Kinetics of T-cell development of umbilical cord blood transplantation in severe T-cell immunodeficiency disorders

Alan P. Knutsen, MD,[a] and Donna A. Wall, MD[b] *St Louis, Mo*

Background: Hematopoietic stem-cell transplantation is the treatment of choice for severe primary T-cell immunodeficiencies. When an HLA-identical sibling donor is not available, an alternative donor stem-cell source is needed. In primary T-cell immunodeficiencies, T-cell–depleted HLA-haploidentical bone marrow transplantation has been particularly successful in reconstituting the T-cell immune system in many of the severe combined immunodeficiency syndrome types. However, there are some problems associated with this preparation as a stem donor source, such as increased resistance to engraftment, a long period of time for T-cell engraftment to occur, and failure to engraft B cells and B-cell functions. These problems can be especially troublesome if the patient is infected before the transplantation.

Objective: Umbilical cord blood was evaluated as a stem-cell source for immune reconstitution in children with severe primary T-cell immunodeficiency disorders, such as severe combined immunodeficiency syndrome, reticular dysgenesis, thymic dysplasia, and combined immunodeficiency disease, when a matched sibling donor was unavailable.

Methods: From January 1996 through July 1997, 6 children received unrelated cord blood stem-cell transplantation after a preparative regimen for the treatment of combined immunodeficiency diseases. The patients ranged in age from 2 weeks to 6 years. The cord blood units were 3 of 6 HLA antigen matches in 2 children, 4 of 6 HLA antigen matches in 3 children, and 5 of 6 HLA antigen matches in 1 child, with molecular HLA-DR mismatch in 3 of the children.

Results: The average time for neutrophil engraftment (absolute neutrophil count, >500/mm^3) was 12 days (range, 10 to 15 days), and the average time for platelet engraftment (platelet count, >20,000/mm^3) was 36 days (range, 24 to 50 days). In a patient with reticular dysgenesis, the first transplant failed to engraft but fully engrafted after a second unrelated donor cord blood transplantation. Five of 6 patients exhibited grade I graft-versus-host disease (GvHD), although 1 child experienced grade IV skin and gut GvHD. Immunologic reconstitution demonstrated that cord blood stem-cell transplantation resulted in consistent and stable T-cell, B-cell, and natural killer–cell development. The kinetics of recovery of phenotypic expression and function of T cells occurred between 60 to 100 days and that of natural killer cells at approximately 180 days. B cells engrafted early, and a study of functional B-cell antibody responses revealed that 2 of 2 patients in whom intravenous immune globulin was discontinued have low detectable antibody responses to tetanus and diphtheria toxoid immunizations more than 1 year after the transplantation.

Conclusions: Unrelated umbilical donor cord blood is an excellent source of stem cells for transplantation of children with immune deficiency disorders. Benefits include rapid and reliable recovery of immune function, low risk of GvHD, and low viral transmission rate. (J Allergy Clin Immunol 1999;103:823-32.)

Key words: *Umbilical cord blood transplantation, severe combined immunodeficiency, combined immunodeficiency, graft-versus-host disease*

Hematopoietic stem-cell transplantation is the treatment of choice for a broad spectrum of severe primary T-cell immunodeficiencies, including severe combined immunodeficiency syndrome (SCID), reticular dysgenesis, thymic dysplasia (Nezelof's syndrome), and combined immunodeficiency disease.[1-8] If available, an HLA-identical sibling is the donor of choice.[3,5] Frequently this is not an option, and an alternative stem-cell source is needed. In primary T-cell immunodeficiencies, T-cell–depleted HLA-haploidentical bone marrow transplantation has been particularly successful in reconstituting the T-cell immune system in many of the SCID types.[1-8] However, there are some problems associated with this preparation as a stem donor source. First, there is increased resistance to engraftment or nonengraftment in some of the T-cell defects (such as adenosine deaminase deficiency, SCID with elevated natural killer [NK] cells, and thymic dysplasia). Second, there may be a long period of time for T-cell engraftment to occur (up to 3 to 6 months), resulting in only weak T-cell function before eventual graft rejection. Third, B-cell antibody function frequently remains abnormal. Graft failure and the prolonged time to T-cell engraftment is a particular problem if the infant was already infected before the time of the transplantation. Because of the problems associated with T-cell–depleted haploidentical bone marrow transplantation, unrelated donor bone marrow transplantation has been evaluated as an alternative stem-cell source.[9] Such transplantations are often complicated because of the difficulties in the timely location of a suitable donor for the

From [a]the Divisions of Allergy/Immunology and [b]Hematology/Oncology/Bone Marrow Transplantation, Department of Pediatrics, Saint Louis University Health Sciences Center and Cardinal Glennon Children's Hospital, St Louis.

Received for publication June 12, 1998; revised Nov 5, 1998; accepted for publication Nov 6, 1998.

Reprint requests: Alan P. Knutsen, MD, Allergy/Immunology, Pediatric Research Institute, 3662 Park Ave, St Louis, MO 63110.

0091-6749/99 $8.00 + 0 **1/1/95765**

Abbreviations used

Con A:	Concanavalin A
GvHD:	Graft-versus-host-disease
Jak3:	Janus kinase 3
NK:	Natural killer
PWM:	Pokeweed mitogen
SCID:	Severe combined immunodeficiency syndrome
UCB:	Umbilical cord blood

children with SCID. In addition, matched unrelated bone marrow transplantation is associated with a significant risk of acute and chronic graft-versus-host disease (GvHD). There is also the associated risk of transferring viruses (eg, EBV and cytomegalovirus) with the bone marrow infusion, resulting in a primary infection in the immunocompromised child that could lead to disseminated infection or lymphoproliferative disorder.

Recently, umbilical cord blood (UCB) transplantation was evaluated as an alternative stem-cell source when a genotypic HLA-matched donor was not available in other clinical settings. UCB transplantation was first used in an HLA-matched sibling setting in 1988 and resulted in a durable engraftment.[10] Both related and unrelated donor cord blood transplantations have been performed.[11-16] Several cord blood banks are now able to provide unrelated HLA-matched donor cord blood for transplantation.[16,17] In deciding on the hematopoietic stem-cell sources, it is important to consider issues unique in the treatment of immunodeficiency disorders. First, because these children frequently are infected before the transplantation, it is important that the transplantation result in rapid recovery of both myeloid (if ablative regimen used) and lymphoid function. Second, both acute and chronic GvHD must be minimized. Third, functional B-cell development is important. Fourth, transmission of viral infections with the stem-cell source, such as EBV and cytomegalovirus, must be minimized. Thus the optimal hematopoietic stem-cell source should produce a high rate of rapid, durable engraftment with a minimum of GvHD and have a low viral transmission rate. In this study we report the results of our first 6 patients, who received unrelated UCB transplants for severe primary T-cell immunodeficiency disorders.

METHODS

Patients

Patients seen at the Stem Cell Transplant Program at Cardinal Glennon Children's Hospital with severe T-cell immunodeficiency were considered for unrelated donor cord blood transplantation if they did not have an HLA-matched donor available. HLA matching between UCB donor and patient was based on serologic class I HLA typing and high-resolution molecular class II typing. Priority was given for HLA-DR matching at the high-resolution molecular level. This study was approved by the Institutional Review Board at Saint Louis University Health Sciences Center.

Preparative regimen and GvHD prophylaxis

The transplantation regimen used was part of a collaboration with Dr Joanne Kurtzberg (Duke Cord Blood Collaborative Group). Conditioning for all patients was done with busulfan 1 mg/kg (1.25 mg/kg if the patient was less than 2 years old) orally every 6 hours on days 9 through 6 before transplantation. The dosage of busulfan was adjusted on the basis of first-dose kinetics (steady-state level, 400-600 ng/mL). This was followed by cyclophosphamide 50 mg/kg intravenously on days 5 through 2 before the transplantation and antithymocyte globulin 30 mg/kg intravenously on days 3 through 1 before the transplantation. UCB infusion was on day 0. One child did not experience engraftment and had early trilineage autologous recovery of hematopoiesis. She was subsequently treated with a salvage preparative regimen with fractionated total body irradiation (1000 cGy), cyclophosphamide, and antithymocyte globulin followed by a second infusion of unrelated donor cord blood from a second unrelated donor. Prophylaxis for acute GvHD included continuous infusion of cyclosporine A beginning on day 2, targeting whole blood levels to be 250 to 350 ng/mL, and methylprednisolone 10 mg/kg/d on days 5 through 7, 5 mg/kg/d on days 8 through 10, and 3 mg/kg/d on days 11 through 13, followed by a 10% weekly reduction taper. The patients were evaluated daily during hospitalization for acute GvHD and at least weekly after hospital discharge for the first 100 days after transplantation. The diagnosis of acute GvHD was made by clinical evaluation. Corticosteroids were generally discontinued by day 60 after the transplantation, and cyclosporine A was discontinued between days 100 and 365, depending on clinical evidence of GvHD.

Preparation of UCB

Previous reports detailed the collecting and processing of UCB.[16,17] Cryopreserved donor cord blood units were obtained from the New York Placental Blood Program or the St Louis Cord Blood Bank. All units used were seronegative for cytomegalovirus, EBV, hepatitis viruses, and HIV types 1 and 2. Hemoglobin electrophoresis was performed on units at risk for sickle-cell disease and thalassemia. The thawing procedure used was developed by Rubinstein et al.[17] Briefly, the UCB was placed in a sterile bag and thawed in a 38°C water bath with gentle agitation. After thawing, an equal volume of dextran/albumin solution was added over 10 minutes to the UCB; it was centrifuged at 250*g* for 10 minutes at 10°C, and the supernatant was removed. The buffy coat was resuspended in dextran/albumin and immediately infused into the patient over 5 to 10 minutes.

Supportive care

All the patients were hospitalized in single rooms with high-efficiency particulate air filtration systems. Prophylactic acyclovir was used if the patient had been diagnosed as *Herpes simplex* virus or cytomegalovirus positive before the treatment. Fluconazole was used for prevention of fungal infections. Patients were administered intravenous immune globulin 500 mg/kg intravenously weekly through day 100 after the transplantation and then monthly for the first year. Granulocyte colony-stimulating factor at 10 mg/kg/d subcutaneously was administered from the day of the transplantation until the absolute neutrophil count of more than 2000/mm^3 was attained. Patient 2 received irradiated granulocyte infusions during the neutropenic nadir to assist the management of necrotizing *Pseudomonas* pneumonitis that was present before the transplantation.

Engraftment

Hematologic recovery was defined as achieving an absolute neutrophil count of more than 500/mm^3 (first of 2 consecutive days) and a platelet count of more than 20,000/mm^3 (first of 7 consecutive days without the need for transfusion). Donor-cell engraftment was assessed by RFLP, quantitating donor-recipient chimerism.

TABLE I. Initial immunologic studies of patients with severe primary T-cell immunodeficiency disorders before UCB transplantation

Study	Patient						Normal values
	UPN 036	CG 52	CG 67	CG 74	CG 64	CG 71	
Diagnosis	Nezelof's syndrome	SCID, Jak3	AR, SCID	AR, SCID	Reticular dysgenesis	CID with ↓CD4	
Sex	F	F	F	F	F	M	
Age at diagnosis/transplantation	15/15 mo	5/10 mo	NB/2 wk	5/6 mo	2 wk/1 & 3 mo	5/6 yr	
Lymphocytes (mm^3)	1598	610	312	1120	574	1638	3800 -9900
$CD3^+$ cells (mm^3)	304	0	0	101	75	901	2400 - 6900
%	19	0	0	9	13	55	50 - 77
$CD4^+$ cells (mm^3)	96	12	9	134	34	278	1400 - 5100
%	6	2	3	12	6	17	33-58
$CD8^+$ cells ($mm^{3)}$	128	6	144	45	98	606	600 - 2200
%	8	1	46	4	17	37	13-26
$CD20^+$ cells (mm^3)	927	567	16	0	80	491	700 - 2500
%	58	93	5	0	14	30	13 - 35
$CD56^+$ cells (mm^3)	288	73	275	594	264	246	200 -1200
%	18	12	88	53	46	22	2-13
PHA (cpm)	14,315	–944	179	18,819	3020	30,813	257,076 ± 2.6
Normal response (%)	5.1	2.0	0.1	5.9	1.0	13.7	≥50
Con A (cpm)	17,014	–218	287	43,035	–386	16,531	163,503 ± 3.1
Normal response (%)	9.0	1.0	0.1	18.3	0.1	7.8	≥50
PWM (cpm)	3867	–1815	450	367	–3973	26,096	116,647 ± 2.9
Normal response (%)	3.0	0.8	0.1	0.2	3.3	11.2	≥50
C albicans (cpm)	2922	191	—	–2194	—	7245	16,846
SI	1.6	1.1	—	0.8	—	1.7	≥3
Tetanus toxoid (cpm)	1943	1863	—	–2592	—	8126	17,482
SI	1.4	2.0	—	0.7	—	1.8	≥3
MLC (cpm)	5773	1929	1997	2290	–227	–1237	119,893 ± 1.1
Normal response (%)	4.2	1.2	1.2	1.7	0.2	1.6	≥30
NK-cell cytotoxicity (%)lysis	1.5	—	0	72.2	0	27.5	41 ± 3
IgG (mg/dL)	303	56	950	<46	1150	237	268 - 717
IgA (mg/dL)	175	<7	<7	<5	<7	41	12 - 31
IgM (mg/dL)	55	34	<5	<5	<25	44	18 - 191
IgE (IU/mL)	8	15	<5	<5	<5	108	3 - 15

Normal lymphocyte population for 9- to 15-month-old patients is expressed as the range for 5th and 95th percentiles.[37] Normal lymphoproliferative responses are expressed as mean ± SE, and NK cytotoxicity is expressed as mean ± SE in adult control mean.[20] Normal immunoglobulin serum concentrations for 7- to 9-month-old patients are expressed as the range for 5th and 95th percentiles.

UPN, Unique patient number; *AR*, autosomal recessive; *NB*, newborn; *MLC*, lymphoproliferative response to B-cell alloantigens; SI, stimulation index.

Immunologic studies

T-, NK-, and B-cell enumeration and lymphocyte stimulation studies were performed as previously described.[18-20] Cytofluorographic analyses of lymphocyte subpopulations were determined by reacting murine monoclonal antibodies conjugated either with FITC or phycoerythrin and then analyzed by flow cytometry (FACScan; Becton Dickinson, San Jose, Calif). Lymphoproliferative responses were performed to PHA (Difco, Detroit, Mich), concanavalin A (Con A; Sigma, St Louis, Mo), pokeweed mitogen (PWM; Gibco BRL, Grand Island, NY), *Candida albicans* (Greer Laboratories, Lenoir, NC), tetanus toxoid (Connaught Laboratories, Willowdale, Ontario, Canada), and alloantigens (mixed B-cell pool). NK-cell activity was assayed with PBMCs isolated from heparinized venous blood by Ficoll-Hypaque density gradient sedimentation as effector cells against chromium ^{51}Cr–labeled K562 cell–line target at an effector:target ratio of 100:1.[3] Normal control subjects consisted of healthy adult laboratory personnel volunteers. Serum IgG, IgA, and IgM quantification was performed with nepholometry by the clinical laboratory in the Department of Pathology.[21] Antidiphtheria and antitetanus antibody titers were measured by tanned-cell hemagglutination.[18,21] Assay for adenosine deaminase and purine nucleoside phosphorylase activity in red blood cells were performed by M. S. Hershfield at Duke Medical Center.[3] Studies for identification of common cytokine receptor γ-chain and Janus kinase 3 (Jak3) deficiencies were performed by J. M. Puck at the National Institutes of Health and by J. Roberts at Duke Medical Center, respectively, by Western blot and DNA sequencing analyses on EBV-transformed B-cell lines.[3,22]

RESULTS

Patient characteristics

Six patients received unrelated donor UCB transplantations for primary T-cell immunodeficiency disorders (Table I) at Cardinal Glennon Children's Hospital of Saint Louis University Health Sciences Center from January 1996 through July 1997. Primary severe T-cell immunodeficiency disorders were diagnosed as Jak3 deficiency (1 patient) or autosomal recessive (2 patients) forms of SCID, Nezelof's syndrome (1 patient), reticular dysgenesis (1 patient), and combined immunodeficiency

TABLE II. Histocompatibility matching and cell dose of UCB, GvHD, and hematologic engraftment in patients with severe primary T-cell immunodeficiency disorders who underwent UCB transplantation

	Patients					
Study	**UPN 036**	**CG 052**	**CG 67**	**CG 74**	**CG 64**	**CG 71**
Degree of HLA match (n=6)	4	4	5	3	3	4
Location of mismatch	A, B	A, B	A	A, B, B	A, B, DR^{mol}	A, DR
UCB transplantation cell dose ($\times 10^8$ nucleated cells/kg)	1.3	2.0	1.1	1.6	1.4	1.2
GvHD (grade)	I	I	I	I	I	IV
Skin	+	+	+	+	+	+
GI	-	-	-	-	-	+
Engraftment (days)						
CD3 (> 1000 cells/mm^3)	239	218	101	189	246	—
PHA (> 50% of normal response)	134	71	70	97	246	153
ANC (> 500 cells/mm^3)	15	10	12	12	11	12
Platelets (> 20,000 cell/mm^3)	42	50	34	28	38	24

UPN, Unique patient number; *DR^{mol}*, molecular HLA-DR; *GI*, gastrointestinal; *ANC*, absolute neutrophil count.

TABLE III. Kinetics of immunologic reconstitution in patients with severe primary T-cell immunodeficiency disorders after UCB transplantation

	Lymphocyte populations (cells/mm^3)					Lymphoproliferative responses (% of normal response)					
	ALC	**$CD3^+$**	**$CD4^+$**	**$CD8^+$**	**$CD20^+$**	**$CD56^+$**	**PHA**	**Con A**	**PWM**	**Alloantigen**	**NK (% lysis)**
Pretransplantation(n=6)	975 ± 230	230 ± 142	94 ± 42	171 ± 89	347 ± 153	290 ± 69	5 ± 2	6 ± 3	3 ± 2	2 ± 1	20 ± 14
27 (n=3)	974 ± 941	152 ± 152	105 ± 104	56 ± 44	10 ± 10	62 ± 41	12 ± 1	9 ± 2	12 ± 2	28 ± 13	
65 (n=4)	2097 ± 679	245 ± 37	220 ± 44	101 ± 42	451 ± 351	157 ± 53	43 ± 12	75 ± 13	30 ± 13	40 ± 17	
100 (n=5)	2761 ± 755	616 ± 170	577 ± 157	141 ± 51	1527 ± 629	242 ± 80	48 ± 14	56 ± 15	29 ± 12	73 ± 24	0 ± 0
178 (n=5)	2499 ± 806	1083 ± 426	709 ± 236	562 ± 229	834 ± 378	537 ± 165	81 ± 15	73 ± 18	73 ± 15	71 ± 13	39 ± 7
242 (n=4)	5845 ± 2804	2905 ± 1516	1986 ± 827	918 ± 558	1690 ± 697	462 ± 108	69 ± 8	61 ± 16	40 ± 14	51 ± 11	
361 (n=3)	4672 ± 798	2875 ± 908	1960 ± 480	1014 ± 479	1357 ± 311	245 ± 32	108 ± 4	114 ± 10	121 ± 17	83 ± 22	42 ± 29
Mean (normal, range)	5500 (2600 - 10400)	3400 (1600 - 6700)	2300 (1000 - 4600)	1100 (400 - 2100)	1400 (600 - 2700)	400 (200-1200)	>50	>50	>50	>50	41 ± 3

Normal lymphocyte population for 9- to 15-month-old patients is expressed as median and percentile (5th and 95th percentiles).[37] Normal lymphoproliferative responses are expressed as mean ± SE, and NK cytotoxicity is expressed as mean ± SE in adult control mean.[20]

disorder with CD4 lymphopenia (1 patient). Three of the patients were ill at the time of transplantation with *Pseudomonas*-necrotizing pneumonitis that required lobectomy (patient CG 52), respiratory syncytial virus (RSV) pneumonia (patient CG 74), and bacterial and fungal pneumonitis and bronchiectasis (patient CG 71). The results of pretransplantation immunologic evaluation are presented in Table I. Significantly decreased $CD3^+$ T-cell percentages, numbers, and lymphoproliferative responses to mitogens (PHA, Con A, PWM), antigens (*C albicans*, tetanus toxoid), and alloantigen stimulations were present in the patients with SCID and Nezelof's syndrome. The patient with combined immunodeficiency disease and CD4 lymphopenia (patient CG 71) had a normal percentage and number of $CD3^+$ T cells but decreased percentages and numbers of $CD4^+$ T cells, with markedly decreased T-cell function. $CD56^+$ NK cells were elevated in the patients with autosomal recessive SCID (patients CG 67 and CG 74) and reticular dysgenesis (patient CG 64); however, only 1 of the patients with autosomal recessive SCID had increased NK-cell cytolytic function.

Hematopoietic engraftment

All patients had documentation of 100% donor chimerism (after the second transplantation in the child with reticular dysgenesis) determined by RFLP analysis once peripheral blood neutrophil and lymphocyte numbers recovered, usually at 1 month after transplantation. All patients in the group reached an absolute neutrophil count of more than 500/mm^3; the mean time to engraftment was 12 days (range, 11 to 15 days; Table II). The patient with reticular dysgenesis (patient CG 64) experienced rejection of the initial UBC transplant, with early trilineage recovery of autologous cells. She was prepared again as described and engrafted with the second cord

blood infusion. The mean time to platelet engraftment was 36 days (range, 24 to 50 days; Table II).

GvHD

As seen in Table II, the degree of acute GvHD among the patients in the series ranged from grade I in 5 patients to grade IV in 1 patient. Two patients had limited GvHD, consisting of mild skin rash and diarrhea that resolved with corticosteroid pulse during the cyclosporine taper. Patient CG 71, who had a serologic HLA-DR mismatch, experienced progression of GvHD while receiving steroid therapy but responded to antithymocyte globulin. Patient CG 71 eventually died of infectious complications. None of the surviving patients have experienced the development of chronic GvHD, with follow-up at 12 to 30 months.

Lymphoid development

The kinetics of development of the T-, B-, and NK-cell lymphocyte populations are presented in Table III. The mean absolute lymphocyte count increased at approximately 2 months, predominantly as the result of increases of $CD3^+$ T cells and $CD20^+$ B cells. $CD3^+$ T cells steadily increased between 100 and 240 days and thereafter remained stable in an age-adjusted normal range. The $CD4^+$ and $CD8^+$ T cells increased proportionally. $CD56^+$ NK cells increased at 180 days both in number and in function. Examination of the kinetics of T-cell development for the individual patients demonstrated a similar pattern and rate of development in all the patients, beginning at 100 days and steadily increasing (Fig 1). Similarly, lymphoproliferative responses to mitogens and alloantigens were detectable as early as 60 days, preceding the marked increase of T cells and steadily increasing to normal (defined as more than 50% of normal response) by 180 days, and remained stable (Fig 2). The development of NK cells occurred consistently by 180 days, both for $CD56^+$ NK-cell phenotype and NK-cell cytolytic function, as determined by percent lysis of K562 cells (Fig 3).

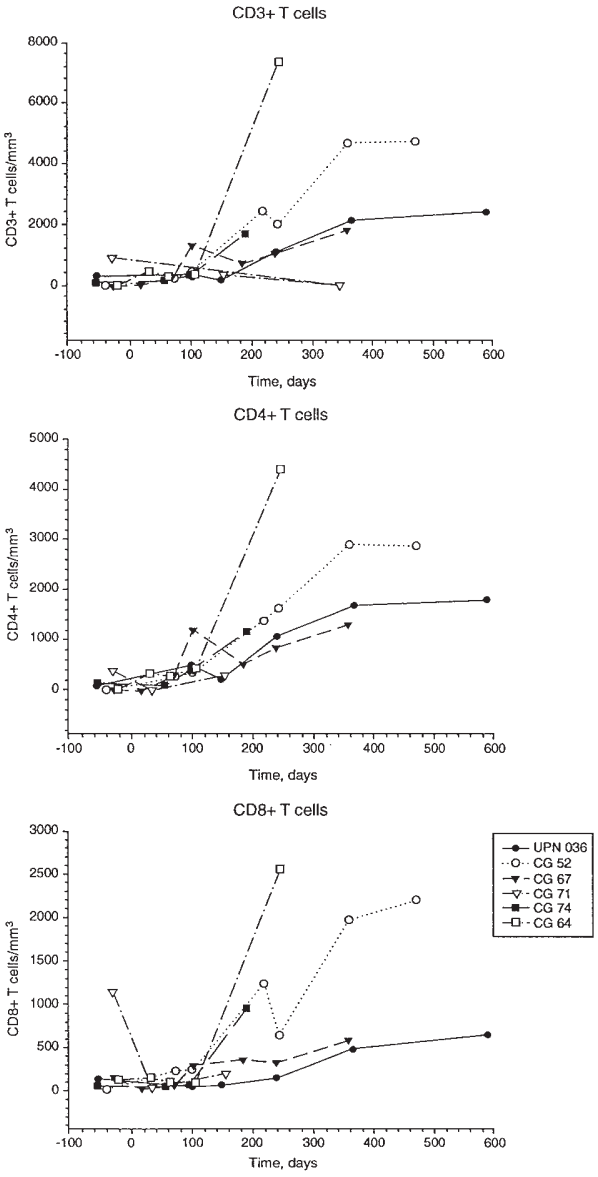

FIG 1. Kinetics of $CD3^+$, $CD4^+$, and $CD8^+$ T-cell engraftment for individual patients with severe T-cell immunodeficiency disorders. For each patient (with the exception of patient CG 71 who had grade IV GvHD), $CD3^+$ T cells appeared at approximately 100 days and steadily increased thereafter. $CD4^+$ and $CD8^+$ T cells increased proportionally. *UPN*, Unique patient number.

DISCUSSION

The optimal transplantation management of children with serious T-cell diseases entails the following issues: (1) a donor source when an HLA-matched relative donor is not available; (2) the specific T-cell immunodeficiency; (3) the preexisting infections and the relative benefit of transfer of competent mature T cells in the graft; (4) the use of immunosuppressive and/or myeloablative conditioning regimen before the transplantation; (5) the development of B-cell function or engraftment; and (6) the use of GvHD prophylaxis after transplantation.

In immune reconstitution of patients with severe T-cell diseases, the ideal stem-cell source is unmanipulated genotypic HLA-matched sibling bone marrow. However, when this is not available, there are 3 alternative hematopoietic stem-cell sources: (1) T-cell–depleted HLA haploidentical parental bone marrow; (2) phenotypic HLA-matched unrelated bone marrow; and (3) phenotypic HLA-matched unrelated UCB. Beginning in 1981, the development of bone marrow stem-cell transplantation using parental HLA haplotype-disparate T-cell–depleted bone marrow grafts has been highly successful and is the first choice in immune reconstitution of the T-cell system in patients with SCID who do not have an HLA-matched donor. This type of transplantation has also been highly instructive in elucidating the immunobiologic nature of engraftment, graft resistance, graft/host tolerance, and the capacity of donor T cells to develop

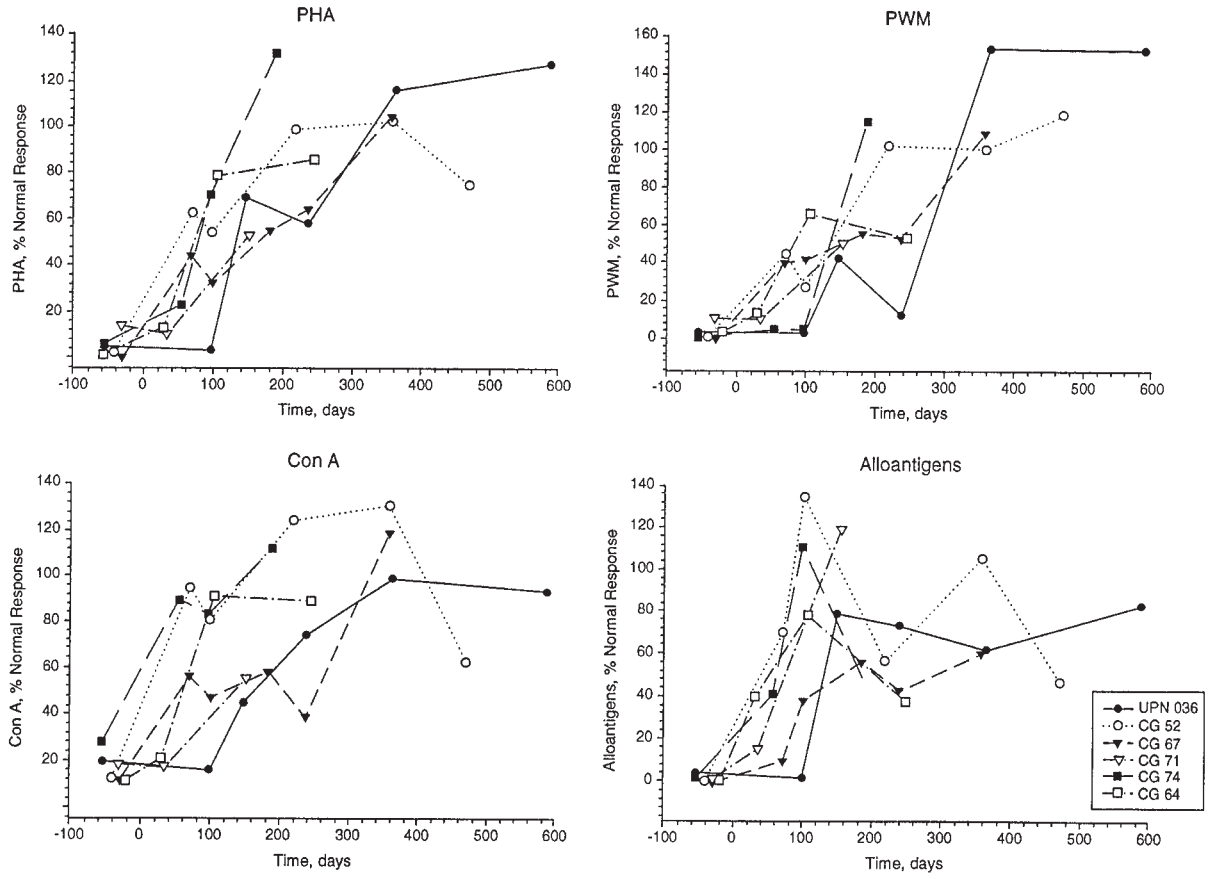

FIG 2. Kinetics of lymphoproliferative responses to mitogen (PHA, Con A, and PWM) and alloantigen engraftment for the individual patients with severe T-cell immunodeficiency disorders reported as percent normal response. Lymphoproliferative responses were detectable to mitogens and alloantigens as early as 60 days, preceding the marked increase of T cells and steadily increasing to normal (defined as more than 50% of normal response) by 180 days and remained stable thereafter. *UPN,* Unique patient number.

antigen-specific T-cell reactivity in an allogeneic host environment.[1] However, outcomes of T-cell–depleted HLA-haploidentical bone marrow transplantations are disappointing in certain T-cell immune deficiency disorders, such as adenosine deaminase–deficient SCID, thymic dysplasia (Nezelof's syndrome), Wiskott-Aldrich syndrome and combined immunodeficiency disease. In this study UCB was used as the hematopoietic stem-cell and T-cell source to immune reconstitute patients with severe T-cell immunodeficiency disorders. UCB is recognized as a source of $CD34^+$ hematopoietic progenitor cells that can result in durable engraftment with potentially lower degrees of GvHD and lower viral transmission than is expected from unrelated donor bone marrow.[23] Also potentially advantageous is the ability to infuse T cells with the graft, which may result in earlier immune recovery. Previous studies have indicated that 1 potential disadvantage of the use of UCB as a stem-cell source is that the time required for hematopoietic engraftment may be longer than that observed after bone marrow transplantation. Wagner et al[11] have previously reported successful T-cell development and engraftment in children with a variety of immunodeficiencies who underwent transplantation with UCB, including SCID, Wiskott-Aldrich syndrome, and X-linked lymphoproliferative syndrome. Our group and Hans Ochs' group have each successfully performed a transplantation for 1 patient with Wiskott-Aldrich syndrome using UCB.

In our series of infants with severe T-cell immunodeficiency disorders, we were able to infuse a minimum cell dose of 1×10^8 cord blood cells per kilogram, with a resulting neutrophil recovery time that is the same as that seen for bone marrow. The Eurocord Transplant Group[4] supports our finding, noting that infusion of 0.37×10^8 nucleated blood cells per kilogram leads to reliable neutrophil engraftment. Platelet recovery time was in the range that has been reported for cord blood transplantation and the same as that noted for unrelated HLA-matched bone marrow transplantation. Clearly with larger children and adults this cell dose is not currently possible. With continued development of technology for stem-cell expansion, however, UCB transplantation may soon be a potentially viable choice in the treatment of larger children and adults. More experience will be need-

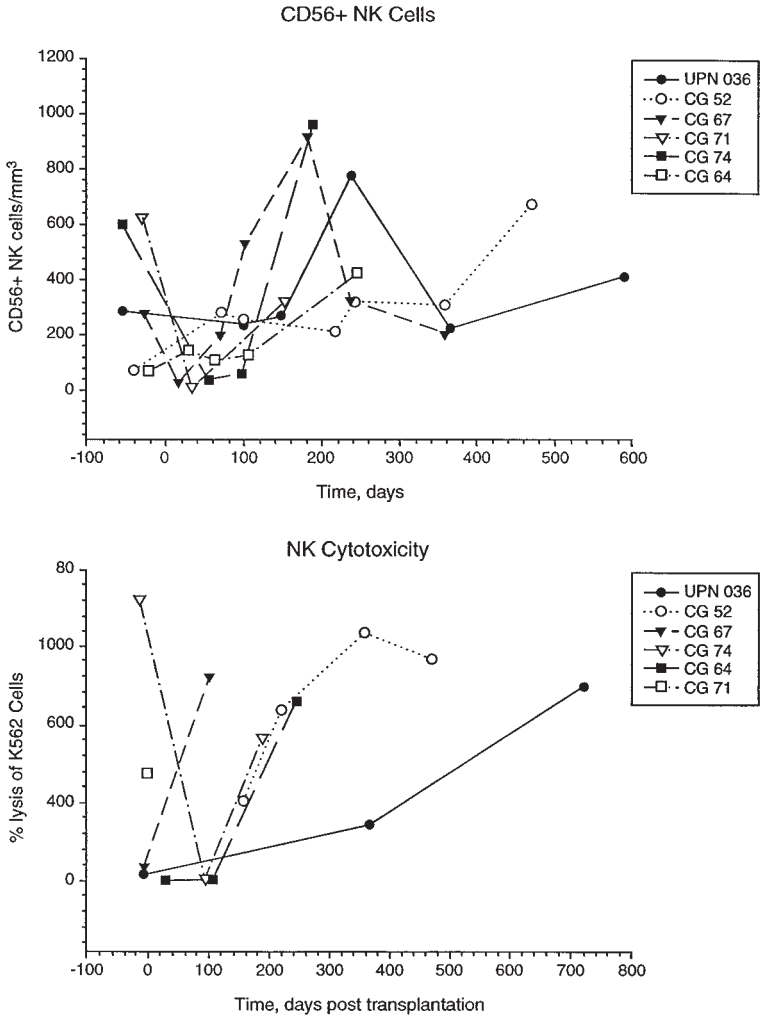

FIG 3. Kinetics of NK-cell engraftment for individual patients with severe T-cell immunodeficiency disorders. Engraftment of NK cells seemed to occur consistently by 180 days (with the exception of patient CG 71 who had grade IV GvHD), both for $CD56^+$ NK-cell phenotype and NK-cell cytotoxic function as measured by percent lysis of K562 cells. *UPN*, Unique patient number.

ed to directly answer whether UCB transplantation will result in equivalent or improved outcome compared with T-cell–depleted haploidentical bone marrow transplantation.

One important issue in the management of children with severe T-cell disorders is whether to use a preparative regimen before stem-cell infusion for immunosuppression to prevent rejection and/or myeloablation to make space that would allow donor T-, B-, and monocytic-cell engraftment. Indeed, this is an important question for all of the hematopoietic stem-cell options and not just for UCB. O'Reilly et al[1] and Fischer et al[24] reported graft failures in 30% and 50% of the patients who underwent T-cell–depleted bone marrow graft transplantation without prior cytoreduction in the United States and Europe, respectively. Four mechanisms have been proposed to explain graft resistance: (1) alloreactive host T cells; (2) host NK cells; (3) transplacentally derived maternal T cells; and (4) accessory cell dysfunction. In both murine models[25,26] and in human patients with SCID,[27-30] normal to high NK-cell activity has a higher incidence of graft failure or delayed immunologic reconstitution and is perhaps a foremost cause for graft rejection. The nature of the preparative regimen and the relative importance of immunosuppression versus ablation has not as yet been fully defined and may be dependent on the nature of the hematopoietic stem-cell graft. Furthermore, children with severe T-cell diseases may have serious infections and cannot eliminate the infection before the transplantation. This was observed in this cohort of patients (eg, necrotizing *Pseudomonas* pneumonia, RSV pneumonia, and chronic bronchiectatic lung disease). In these children, dependable early recovery of immunocompetent function was important. This is not necessarily so when T-cell–depleted haploidentical bone marrow transplantation is performed.[1,2,24] The pretransplantation

preparative regimen used in this study was a conventional regimen that is used in young children who are undergoing hematopoietic stem-cell transplantation for malignant and nonmalignant hematopoietic disorders. It was chosen because it has known myeloablative and immunosuppressive properties. Our goal was to ensure that there would be adequate immunosuppression and hematopoietic space to ensure trilineage engraftment of progenitor cells and, at the same time, infusion of immunocompetent mature T cells. The role of a myeloablation in the preparative regimen in transplantation for SCID remains controversial. Other groups have obtained stable immune reconstitution by using UCB transplantation in patients with thymic dysplasia and SCID without ablative therapy (R. Buckley, personal communication, 1997). However, there are some subsets of severe T-cell immunodeficiency disorders that require an immunosuppression preparative regimen, such as reticular dysgenesis, combined immunodeficiency disease, and thymic dysplasia. Some groups would also use a preparative regimen in SCID with high NK function.[1] In this study the child with reticular dysgenesis did not experience engraftment on the first transplantation despite treatment with a busulfan-containing preparative regimen and a high cell dose in the cord blood infusion (3.7×10^8 nucleated cells/kg). She had prompt trilineage recovery of autologous hematopoiesis after the myeloablative regimen, with no evidence of donor hematopoiesis on RFLP. This graft rejection occurred despite her neutropenia and lymphopenia but with NK-cell function before the transplantation. De Santes et al[31] reported difficulty in attaining engraftment in 2 infants with reticular dysgenesis but achieved success by using a radiation therapy–based preparative regimen. Importantly, we were able to attain engraftment with a second cord blood transplantation, supporting the belief that the difficulty in engraftment was not due to an intrinsic difference in the graft, such as lack of stromal cells, but rather was due to undefined host factors. The optimal combination of ablative agents (eg, busulfan, with immunosuppressive agents [such as antithymocyte globulin], cyclophosphamide, and/or newer agents [such as fludarabine]) has not been systematically studied and should be the focus of future clinical trial.[32] The risks of this preparative regimen include sterility; liver, heart, and lung toxicity; and the risk of malignancy. This needs to be balanced with the morbidity and mortality rates associated with graft rejection and repeated transplantation, poor T-cell engraftment, poor B-cell function, and the ultimate goal for the graft to provide complete T-, B-, and NK-cell function. The choice of the preparative regimen is further complicated by the heterogeneity of the NK- and B-cell function that can be expected to develop in the various forms of SCID when successful T-cell engraftment occurs.[1,3,24] In the preparative regimen used in this study, both lymphoid and myeloid lineages are of the same donor HLA-DR, and we hypothesized that T-cell, B-cell, and antigen-presenting cell cooperation would be optimal. In addition, these patients received posttransplantation GvHD prophylaxis of cyclosporine and corticosteroids, which would affect the function of mature T cells in the umbilical cord preparation. Because the incidence of acute GvHD has been low, it is not clear how much GvHD prophylaxis is needed.

In T cell–depleted bone marrow transplantation whether patients receive cytoreduction or not, mature T cells with functional responses to mitogens and alloantigens appear by 3 to 7 months after transplantation.[1] The quality of antigen-specific T-cell responses, although present, is still suspect. In murine and human studies bone marrow–derived CD34+ progenitor cells migrate to the thymus, where T-cell maturation and T-cell tolerance and restriction occurs.[1] Both thymic epithelial cells and thymic accessory cells are important in imprinting self-recognition and tolerance on developing T cells. HLA requirements between stem cells and thymic tissue for T-cell and antigen-presenting cell restriction are less clear. Conventional hypotheses postulate that some degree of HLA identity between the stem cell and the thymus must be present for subsequent T-cell and antigen-presenting cell restriction. However, in T-cell–depleted haploidentical bone marrow transplantation when patients are not treated with a cytoreduction preparative regimen, the donor thymocytes share only a single haplotype with host thymic epithelial cells and accessory cells. When a cytoreduction regimen is used, the thymic accessory cells (eg, dendritic cells and monocytes) are also of donor origin. In patients with SCID who have not received cytoreductive pretreatment, T cells are of donor origin, and monocytes and B cells are of host origin. In this setting Roberts et al[33] and Roncarolo et al[34] reported that donor T cells proliferated in response to tetanus toxoid–antigen stimulation only when presented by EBV-transformed B cells bearing the shared HLA-DR haplotype or the haplotype unique to the host. Geha and Rosen[35] further elucidated this in a patient who received cytoreduction and a T-cell–depleted bone marrow transplantation. Four years after the transplantation, both T cells and monocytes were of donor origin. Although tetanus toxoid–specific T-cell responses restricted to host-specific HLA-DR haplotype were still detectable, the major restriction was to donor haplotypes. Furthermore, when the patient was immunized to the neoantigen keyhole limpet hemocyanin, HLA restriction was of the donor and shared haplotype. These studies suggested that thymic dendritic cells and monocytes may be the predominant population important for positive selection. This poses the question of whether donor T cells that recognize antigen in an haploidentical host may have some limitation of intensity and diversity to the multitude of antigens infecting the host. This has led investigators to speculate whether both parents should be used as donors in T-cell–depleted haploidentical bone marrow transplantation. In this series early appearance of T-cell immunocompetence measured by lymphoproliferative responses to mitogens and alloantigens was observed, comparable to that seen in T-cell depleted haploidentical bone marrow transplantation. In addition, donor NK-cell number and function appeared

consistently at approximately 6 months. T- and NK-cell development correlated with the clinical observation of few late infections. The kinetics of donor T- and NK-cell development is similar to that observed with T-cell–depleted haploidentical bone marrow transplantation in patients with SCID, regardless of the preparative regimen used in these studies.[1-8] We would hypothesize that in our patients the new T cells are donor HLA-DR restricted but tolerant to host and donor HLA-DR antigens. As antigen-specific T-cells responses develop in these patients, this hypothesis will be evaluated. In addition, donor cord blood B-cell engraftment was observed with UCB transplantation; whereas with T-cell–depleted haploidentical bone marrow transplantation, functional humoral immunity develops in approximately 50% of the patients who retain host B cells, probably as the result of intrinsic B-cell defects.[1] In T-cell–depleted bone marrow transplantation, cytoreduction treatment improves engraftment of donor B cells and the antibody responses. Preliminary evaluation of B-cell function in these patients demonstrates that engraftment of donor B cells and tetanus and diphtheria toxoid antibody responses have appeared in 2 patients more than 1 year after the transplantation.

In our series all of the patients exhibited some degree of acute GvHD, but the severity of GvHD was typically grade I, mild, and resolved with increased corticosteroid treatment and did not evolve into significant chronic GvHD. The occurrence of grade I GvHD did not correlate with the degree of HLA class I mismatch. However, grade IV GvHD did occur in the only patient with a serologic HLA-DR mismatch, and this patient died of infectious complications. This is a contrast to HLA-matched related and unrelated donor bone marrow transplantation, where there is a clear correlation between the degree of mismatch between the donor and recipient and the degree of GvHD.[9,36] The relative immaturity of cord blood T cells and/or their ability to become tolerant to host HLA antigens has been postulated as the reasons for the reduced risk and severity of GvHD from that which would be expected given the degree of HLA mismatch. The lack of viral contamination of the products is also advantageous in these children. No cytomegalovirus- or EBV-related infections occurred in this series. The lack of cytomegalovirus contamination obviated the need for viral monitoring and prophylaxis.

In summary, in unrelated donor transplantation for children with primary immunodeficiency disorders, it is transplantation-related death that is the greatest risk for these children, including graft rejection, preexisting and acquired opportunistic infections, GvHD, and toxicity from the myeloablative preparative regimen. The major morbidity in this series of patients was related to the transplantation preparative regimen, with remarkably few infectious problems. We conclude that in the unrelated donor stem-cell transplantation setting, UCB should be considered as an alternative source for children with SCID.

We thank Drs Pablo Rubinstein and Joanne Kurtzberg for their support of this study and the field of cord blood transplantation and the stem-cell transplantation team at Cardinal Glennon Children's Hospital who made this study possible.

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