Hematopoietic stem cell transplantation for CD3δ deficiency

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Background: CD3δ deficiency is a fatal form of severe combined immunodeficiency that can be cured by hematopoietic stem cell transplantation (HSCT). The presence of a thymus loaded with T-cell progenitors in patients with CD3δ deficiency may require special considerations in choosing the regimen of conditioning and the type of HSCT.
Objectives: To study the outcome of CD3δ deficiency by using various modalities of stem cell transplantation.
Methods: We analyzed data on 13 patients with CD3δ deficiency who underwent HSCT in 7 centers. HSCT was performed by using different sources of donor stem cells as well as various conditioning regimens.
Results: One patient received stem cells from a matched related donor and survived after a second transplant, needing substantial conditioning in order to engraft. Only 2 of 7 other patients who received a mismatched related donor transplant survived; 2 of them had no conditioning, whereas the others received various combinations of conditioning regimens. Engraftment of T cells in the survivors appears incomplete. Three other patients who received stem cells from a matched unrelated donor survived and enjoyed full immune reconstitution. Two patients received unrelated cord blood without conditioning. One of them has had a partial but stable engraftment, whereas the other engrafted well but is only 12 months after HSCT. We also report here for the first time that patients with CD3δ deficiency can present with typical features of Omenn syndrome.
Conclusions: HSCT is a successful treatment for patients with CD3δ deficiency. The small number of patients in this report prevents definitive statements on the importance of survival factors, but several are suggested: (1) HLA-matched donor transplants are associated with superior reconstitution and survival than are mismatched donor transplants; (2) substantial conditioning appears necessary; and (3) early diagnosis and absence of opportunistic infections may affect outcome.
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Key words: *CD3δ, severe combined immunodeficiency, bone marrow transplant, stem cell transplant, myeloablative conditioning, engraftment*

Severe combined immunodeficiency (SCID) consists of a group of inherited disorders characterized by profound T-cell impairment, leading to death in infancy unless treated with hematopoietic stem cell transplantation (HSCT). Optimal outcomes are achieved by using a matched related donor (MRD). When such donors are unavailable, alternative donors such as mismatched related donors (MMRDs) or matched unrelated donors (MUDs) have been used.[1-4] The overall experience in the last 20 years has shown that the survival rate with MMRD was around 50% whereas the survival rate after MUD HSCT was 80% or more.[1] However, the survival rates recorded in small single-center (and sometimes with short follow-up) studies using haploidentical donors varied dramatically from 87%[5] to 30%.[6] In contrast, the survival rates of patients after MUD HSCT were consistently between 65% and 85% in single-center as well as multicenter studies.[1,4,7] These results highlight the importance of donor-to-recipient HLA proximity to the success of HSCT.

Other factors influencing outcome include the type of SCID. Patients with circulating B cells (T^-B^+ SCID) were reported to have a superior outcome than patients with no circulating B cells (T^-B^-).[8] Patients with HLA-Class II deficiency also have inferior survival rates after bone marrow transplant (BMT) when compared with patients with other subtypes of SCID.[9] The survival rate of patients with adenosine deaminase deficiency has also been reported to be lower than that of patients with other types of profound T-cell deficiencies, especially if related identical donor has not been used.[10]

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Abbreviations used
ATG: Antithymocyte globulin
BMT: Bone marrow transplant
CMV: Cytomegalovirus
GvHD: Graft versus host disease
HSCT: Hematopoietic stem cell transplantation
MMRD: Mismatched related donor
MMUD: Mismatched unrelated donor
MRD: Matched related donor
MUD: Matched unrelated donor
NK: Natural killer
SCID: Severe combined immunodeficiency
TCR: T-cell receptor
TREC: T-cell receptor excision circle

These genotype-specific differences in outcome highlight the need for similar studies in each subgroup of patients with SCID. We studied here the outcome of patients with CD3δ deficiency, first described by Dadi et al[11] in 2003 with a peculiar presentation. While the numbers of circulating T cells carrying the αβ T-cell receptor (TCR) or the γδ TCR were markedly reduced, the thymus was heavily populated with T-cell progenitor cells. These cells appeared to contain increased levels of precursor TCR α but reduced levels of CD4, CD8α, and CD8β consistent with an arrest of differentiation in the $CD4^-CD8^-$double-negative stage of T-cell development.[11-13] Other types of SCID have typically completely dysplastic thymuses highlighted by a marked depletion of thymocytes in addition to lack of Hassall's corpuscles,[14] raising the possibility that the abundance of T-cell precursors in CD3δ recipients might influence the outcome of HSCT, especially if conditioning is not used.

Because of the extremely rare occurrence of CD3δ deficiency, we have gathered the total number of known children with CD3δ deficiency treated with HSCT in order to gain more information on their survival with various modes of transplantation.

METHODS

Patients

We analyzed the data of 13 patients with CD3δ deficiency who underwent HSCT from 7 medical centers around the world, including North America, Japan, Spain, and Germany. All patients had a molecular diagnosis of CD3δ deficiency. Questionnaires were filled by the managing physician and included epidemiological data, clinical presenting symptoms, immune work up, HSCT data, and outcome.

Evaluation of cellular and humeral immunity

Cell surface markers of peripheral blood cells were evaluated by using flow cytometry (Epics V; Coulter Electronics, Hialeah, Fla, or Becton Dickenson, Franklin Lakes, NJ). Lymphocyte proliferation in response to PHA stimulation, using tritiated thymidine incorporation, was determined as previously described.[1] The amount of signal joint T-cell receptor excision circles (TRECs) adjusted to $CD4^+$ and $CD8^+$ T subsets was determined by real-time quantitative PCR as previously described. The number of TRECs in a given sample was compared with a value obtained with 10-fold serial dilutions of an internal standard provided by Dr Daniel Douek (Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, Md). Representatives of specific T-cell receptor Vβ families were detected and were quantified by using quantitative PCR and flow cytometry (Beckman Coulter, Elite, Mississauga, Ontario, Canada) as previously described.[1]

Stem cell transplantation procedures

Procedures varied between clinical centers in this report and included transplants with MRD (9/10 antigen match or better), MMRD, MUD (9/10 antigen match or better), or mismatched unrelated donor (MMUD). Stem cell sources included peripheral blood cells, bone marrow, or cord blood. Conditioning and graft versus host disease (GvHD) prophylaxis, when given, also varied widely among centers.

Engraftment and chimerism studies

Neutrophil engraftment was considered to have occurred on the first of 3 consecutive days in which the absolute neutrophil count exceeded 0.5×10^9/L. Platelet engraftment was considered to have occurred on the first day of 7 consecutive days in which the platelet count exceeded 20×10^9/L without platelet transfusion. Red cells engraftment was considered to have occurred on the last day that red blood cell transfusions were required.

Assessment of TCR repertoire

TCR Vβ families within $CD4^+$ or $CD8^+$ subpopulations were assessed by using flow cytometry (Beckman Coulter Immunotech, Mississauga, Ontario, Canada).[15]

RESULTS

Patients

Thirteen patients treated in 7 medical centers were included in this study. Seven patients were the first to be diagnosed with SCID in their family. Their age at the time of diagnosis ranged from 1 week to 14 months (Table I). The remaining 6 patients were diagnosed soon after birth because they were siblings or cousins of already-diagnosed patients. Patients came from 3 ethnic groups: Caucasian in Mennonite communities (North American or European), Asian (Japanese), and Hispanic. All patients were found to have homozygous mutations in the *CD3δ* gene. Three mutations that segregate according to ethnic origin suggesting a different founder effect in each group have been identified (Table I). Interestingly, the patient treated in Germany harbored the same mutation that was detected in the Mennonites. This suggests a common ancestry and predicts that the mutation precedes the migration of Mennonites from eastern Europe more than 400 years ago.

Patients presented with typical features of SCID (Table I), such as pneumonitis due to *Pneumocystis jiroveci* (patients 4 and 9) or cytomegalovirus (CMV) infections (patients 3, 4, and 7), human herpesvirus 6 (patient 6), or *Aspergillus* (patient 7). Five patients had chronic diarrhea (patients 2, 3, 4, 6, and 9) with isolates of adenovirus, rotavirus, or salmonella. Four patients had failure to thrive (patients 2, 6, 7, and 9), 2 had oral thrush (pateints 5 and 7), and 1 patient had systemic candidiasis (patient 9). Patient 2 had *Cryptosporidium* infection in addition to viral pneumonitis and failure to thrive. Patients 2 and 3 also had erythrodema as well as lymphadenopathy consistent with Omenn syndrome, suggesting that the *CD3δ* mutations in these patients allowed for a leaky thymus.

Unlike other types of SCID, the thymus in CD3δ was detected by using ultrasound and appeared heavily populated with early T-cell progenitors, in contrast to the marked peripheral lymphopenia. This pattern could be clearly seen in patients who were diagnosed early before stress or immunosuppression affected the size of the thymus.[11,16]

Evaluation of the immune system before HSCT

All patients with the exception of patients 2 and 3 had extremely low (<500 cells/μL) numbers of circulating $CD3^+$

TABLE I. Demographic data, clinical presentation, and genetic analysis

Patient	Age at diagnosis	Gender/ethnicity	Pre-BMT features	Mutation analysis
1	Birth	F/Mennonite	None, cousin with SCID	c.202C>T
2	14 mo	[a]M/Ecuadorian	Cryptosporidium, Salmonella Group C, FTT, pneumonitis, Omenn syndrome	c.274+5G>A
3	5 mo	[a]M/Ecuadorian	CMV pneumonitis, chronic diarrhea with adenovirus Omenn syndrome	c.274+5G>A
4	3 mo	[b]F/Japanese	PJP pneumonitis, CMV, chronic diarrhea	c.275-2A>G
5	1 wk	M/Mennonite	Cutaneous candidiasis, oral thrush, otitis externa	c.202C>T
6	10 mo	F/Mennonite	Rotavirus, HHV6 pneumonitis, FTT	c.202C>T
7	13 mo	[c]M/Mennonite	Pulmonary aspergillosis, CMV hepatitis, FTT, oral thrush, Otitis	c.202C>T
8	6 d	[c]F/Mennonite	None, sibling with SCID	c.202C>T
9	9 mo	[d]F/Mennonite	PJP pneumonitis, chronic diarrhea, candida sepsis, FTT	c.202C>T
10	Birth	[d]F/Mennonite	None, sibling with SCID	c.202C>T
11	Birth	[d]M/Mennonite	None, cousin with SCID	c.202C>T
12	Birth	[b]M/Japanese	None, sibling with SCID	c.275-2A>G
13	Birth	[b]M/Japanese	None, sibling with SCID	c.275-2A>G

FTT, Failure to thrive; *PJP*, *Pneumocystis jiroveci* pneumonia.
[a,b,c,d]Related patients as indicated.

TABLE II. Humoral and cell-mediated immunity at presentation

	Patient													
	1	2	3	4	5	6	7	8	9	10	11	12	13	Normal range
Absolute lymphocyte count (cells/μL)	1,050	3,800	3,600	758	3,500	640	972	1,530	1,100	3,400	580	996	1,912	4,000-10,500
Lymphocyte markers (%)														
$CD3^+$	0	14	30	1.7	1	0	27	1	2	0.4	0	0.18	0	51-77
$CD4^+$	0	7	9	ND	0	0	4	5	2	0	0	ND	0	35-56
$CD8^+$	0	3	9	ND	0	0	15	<1	0.4	0.1	0	ND	23	12-28
$CD19^+$	66	54	53	67	51	86	ND	65	89	63	71	42	72	6-41
$CD56^+$	31	29	18	23	44	8	ND	33	7	33	24	57	27	4-18
PHA	ND	2*	2.3*	ND	ND	0*	0†	0†	0*	0.6*	1*	ND	ND	
TREC (copies/0.5 μg DNA)	ND	ND	ND	ND	ND	ND	ND	ND	UD	UD	UD	ND	ND	>400
IgE (U/L)	ND	4,000	2,790	ND	120	ND	ND	ND	<5	ND	ND	ND	ND	<12
Eosinophils (cell/uL)	0	5,200‡	5,100	90	7,000	ND	10	40	740	590	40	234	241	50-700

ND, Not determined; *UD*, undetected.
*Percent of control stimulation index (normal >50%).
†Counts per million (normal >25,000).
‡Normal 70 to 500 cells/uL.

T cells as originally described (Table II). Patients 2 and 3 had a restricted T-cell repertoire with clonally expanded T cells (Fig 1), commonly associated with Omenn syndrome and normal numbers of γδ T cells. In contrast, $CD19^+$ B cells and $CD56^+$ natural killer (NK) cells were comparable in number to controls. Mitogenic responses to *in vitro* stimulation with PHA were markedly depressed in all 8 patients tested. TRECs, a reflection of thymopoiesis, were undetectable in all 4 patients tested. This was consistent with a maturational arrest of T cells at an early stage of differentiation. Indeed, a thymus biopsy performed in 1 patient as well as analysis of a thymus at autopsy on another patient revealed an arrest at the CD4/CD8 double-negative stage of thymocyte development.[11,16]

Serum immunoglobulins appeared normal for age in all patients (data not shown); however, IgE was markedly increased in patients 2 and 3, but only moderately elevated in patient 5 (Table II). Furthermore, eosinophil counts were increased in all 3 patients. In patients 2 and 3, the T-cell repertoire was assessed by using flow cytometry to determine the representation of a panel of TCR Vβ families. In both patients, the TCR repertoire was restricted while displaying overrepresentation of several Vβ families (Fig 1).

HSCT procedure

The mean age of patients at transplant was 7months, ranging from 1 to 23 months (Table III). Five patients received stem cells from MUDs, 3 from a bone marrow harvest (MUD-BM). Of these 3, 2 received a 6/6 HLA identical donor, while in the remaining patient a 9/10 antigen-matched donor was used. Of the 2 other patients who received stem cells from unrelated donors, one was a 10/10 matched cord blood (MUD-CB) and the other had a 4/6 antigen mismatched cord blood (MMUD-CB). Eight patients received stem cells from related donors, of which 1 patient had a 9/10 (non-DR) minor mismatch donor (MRD) bone marrow transplant. Seven other patients received stem cells from true haploidentical-related donors (MMRD). In 5 of these 7 cases, peripheral blood stem cells were used while the other 2 received

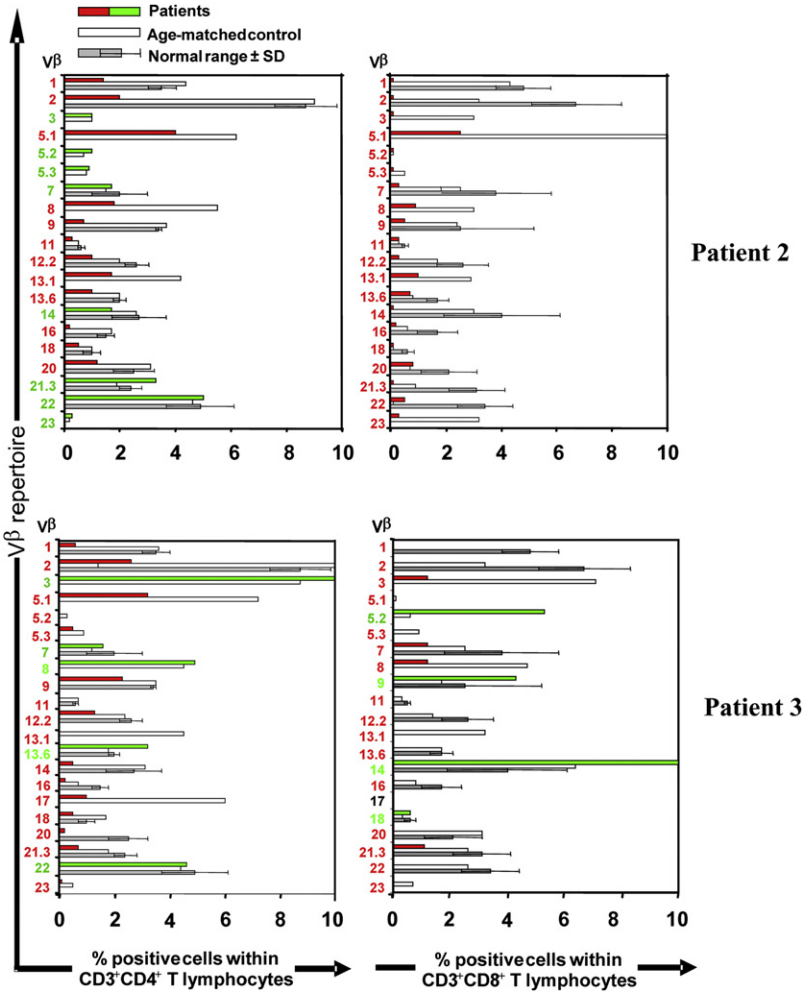

FIG 1. TCR Vβ repertoire with T-cell subsets. TCR Vβ repertoire within $CD4^+$ and $CD8^+$ T populations was determined by means of flow cytometry using a collection of anti-TCR Vβ antibodies. Data shown within range or overrepresented *(green)* or underrepresented *(red)* in comparison with a normal age-matched control *(empty bars)* and the normal range *(gray bars ± SD)*.[15]

bone marrow. In 1 patient, depletion was achieved by anti-CD2 and anti-CD8 antibodies; in the others, $CD34^+$ cells were extracted with or without CD3 depletion. Two patients received stem cells after depletion of bone marrow with soybean agglutinin and E-rossetting (patients 6 and 7).

Conditioning was applied in 10 of 13 patients. Patients with MUD-BM received myeloablation with busulfan and cyclophosphamide. The 2 patients who received cord blood were not conditioned. Of the 7 patients who received MMRD, 1 had no conditioning (patient 4) while the rest received fludarabine, busulfan, and antithymocyte globulin (ATG) (patient 2); fludarabine, melphalan, and ATG (patient 3); busulfan, fludarabine, and ATG (patient 5); fludarabine and Campath (patient 6); ATG (patient 7); or cyclophosphamide with ATG (patient 8). The patient who had a 9/10 MRD-HSCT was conditioned with fludarabine (patient 1). Five patients failed to engraft after the first HSCT. Patient 1 received a second transplant from the same donor after more aggressive conditioning with fludarabine, Campath, and melphalan. Four other patients who received HSCT from an MMRD (haploidentical) needed a second (patients 6 and 8) or even a third (patients 5 and 7) transplant (Table III).

GvHD prophylaxis was used in all but 3 protocols (patients 3, 7, and 8). Patients with MUD-BM received prednisone and cyclosporine A, whereas the 2 patients who received cord blood were treated with methotrexate and tacrolimus. In the MMRD transplants, prednisone was used as the sole medication in patients 2 and 4, cyclosporine A was used as the sole drug in patient 5, while the combination of methotrexate and tacrolimus was used in patient 6. The MRD recipient got tacrolimus (patient 1).

Engraftment of the hematopoietic system

In the MUD-BM group, engraftment was rapid with normal neutrophil counts achieved by 8 to 12 days posttransplant, platelets supplementation was no longer needed 12 to 35 days after HSCT, and red cell transfusion was not needed after 7 to 30 days (Table IV). The second transplant in pateint 1 resulted in a rapid engraftment of neutrophils and platelets within 20 and 24 days posttransplant, respectively. Multilineage engraftment in patients 6, 7, and 8 was not achieved, and there was no chimerism data for patients 4, 12, and 13 (Table IV). Achievement of chimerism was virtually 100% in patients 3, 5, 9, 10, and 11, all of whom received a myeloablative conditioning regimen

TABLE III. Stem cell transplantation procedures

Patient	Age at 1st BMT	Type of BMT			HLA match	Conditioning			GvHD prophylaxis
		1st HSCT	2nd HSCT	3rd HSCT		1st HSCT	2nd HSCT	3rd HSCT	
1	35 d	MRD, BM	MRD, BM, same donor		9/10	Flu 16 mg/kg	Flu 150 mg/m^2, Cam 0.8 mg/kg, Melf 140 mg/m^2		Tacrolimus
2	23 mo	MMRD, PBSC, CD34$^+$			Haplo 5/10	Flu 150 mg/m^2, Bu 140 mg/m^2, ATG 12.5 mg/kg			Prednisone
3	8 mo	MMRD, PBSC, CD34$^+$			Haplo 3/6	Flu 150 mg/m^2, Melf 280/m^2, ATG 12.5 mg/kg			None
4	4 mo	MMRD, PBSC, CD2$^-$/CD8$^-$			Haplo 3/6	None			Prednisone
5	6 mo	MMRD, PBSC, CD34$^+$ CD3$^-$	MMRD, PBSC, CD34+/CD3$^-$, Cryopreserved, same donor	MMRD, BM, CD3$^-$/CD19$^-$, same donor	Haplo 5/10	Bu 16 mg/kg, Flu 160 mg/m^2, ATG 10 mg/kg			CyA
6	12 mo	MMRD, PBSC	MMRD, PBSC same donor		Haplo 3/6	Flu 4 mg/kg, Cam 24 mg	Bu 16 mg/kg, Flu 4 mg/kg, Cam 24 mg		MTX, tacrolimus (2nd HSCT)
7	14 mo	MMRD, BM S+E maternal	MMRD, BM S+E paternal	MMRD, BM S+E paternal	Haplo 3/6	ATG 30 mg/kg	ATG 80 mg/kg, Cyclo 100 mg/kg	ATG 80 mg, Cyclo 120 mg/kg, TBI	None
8	2 mo	MMRD, BM S+E	MMRD, BM, S+E same donor		Haplo 3/6	Cyclo 200 mg/kg ATG 80 mg/kg			None
9	16 mo	MUD, BM			6/6	Bu 16 mg/kg, Cyclo200 mg/kg			Prednisone, CyA
10	4 mo	MUD, BM			6/6	Bu 16 mg/kg, Cyclo 200 mg/kg			Prednisone, CyA
11	4 mo	MUD, BM			9/10	Bu 16 mg/kg, Cyclo 200 mg/kg			Prednisone, CyA
12	1 mo	MMUD, cord			4/6	None			MTX, tacrolimus
13	25 d	MUD, cord			6/6, 10/10	None			MTX, tacrolimus

BM, Bone marrow; *Bu*, Busulfan; *Cam*, Campath; *CyA*, cyclosporine A; *Cyclo*, cyclophophomide; *Flu*, fludarabine; *HI*, haploidentical; *melf*, melfalane; *MTX*, methotrexate; *MRD*, matched related donor (matched was defined as 9/10 antigen or better); *PBL*, peripheral blood; *PBSC*, peripheral blood stem cell; *TBI*, total body radiation; *S+E*, soybean agglutinin and sheep erythrocyte rosettting.

with either busulfan or fludarabine. Engraftment of the second transplant in patient 1 also showed 100% donor cells 1 year after the second HSCT. Patients 2 and 8 had only 7% donor cells among T lymphocytes (Table IV).

Immune reconstitution

Eight of 13 patients survived and had evidence of immune reconstitution (Table V). Patients who received MUD-BM transplant showed full reconstitution up to 20 years after HSCT (patients 9, 10, and 11). The numbers of CD3$^+$ T cells, CD19$^+$ B cells, and CD56$^+$ NK cells were normal as were the proportions of CD4$^+$ and CD8$^+$ cells. Mitogenic responses to PHA (Table V) and anti-CD3 (not shown), T-cell repertoire as determined by detection of TCR Vβ families, and TREC levels were all comparable to those in normal controls. Humoral immunity was also normal in all 3 patients including a robust ability to produce specific antibodies. Immune reconstitution in the MUD-CB case (patient 13) did not achieve normal levels of circulating CD3 and CD4 cells 1 year posttransplant. However, PHA responses were normal (Table V) and immunoglobulin levels appear normal. TCR Vβ, TRECs, and specific antibody production in response to vaccinations were not assessed in this patient. The patient who received an MMUD-CB (patient 12) had a low number of circulating CD3$^+$ and CD4$^+$ T cells but normal responses to PHA 4 years after the transplant. Immunoglobulin levels appear normal, but antibody responses to vaccines have not been assessed. The 2 survivors of a haploidentical MMRD transplant had low CD3$^+$ cells 16 months (patient 2) or 16 years (patient 8) after transplant. PHA responses were normal, but TCR repertoire and TRECs were not analyzed, making it difficult to fully appreciate immune reconstitution in patient 8. Meanwhile, patient 2 had an abnormal PHA response, but normal T cell repertoire as well as normal proportions of CD4$^+$RO and CD4$^+$RA cells. Serum immunoglobulin levels and specific antibodies were normal in both patients (Table V).

Patient 1 who received MRD has shown signs of engraftment with lower than normal numbers of circulating CD3$^+$ as well as CD4$^+$ T cells. Responses to stimulation with PHA were not

TABLE IV. Engraftment and outcome

Patient	Neutrophils >500 (days after transplant)	Platelets (days after transplant)	Red cells (last transfusion/days)	Chimerism (% donor)	Time from BMT	Outcome	Cause of death
1	20	24	30	100	13 mo	Alive and well	
2	10	12	ND	7% $CD3^+$ 50% $CD19^+$	1 y	Alive and well	
3	12	13	26	100	28 d	Died	CMV liver failure
4	ND	ND	ND	ND	8 mo	Died	CMV encephalitis
5	18	29	21	100	14 mo	Died	GvHD
6	ND	ND	ND	Failed to engraft		Died	GvHD, DIC, HHV6
7	ND	ND	ND	Engrafted	14 mo	Died	CMV encephalitis, infection, respiratory failure
8	ND	ND	ND	7	17 y	Alive and well	
9	12	12	7	>95	18 y	Alive and well	
10	12	14	30	>95	7 y	Alive and well	
11	8	35	25	>95	3 y	Alive and well	
12	ND	ND	ND	ND	4 y	Alive and well	
13	ND	ND	ND	ND	1 y	Alive and well	

TABLE V. Immune reconstitution after HSCT

	Patient								Normal range
	1	2	8	9	10	11	12	13	
Absolute lymphocyte count (cells/μL)	2,724	2,900	930	2,440	2,450	3,400	1,241	2,220	1,200-6,000
Time of evaluation after BMT	1 y	24 mo	16 y	20 y	7 y	3 y	4 y	1 y	
Lymphocyte markers (%)									
$CD3^+$	ND	61	42	75	74	84	34	22.5	51-77
$CD4^+$	21	37	32	47	36	47	24	18	35-56
$CD8^+$	23	20	9	26	27	31	20	6	21-28
$CD56^+$	ND	5	43	12	11	4	23	21	6-41
$CD19^+$	38	32	13	10	14	8	41	58.1	4-18
TCR Vβ	ND	Normal	ND	Normal	Normal	Normal	ND	ND	
PHA	ND	36*	105,543†	68*	69*	65*	285‡	307‡	
TREC (copies/0.5 μDNA)	ND	ND	ND	Normal	Normal	Normal	ND	ND	>400
Serum Ig (g/L)									
IgG	7.3	11.4	12.50	10	8.5	7.2	7.71	8.5	2.3-14.1
IgA	0.13	0.89	2.47	0.9	1.5	0.4	1.51	3	0-0.8
IgM	1.38	1.31	0.8	1.5	0.9	0.5	1.19	7.5	0-1.7
Tetanus (IU/mL)	ND	ND	Positive	2.26	>7	3.5	ND	ND	>0.13
Polio	ND	ND	Positive	1:64	ND	ND	ND	ND	>1:16
Isohemagglutinin	ND	ND	1:32	1:128	1:64	1:02	ND	ND	>1:64

ND, Not done.
*Percent of control stimulation index (normal >50%).
†Counts per million (normal >25,000).
‡Stimulation index (normal 254-388).

performed. Serum immunoglobulin as well as specific antibody levels were normal.

Complications after HSCT

Acute GvHD occurred in most patients. Patient 5 had a severe fatal course of GvHD with liver involvement. Patients 9, 10, 11, and 12 had mainly skin and gut GvHD graded II to III that was reversed by steroid treatment. Chronic GvHD developed in 2 patients. Patient 1 had a more severe course and is still receiving immunosuppression. Patient 9 has residual vitiligo and hyperpigmentation. Infectious complications after HSCT included CMV infection in 3 patients that proved fatal, although all these patients presented with CMV disease at the time of diagnosis; another patient suffered HHV-6 infection, while 1 patient had a perineal abscess with *Enterobacter cloache.* Other complications included autoimmune oophoritis in 1 patient.

Long-term outcome after HSCT

The survival rate was 62%, with 8 of 13 patients alive to date. Five patients who received an HLA haploidentical depleted donor transplant had died: patient 3 died of CMV-related hepatic failure. Patient 4 had failed to engraft and died at 8 months from CMV encephalitis, while patient 5 died at 14 months from sequels of severe GvHD after 3 transplants. Patient 6 died of GvHD and disseminated intravascular coagulation, while patient 7 died of CMV encephalitis and respiratory failure. Patients 2, 9, 10, 11,

and 12 remain well with no treatment. Patient 9, now 20 years after HSCT, was able to complete her pregnancy and deliver a healthy baby girl.

DISCUSSION

Patients with CD3δ deficiency typically present with a profound T-cell lymphopenia, lacking both T cell carrying the αβ or the γδ TCR.[11] This is in contrast to mice that underwent *CD3δ* gene deletion, who are markedly leaky for γδ T cells.[1-4] Unlike other types of SCID, the thymus in CD3δ deficiency is easily detected by ultrasound or chest radiography.[11,17] Thymic tissue shows marked accumulation of early T-cell progenitors, mostly CD4/CD8 double-negative cells, and a lack of Hassall's corpuscles. As expected, the thymus appeared more depleted of thymocytes in autopsies of patients because of stress and/or the use of immunosuppressive drugs.[14] To date, the best hope for cure in these patients remains an HSCT.

However, the presence of residual T-cell progenitors as well as normally developed B cells and NK cells raised a dilemma of what strategy should be used for stem cell therapy.

We have therefore studied a group of patients with CD3δ deficiency who received different modalities of HSCT. Although the small number of patients did not allow for a detailed statistical analysis, some trends emerged.

Five of 7 patients who received HSCT from a related haploidentical donor died. Three of 5 presented with CMV disease and 1 presented with human herpesvirus 6 pneumonitis, which possibly influenced their poor outcome. The fourth patient (patient 5), in spite of conditioning with busulfan, fludarabine, and ATG, showed no signs of T-cell engraftment. Subsequently, 2 additional attempts at immune reconstitution made using stem cells without conditioning failed, and the patient died of GvHD. This case highlights the difficulty in engrafting T cells in patients with CD3δ deficiency.

A similar poor outcome has been already observed before, in 3 other patients who died after a haploidentical donor transplantation.[16] Two of them were very young at diagnosis and transplant. The first died 25 days after transplant from disseminated adenovirus at the age of 2 months. The second patient was diagnosed at 6 days but died of disseminated aspergillosis at 6 months after BMT.[16] In sharp contrast, all 5 patients who received an unrelated matched bone marrow or cord blood transplant survived. None of the patients who received MUD-BM were younger than 4 months at transplant, while the 2 who received MUD-CB were young babies. Nevertheless, the largest study of MUD-BMT showed no difference in outcome among patients younger or older than 3.5 months of age, unlike results from a single-center experience using MMRD.[18] Patients who were treated with MUD-BM showed a robust immune reconstitution that has lasted for more than 20 years in 1 patient. Moreover, the conditioning regimen with busulfan and cyclophosphamide did not compromise this patient's ability to conceive. Interestingly, the 2 patients who were transfused with unrelated matched or mismatched cord blood appeared to engraft without conditioning. Immune reconstitution in these patients should be interpreted cautiously because of the relatively short follow-up time. So far, it has been seen that the number of T cells remains low up to 4 years after transplant, although PHA responses and serum immunoglobulins appear normal, but specific antibodies, T-cell repertoire, and TRECs were not assessed. Long-term follow-up should determine whether conditioning is required for effective long-term engraftment in patients with CD3δ deficiency who receive cord blood.

To further support the notion that substantial conditioning was required, 1 patient who received a related HLA-matched donor (9/10 match) BMT after mild conditioning failed to engraft. More aggressive conditioning appeared to have resulted in engraftment. The need for substantial conditioning in patients with SCID who receive a related match donor transplant is unusual and reserved for patients who have a large number of circulating autologous T cells or maternal engraftment. Engraftment in this series was superior in individuals who received myeloablative conditioning regimens. The reason for this phenomenon is not completely understood and should be the subject of future studies. It has been clearly shown though that in the absence of CD3δ, the thymus gland in patients is heavily populated with surviving immature thymocytes. It is possible that one reason for the accumulation of thymocytes is their resilence to apoptosis, which perhaps renders them less sensitive to nonmyeloablative treatments such as ATG.

Interestingly, 2 patients in this series (patients 2 and 3) presented with typical features consistent with Omenn syndrome. The unique mutation in these patients may have resulted in a leaky thymus as well as the delayed diagnosis in patient 2. This is the first description of Omenn syndrome in patients with CD3δ deficiency.

The major complications observed included infections and GvHD. Patients 9, 10, 11, and 12 had grades II to III skin and gut GvHD, which was reversed after treatment with pulse steroids. Two patients developed chronic GvHD (patients 1 and 12), of which one (patient 1) was still being treated with immunosuppression. The most significant infections at the time of diagnosis were with CMV, which were fatal in 3 patients.

We present the largest series of patients with CD3δ deficiency that underwent stem cell transplantation and their outcome. Donor-recipient HLA proximity appeared critical as 6 of 6 patients who received 0-1 antigen mismatched donors as compared with only 2 of 7 patients with more than 2 antigen mismatch survived. MUD-BM transplant with myeloablative conditioning therapy resulted in excellent long-term immune reconstitution. MUD-cord blood also appeared to be an effective treatment although full immune reconstitution and long-term engraftment have not been established yet. The related matched transplant needed substantial conditioning for full engraftment, while the MMRD transplants failed to engraft and led to death of most of these patients, although it must be noted that 4 of 6 recipients of the MMRD transplants versus only 1 of 5 recipients of MUD transplants had CMV or other life-threatening infectious disease at the time of diagnosis and transplant. Also, of the 6 children diagnosed at birth because of a previously affected family member only 1 received an MMRD transplant (and is a long-term survivor) while 4 of the remaining 5 were recipients of an MUD transplant (all survivors). However, we have previously demonstrated that age or severe infections before BMT did not significantly affect survival in patients who received an MUD transplant.[1] This may not be the case in MMRD transplants according to experience in a single center claiming superior outcome in patients who receive MMRD transplants before the age of 3.5 months.[18] Nevertheless, no patient in this series received MUD-BM before the age of 4 months. Moreover, immune reconstitution in the MUD-treated patients was sustained for many years and remains solid, including a full TCR repertoire and

TREC levels, while the 2 survivors after MMRD had lymphopenia as well as a limited T cell engraftment.

In conclusion, with the limitation of a small cohort of patients we observed several factors that might affect survival in case of CD3δ deficiency: (1) HLA-matched donors are preferred to mismatched donors. (2) Substantial conditioning is necessary even in MRD transplants. (3) Early diagnosis and absence of opportunistic infections may affect outcome. Additional patients are needed to confirm these findings and an updated Web-based facility for these and similar rare SCID cases would greatly facilitate therapeutic decisions worldwide.

Clinical implications: We tried to identify the optimal stem cell transplantation strategy for CD3δ deficiency. Several factors appear important for full reconstitution, including close human leukocyte antigen matching, conditioning, and early diagnosis.

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