our study experienced much lower rates of acute GvHD than in the study by Chiesa et al. (grade II to IV acute GvHD 25% versus 50%), though this may have been confounded by the inclusion of patients with malignant disease in the latter study [32]. We found the protection afforded against GvHD by lower dose serotherapy to be similar to high-dose serotherapy. In the study by Chiesa et al., 63% of patients had viral infections and 2 patients died from viral infection [32]. In our study, 35% of patients had pre-existing viral infection, and 35% developed new viral infection after CBT, with no difference between high- and low-dose serotherapy groups. This is important in the PID setting where many patients present with pre-existing viral illness. However, our definition of viral clearance and the small numbers of patients with viral infection at time of transplantation make it difficult to determine the effects of serotherapy dose on clearance of pre-existing viral infections. The influence of giving serotherapy on viral clearance in the context of PID and CBT needs further work with larger studies.

Other single and multicenter studies of CBT for PID tended to use a standard dose of ATG as serotherapy [7-10]. Knutsen et al. demonstrated that in 8 patients with T cell immunodeficiencies who received the same dose of ATG (30 mg/kg/day for 3 days) CD4+ and CD8+ lymphocyte

| Table 3 | Transplantation Outcomes and Complications after Cord Blood Transplantation |
|---------|--------------------------------------------------|
| Serotherapy | P Value |
| High Dose | Low Dose | |
| Viral infection | | |
| No. patients with pre-existing infection at CBT | | |
| No virus | 6 | 8 | .404 |
| 1 viral infection | 3 | 1 | |
| 2+ viral infections | 2 | 0 | |
| No. patients with new infection post CBT | | |
| No virus | 8 | 5 | .336 |
| 1 viral infection | 3 | 2 | |
| 2+ viral infections | 0 | 2 | |
| Viral clearance | | |
| No. patients achieving complete viral clearance | 5 | 4 | |
| No. patients not clearing all virus | 0 | 0 | |
| Acute GvHD | | |
| 0 | 6 | 6 | .464 |
| I | 1 | 2 | |
| II | 4 | 1 | |
| III | 0 | 0 | |
| Immunoglobulin replacement stopped by 24 months | | |
| 7/9 | 9/9 | NA | |
| Achieved vaccine response | | |
| 9/9 | 8/8 | NA | |
| Mortality | | |
| 1 TRM 1 SIDS | 0 | |
| Transplantation survival rate | | |
| 5/9 | 1/9 | NA | |

CBT indicates cord blood transplantation; GvHD, graft-versus-host disease; TRM, transplantation-related mortality; SIDS, sudden infant death syndrome.

![Figure 2](image.png)

Figure 2. Most recent chimerism results for (A) T cell, (B) B cell, and (C) myeloid cell lineages for high- and low-dose serotherapy. There was no significant difference between high and low-dose serotherapy for any of cell lineage ((A) P = .566, (B) P = .816, (C) P = .910).
numbers increased from 3 months and there was evidence of thymopoiesis (using presence of CD4+CD45RA cells as marker) from 12 to 24 months [9]. This evidence of thymopoiesis occurs later than that demonstrated by the low-dose serotherapy group in our study. Overall other studies demonstrated higher rates of GvHD grades II to IV than our study [7-10]. However, these rates of GvHD are more likely to be caused by the greater number of CBT with 2 or more mismatched HLA loci, as compared with our study than the speed of immune reconstitution and thymopoiesis over the post CBT but increased diversity as compared with BMT recipients at 2 years post HSCT [33]. Our current study shows that clinically there is good long-term immune function with independence from immunoglobulin replacement by 2 years post CBT and response to primary vaccinations independent of serotherapy dose. Our study is, however, limited to looking at numerical and not functional immune reconstitution and analysis of the effects of serotherapy on T cell functional reconstitution merits further work.

Our study is limited to comparing high- and low-dose serotherapy regimens only and no comparison was made to patients not receiving serotherapy as part of their conditioning regimen. This is because patients who did not receive serotherapy were preselected based on poor clinical condition at time of transplantation, many of whom did not receive chemotherapeutic conditioning either, or they did not receive serotherapy because they received a matched sibling donor CBT. The only factor that differed significantly between the high and low-dose serotherapy groups was that low-dose serotherapy CBT occurred more recently. This was due to a systematic change in the serotherapy policy at our unit. This would have allowed for introduction of more up-to-date supportive measures in the low-dose serotherapy group, potentially influencing survival but not immune reconstitution.

Our results show that low-dose serotherapy improves speed of immune reconstitution and thymopoiesis over the first year after CBT without an increase in incidence of GvHD, as compared with high dose serotherapy. Our results also demonstrate lower rates of GvHD than in other studies. The extent that giving serotherapy affects the risk of delayed viral clearance is unclear from the current study and further research is needed.

**ACKNOWLEDGMENTS**

**Financial disclosure:** The authors have nothing to disclose.

**Conflict of interest statement:** There are no conflicts of interest to report.

**Authorship statement:** A.R.G. and M.A.S. designed the study; J.P.L., P.T.G.E., M.A.S., and, A.R.G. analyzed the data; J.P.L. wrote the paper; J.P.L., M.A.S., and A.R.G. were involved in reviewing the draft; Z.N., S.H., T.J.F., A.J.C., M.A.S., and A.R.G. were involved in acquisition of the data; and D.B. and A.J. provided laboratory tests.

**SUPPLEMENTARY DATA**

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2013.11.005.

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