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ORIGINAL ARTICLE

Hematopoietic stem cell transplantation for 30 patients with primary immunodeficiency diseases: 20 years experience of a single team

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We retrospectively analyzed our results of 30 patients with three distinctive primary immunodeficiency diseases (PIDs) – severe combined immunodeficiency (SCID, $n = 11$), Wiskott–Aldrich syndrome (WAS, $n = 11$) and X-linked hyper-immunoglobulin M (IgM) syndrome (XHIM, $n = 8$) – who underwent hematopoietic SCT (HSCT) during the past 20 years. Until 1995, all donors were HLA-haploidentical relatives with T-cell depletion (TCD) ($n = 8$). Since 1996, the donors have been HLA-matched related donors (MRD) ($n = 8$), unrelated BM (UR-BM) ($n = 7$) and unrelated cord blood (UR-CB) ($n = 7$). Twenty-seven of 30 patients had various pre-existing infections with or without organ damages before HSCT. Conditioning regimen and GVHD prophylaxis were determined according to disease, donor and pretransplant status. Although one of eight patients transplanted with TCD is alive with full engraftment, the other seven died. On the other hand, 18 of 22 patients transplanted without TCD are alive and well, including six of eight transplanted from MRD, seven of seven from UR-BM and five of seven from UR-CB. All 19 survivors did not require Ig supplementation after HSCT. These results indicate that UR-CBT as well as UR-BMT provides good results for PID comparable to MRD-SCT, and that early diagnosis, HSCT at early stage, careful supportive therapy and monitoring for various pathogens are important for the successful HSCT.
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Introduction

Primary immunodeficiency diseases (PIDs) are often accompanied with life-threatening infections. Hematopoietic SCT (HSCT) can be a treatment of choice to cure most of the lethal forms of immunodeficiencies, including severe combined immunodeficiency (SCID), Wiskott–Aldrich syndrome (WAS) and X-linked hyper immunoglobulin M (IgM) syndrome (XHIM). An HLA-matched related donor (MRD), the best hematopoietic stem cell source, may not always be available. T-cell depletion (TCD) of BM from HLA-haploidentical donors made this possible as another stem cell source for patients without MRD, to prevent GVHD and to lead successful transplantation.[1] Methods of TCD varied among different HSCT centers and the results of HSCT also significantly varied.[1–4] BMT with TCD from haploidentical donor often leads to the development of opportunistic infections, such as CMV disease, or lymphoproliferative disorders owing to EBV (EBV-LPD).[5] In the 1990s, HSCT using three alternative sources of stem cell developed: BM from matched unrelated donor (UR-BM),[6] cord blood from unrelated donor (UR-CB)[7] and positively selected $CD34^+$ cells from HLA-mismatched related donor.[8]

In this study, we retrospectively analyzed the results of 31 consecutive HSCTs for 30 patients with three distinctive immunodeficiency diseases: SCID, WAS and XHIM in a single team experience over 20 years.

Patients and methods

Patients (Table 1)

Between July 1984 and February 2005, 30 children with PID, including 11 patients with SCID, 11 with WAS and eight with XHIM, underwent 28 HSCTs at Tokyo Medical and Dental University and three HSCTs (P13, P22, P30) at the National Defense Medical College, Japan. All 30 patients were male, and the mean and median ages of these patients at HSCT were 5 years 11 months and 10 years 1 month, respectively (range 5 months to 19 years).

Table 1 Pretransplant problems and transplantation data of 30 patients

Pt. no.	*Diagnosis*	*Year of SCT*	*Age at SCT*	*Pretransplant infections/organ damages*				*HLA disparity (pheno/geno)*	*Donor/ source*	*TCD*	*Conditioning regimen*	*GVHD prophylaxis*
				Bacterial	*Fungal/protozoal*	*Viral*	*Other organ damages*					
1	SCID (X)	1984	0y10m		PCP		Diarrhea	Haplo	Father/BM	Yes	No	CsA
2	SCID (X)	1985	0y5m	Impetigo	*Candida*	CMV	Diarrhea, LD	Haplo	Father/BM	Yes	No	CsA/MTX/PSL
3	SCID (X)	1990	0y9m	BCG		RSV	FTT, diarrhea	Haplo	Father/BM	Yes	No	CsA
4	SCID (X)	1993	0y11m	BCG, impetigo	PCP	VZV	FTT	Haplo	Father/BM	Yes	No	CsA/MTX
5	SCID (T-B-)	1995	0y5m	Impetigo		CMV	FTT, LD	Haplo	Father/BM	Yes	No	MTX
6-1	WAS	1991	3y1m	OM, abscess, Pn	PCP	HSV	FA	Haplo	Father/BM	Yes	BU/CY/AraC	CsA/PSL
6-2	WAS	1991	3y6m					Haplo	Father/BM	Yes	TBI/CY/AraC	CsA/PSL
7	WAS	1992	3y7m	Impetigo, Campylo		VZV		Haplo	Uncle/BM	Yes	TBI/CY/AraC	CsA/MTX
8	WAS	1994	0y10m	OM				Haplo	Father/BM	Yes	TBI/CY/AraC	CsA/MTX
9	SCID (X)	1996	1y3m		PCP	CMV	Diarrhea	0/1	UR/BM	No	BU/CY	CsA/MTX
10	SCID (X)	1998	0y10m		PCP	Rota	FTT, diarrhea	1/3	UR/CB	No	BU/CY	CsA/MTX
11	SCID (X)	1999	0y10m	BCG	PCP			1/1	UR/CB	No	BU/CY	CsA/MTX
12	SCID (X)	2002	0y9m	Impetigo		Rota	FTT, diarrhea, LD, E	1/2	UR/CB	No	BU/CY	CsA/MTX
13	SCID (X)	2004	0y10m		*Candida*			0/0	UR/CB	No	TBI 2 Gy/FLU	FK/MTX
14	SCID (OS)	2004	0y8m	Sepsis (*P. aeru*)			Erythroderma	0/1	UR/CB	No	FLU/LPAM/ATG	CsA/mPSL
15	WAS	1996	5y11m	OM, abscess, Pn	*Candida*	CMV, HSV	Epididymitis	0/0	UR/BM	No	TBI/CY/AraC/ATG	CsA/MTX
16	WAS	1999	19y3m			VZV	JIA, IgAN	0/0	Sib/BM	No	BU/CY	CsA/MTX/PSL
17	WAS	2000	1y6m	OM	*Candida*	HSV	Diarrhea, FA	0/1	UR/BM	No	BU/CY/ATG	CsA/MTX
18	WAS	2002	0y9m					0/0	UR/BM	No	BU/CY/ATG	FK/MTX
19	WAS	2002	1y1m		*Candida*	CMV	ICH	0/1	URBM	No	BU/CY/ATG	FK/MTX/PSL
20	WAS	2004	1y2m	Cellulitis			Pancytopenia	1/1	UR/CB	No	BU/CY/ATG	CsA/MTX/PSL
21	WAS	2004	1y7m					1/1	UR/CB	No	BU/CY/ATG	CsA/MTX
22	WAS	2005	8y6m			CMV		0/0	Sib/BM	No	BU/CY	CsA/MTX
23	XHIM	1998	17y4m		PCP, *C. neo*, *C. parvum*	HSV	FTT, diarrhea, LD osteoporosis	0/0	Sib/PB	No	TBI/CY	CsA/mPSL
24	XHIM	1999	3y11m	OM, Pn	PCP			0/0	Sib/BM	No	BU/CY	CsA/MTX
25	XHIM	1999	14y11m	OM, Pn	PCP	Kaposi	Bronchiectasis	0/0	Sib/BM	No	BU/CY	CsA/MTX
26	XHIM	2000	3y11m		PCP			0/0	UR/BM	No	BU/CY	CsA/MTX
27	XHIM	2000	18y11m	OM, impetigo, Pn	PCP	HSV		0/0	Sib/BM	No	BU/CY	CsA/MTX
28	XHIM	2002	3y7m					0/0	UR/BM	No	BU/CY	CsA/MTX
29	XHIM	2002	19y9m		PCP			0/0	Sib/BM	No	BU/CY	CsA/MTX
30	XHIM	2004	19y9m	OM, Pn	*C. parvum*		Diarrhea, LD, HE	0/0	Sib/BM	No	FLU/LPAM/ATG	CsA/MTX

BCG = BCG vaccination; Campylo = colitis due to *Campylobacter jejuni*; *C. neo* = *Cryptococcus neoformans* infection; *C. parvum* = *Cryptosporidium parvum* infection; E = encephalopathy; FA = food allergy; FTT = failure to thrive; HE = hepatic encephalopathy; HLA disparity (pheno/geno) = number of HLA phenotypical/genotypical mismatch loci; HSV = herpes simplex virus infection; ICH = intracranial hemorrhage; IgAN = IgA nephropathy; JIA = juvenile idiopathic arthritis; Kaposi = Kaposi's sarcoma; LD = liver dysfunction; OM = otitis media; *P. aeru* = *Pseudomonas aeruginosa*; PCP = *Pneumocystis jiroveci (carinii)* pneumonia; Pn = pneumonia; Rota = Rota virus infection; RSV = respiratory syncytial virus infection; SCID (OS) = Omenn syndrome; SCID (T-B-) = SCID with T-negative, B-negative phenotype; SCID (X) = SCID with *IL2RG* mutation; Sib = sibling; VZV = varicella zoster virus infection.

The analysis was performed at May 2005. Pre-existing infections (Table 2) and clinical complications (Table 3) before HSCT were assessed in all patients. Detailed HSCT courses of P2[9–11] and P14[12] were reported previously. HSCTs for the patients with XHIM (P23–29) were reported elsewhere.[13] A short report of two SCID patients (P10, P11) has been published previously.[14] Written informed consent for HSCT was obtained from the parents of each patient.

Diagnosis

The diagnosis was confirmed by mutation analysis of the causative genes in all patients except for one patient (P5) whose phenotype was $T^-B^-NK^+$ SCID. Mutation analysis of *IL2RG* in X-linked SCID (X-SCID) patients (P2–4),[15] *RAG1* in Omenn syndrome (OS) patient (P14),[16] *WASP* in WAS patients (P6, P15, P16, P17, P22 in this study are P44, P18, P3, P46, P13, respectively[17]) and *CD40L* in XHIM patients (P23–29)[13] were reported previously.

Conditioning regimen (Table 1)

For SCID patients (P1–5) from HLA-haploidentical related donors, no conditioning was given. Conditioning for 18 patients, including four patients with SCID (P9–12), eight patients with WAS (P6–8, P16–22) and six patients with XHIM (P24–29) consisted of BU 4 mg/kg/day for 4 days and CY 50 mg/kg/day for 4 days. In all patients transplanted since 2002, BU dosage was determined by pharmacokinetic study in which BU average steady-state plasma concentration was adjusted between 700 and 900 ng/ml.[18] TBI 12 Gy and CY 60 mg/kg/day for 2 days were used for five patients (P6–8, P15, P23). AraC 2–3 g/m^2 for four doses was added for four patients with WAS (P6–8, P15). Three patients (P13, P14, P30) were transplanted with reduced-intensity conditioning (RIC) regimen, including fludarabine (FLU) 25–30 mg/kg/day for 3 to 5 days and either small dose of TBI (2 Gy) (P13) or melphalan (L-PAM) 70 mg/kg/day for 2 days (P14, P30). Rabbit (P15) or equine (P14, P17–21, P30) antithymocyte globulin (ATG) was used to avoid rejection for eight patients.

GVHD prophylaxis (Table 1)

All but five patients (P1, P3, P6, P14, P23) received short-term (days 1, 3 and 6) MTX. CsA was used for all HSCT except for four patients who received tacrolimus (FK) (P13, P18, P19) or only MTX (P5). Seven patients (P2, P6, P14, P16, P19, P20, P23) received prednisolone (PSL) or methyl-PSL (1 mg/kg/day). Whole blood concentrations of CsA and FK were maintained around 200 and 10 ng/ml until day 30, respectively. These drugs were administered intravenously from day −1, until they could be taken orally. It was carefully tapered over 1 month after day 100 in the absence of GVHD.

Hematopoietic stem cell source (Tables 1 and 4)

BM was obtained from HLA-haploidentical related donor in nine, MRD in seven and UR-BM in seven transplants. Unmanipulated PBSC was used in one transplant (P23) and UR-CB was used in seven transplants.

As none of the patients transplanted before 1994 had MRD and unrelated donors were not available during this period, all donors for these patients were haploidentical relatives. Donor–recipient histocompatibility was determined by serology for HLA-A, -B and DR in transplants until 1995 and by DNA typing for HLA-DRB1 thereafter.

Table 2 Pre- (a) and post (b) transplant infections

Diagnosis	*No. of patients*	*Bacterial*	*PCP*	*Fungal and protozoal, other than PCP*	*CMV*	*Viral, other than CMV*
(a) *Pre-transplant infections*						
SCID	11	5	5	2	3	5
WAS	11	11	1	3	3	5
XHIM	8	9	6	3	0	3
(b) *Post transplant infections*						
SCID	11	6	0	0	1	3
WAS	11	4	0	0	3	2
XHIM	8	3	3	5	2	2

Each number in the column represents the number of infections before (pre) and after (post) transplantation.

Table 4 HLA disparity between donor and recipient in UR-BMT and UR-CBT

	UR-BMT	*UR-CBT*
HLA phenotypical disparities		
0	7	2
1	0	5
HLA genotypical disparities		
0	4	1
1	3	4
2	0	1
3	0	1
Total	7	7

Each number in the column represents the number of patients.

Table 3 Pretransplant problems other than infection at HSCT

Diagnosis	*No. of patients*	*FTT*	*Diarrhea/enteritis*	*Liver dysfunction*	*Autoimmune disease*	*Other*
SCID	11	5	6	3	0	2 (E 1, ED 1)
WAS	11	0	2	0	3	3 (FA 2, ICH 1)
XHIM	8	1	2	2	0	4 (OP 1, BE 1, HE 1)

Each number in the column represents the number of the pretransplant problems at HSCT other than infection.
BE = bronchiectasis; E = encephalopathy; ED = erythroderma; FA = food allergy; FTT = failure to thrive; HE = hepatic encephalopathy; ICH = intracranial hemorrhage; OP = osteoporosis.

HLA disparities between donors and recipients in UR-BMT and UR-CBT are shown in Table 4. In seven UR-BM donors, HLA was genotypically full matched in four and genotypically one locus mismatched in three donors. In seven UR-CB donors, HLA was genotypically full matched in one, one locus mismatched in four, two loci mismatched in one and three loci mismatched in one.

T-cell depletion

T cells were depleted from BMs of HLA-haploidentical related donor to avoid severe GVHD reaction. In P1, soybean lectin with E-rosetting was used as reported by others.[19] In P2, monoclonal antibody, B7, which recognizes E-rosette receptor, was used with complement. In P3, P4, P6–8, monoclonal anti-CD2 and anti-CD6 antibodies were used with immunomagnetic beads. In P5, monoclonal anti-CD5 and anti-CD8 antibody coated flasks were used.

Infused cell dose (Tables 5 and 6)

In seven HSCTs with TCD using monoclonal antibody (P3–8), the mean infused nucleated cell count (NCC) was 2.4×10^7/kg body weight (range 1.0–3.5×10^7/kg), the mean removal rate of $CD3^+$ cells was 2.4 log (2.0–2.8 log) and the mean infused $CD3^+$ and $CD34^+$ cells were 1.0×10^5/kg (0.35–2.6×10^5/kg) and 1.1×10^6/kg (0.2–3.2×10^6/kg), respectively (Table 5). In seven BMTs from MRD, seven UR-BMT and seven UR-CBT, the

Table 5 T-cell depletion and recovery of the stem cells

Pt. no.	*NCC (10^7/kg)*		*Removal rate of $CD3^+$ cells (log)*	*Transplanted $CD3^+$ cells (10^5/kg)*	*Transplanted $CD34^+$ cells (10^6/kg)*	*Recovery of $CD34^+$ cells (%)*
	Before	*After*				
3	9.2	3.5	2.5	0.4	3.2	64.1
4	10.7	2.8	2.2	1.1	1.4	90.7
5	2.4	1.0	2.0	1.0	1.1	107.9
6-1	10.9	1.9	2.6	1.2	0.5	15.3
6-2	8.3	2.9	2.1	2.6	0.9	37.1
7	7.9	3.0	2.6	0.6	0.3	18.8
8	11.8	1.7	2.8	0.5	0.2	11.5
Mean	8.7	2.4	2.4	1.1	1.1	49.3
s.d.	3.2	0.9	0.3	0.8	1.0	38.8

Table 6 Outcome of transplantation of 30 patients

Pt. no.	*Diagnosis*	*Nucleated cell dose ($\times 10^8$ cell/kg)*	*Neutrophil engraftment (day)*	*GVHD*		*Post transplant infection*			*Other toxicity*	*Outcome (day)*	*Cause of death*
				Acute	*Chronic*	*Bacterial*	*Fungal/protozoal*	*Viral*			
1	SCID (X)	7.0	NA	4	NA					Dead (32)	GVHD
2	SCID (X)	0.07	NA	0	—	*P. aeru*, Tbc		CMV, VZV		Dead (6213)	CMV
3	SCID (X)	0.35	NA	1	—			EBV, HSV		Dead (1709)	EBV-LPD
4	SCID (X)	0.28	No	NA	NA	*S. epi*				Dead (101)	Sepsis
5	SCID (T-B-)	0.10	NA	1	Extensive	*S. epi*				Dead (342)	Sepsis
6-1	WAS	0.19	No	NA	NA					To 2nd SCT	
6-2	WAS	0.29	Yes (14)	0	—			CMV		Alive (5109+)	
7	WAS	0.30	Yes (16)	1	—			CMV		Dead (398)	CMV
8	WAS	0.17	Yes (21)	1	—	*P. aeru*		EBV		Dead (235)	EBV-LPD
9	SCID (X)	4.1	Yes (12)	2	—					Alive (3135+)	
10	SCID (X)	1.6	Yes (15)	2	—					Alive (2448+)	
11	SCID (X)	0.59	Yes (23)	0	Extensive	BCG			VOD	Alive (2323+)	
12	SCID (X)	0.80	NA	NA	NA				VOD	Dead (5)	VOD
13	SCID (X)	1.1	Yes (9)	0	—					Alive (477+)	
14	SCID (OS)	0.53	Yes (18)	1	—	*M. avium*				Alive (272+)	
15	WAS	3.2	Yes (15)	1	—			CMV	VOD, HC	Alive (3352+)	
16	WAS	3.4	Yes (17)	0	Extensive			VZV		Alive (2015+)	
17	WAS	4.1	Yes (13)	2	—	*S. epi*				Alive (1943+)	
18	WAS	6.8	Yes (12)	0	—				TMA	Alive (1110+)	
19	WAS	3.7	Yes (14)	0	—	*B. cereus*				Alive (1019+)	
20	WAS	0.70	Yes (22)	4	Extensive	*S. epi*			TMA	Dead (216)	TMA
21	WAS	0.63	Yes (19)	0	—					Alive (229+)	
22	WAS	4.2	Yes (11)	0	NA					Alive (98+)	
23	XHIM	NA	Yes (8)	0	NA	*P. aeru*	*C. parvum*	CMV		Dead (55)	Sepsis
24	XHIM	3.3	Yes (14)	0	—			VZV		Alive (2190+)	
25	XHIM	4.8	Yes (16)	4	NA	*P. aeru*	*Candida*, Asper.		VOD	Dead (89)	Asper.
26	XHIM	4.9	Yes (14)	2	—	*C. difficile*	PCP			Alive (1868+)	
27	XHIM	3.8	Yes (12)	2	—		PCP, *Candida*		TMA	Alive (1784+)	
28	XHIM	6.5	Yes (14)	2	Limited		PCP			Alive (1201+)	
29	XHIM	2.0	Yes (14)	0	Extensive				TMA	Alive (1003+)	
30	XHIM	3.0	Yes (13)	1	—		*C. parvum*	CMV, EBV	IVM, ICH	Alive (350+)	

Acute = grade of acute GVHD; Asper. = Aspergillosis; *B. cereus* = sepsis due to *Bacillus cereus*; *C. difficile* = *Clostridium difficile* enteritis; *C. parvum* = disseminated *Cryptosporidium parvum* infection; chronic = type of chronic GVHD; HC = hemorrhagic cystitis; ICH = intracranial hemorrhage; IVM = involuntary movement; *M. avium* = *Mycobacterium avium complex* infection; NA = not available; *P. aeru* = sepsis due to *Pseudomonas aeruginosa*; *S. epi* = sepsis due to *Staphylococcus epidermidis*; Tbc = tuberculosis; TMA = thrombotic microangiopathy.

mean infused NCCs were 3.5×10^8/kg, 4.8×10^8/kg and 8.5×10^7/kg, respectively (Table 6). In one PBSCT from MRD (P23), the infused $CD34^+$ cell count was 9.0×10^6/kg.

Supportive therapy
All patients were nursed in a room with laminar airflow until neutrophil count exceeds 1×10^9/l or more. All patients with conditioning received G-CSF from day 1 until neutrophil counts recovered to more than 1×10^9/l. Antimicrobial prophylaxis during transplantation period consisted of acyclovir 15 mg/kg/day intravenously from day –7 to day 30, cotrimoxazole until 1 year after HSCT and oral amphotericin B or fluconazole until day 100 or more. Four patients (P5, 9, 15, 19) with previous CMV infection received intravenous ganciclovir instead of acyclovir. Vancomycin and polymyxin B were used as intestinal decontamination before mid-1990s and quinolones were used thereafter. All patients received intravenous Ig (IVIG) during the transplantation period maintaining the trough IgG level over 700 mg/dl. Blood samples of all patients were screened for CMV by antigenemia (since 1993) or by PCR (since 2001), and those who tested positive were treated with intravenous ganciclovir and, if necessary, foscarnet.

Post transplantation period
Engraftment was defined as the first day on which neutrophil count exceeded 0.5×10^9/l for 3 consecutive days. Chimerism was assayed on the peripheral blood using HLA typing, XY fluorescence *in situ* hybridization (FISH; for sex-mismatched HSCT) or variable number of tandem repeat (VNTR) analysis (for sex-matched HSCT). In transplants with no conditioning, lineage-specific chimerism analysis by FISH, VNTR or HLA typing was used. GVHD[20] and veno-occlusive disease (VOD)[21] were diagnosed and classified according to the standard criteria.

Statistical analysis
The probabilities of overall survival were calculated by Kaplan–Meier analysis. Overall survival was measured from transplantation until death from any causes. The log-rank test was used to compare cumulative survival between subgroups.

Results

Pretransplant status (Tables 1–3)
Twenty-seven of 30 patients had pre-existing infections with or without organ damages before HSCT. Bacterial infections were observed in half (15 patients) of the patients. These include skin infections ($n = 9$), otitis media ($n = 8$), pneumonia ($n = 6$) and sepsis ($n = 1$) (Table 1). *Pneumocystis jiroveci* (formerly *carinii*) pneumonia (PCP) was diagnosed in 12 patients (five SCID, one WAS, six XHIM patients), and was controlled by cotrimoxazole by conditioning for HSCT. CMV disease was diagnosed in six patients (three SCID, three WAS) and was treated with ganciclovir or ganciclovir in combination with foscarnet (Table 2a). Three SCID patients were vaccinated with Bacillus–Calmette–Guerin (BCG) before diagnosis; however, none experienced disseminated infection before HSCT.

Among SCID patients, five showed failure to thrive, six had intractable diarrhea and three displayed liver dysfunction (Table 3). Two patients with XHIM (P23, P30) suffered from cholangitis, liver dysfunction and prolonged diarrhea caused by *Cryptosporidium parvum* (*C. parvum*) infection. Autoimmune diseases were seen in two WAS patients (P16, P20). P16 had severe juvenile idiopathic arthritis and IgA nephropathy, which required steroid therapy. P20 had severe autoimmune cytopenia (autoimmune neutropenia, anemia and thrombocytopenia).

Engraftment (Table 6, Figure 1)
In nine BMTs transplanted with TCD, two (P4, P6–1) resulted in primary graft failure. P4 was scheduled to receive second BMT from unrelated donor but died from sepsis during the conditioning for second BMT. P6 underwent a second transplant from the same donor and resulted in full engraftment (P6–2). Consequently, seven patients transplanted with TCD experienced sustained engraftment; however, mixed chimerism was induced in all but one (P6) patient. These patients showed poor T-cell function after HSCT. T-cell numbers after HSCT in six of these eight patients with TCD are displayed in Figure 1. All the patients whose T-cell number did not reach 1×10^9/l until 6 months after HSCT died within 5 years. We lost all seven patients with SCID (P1–5) and WAS (P7, P8) with T-cell dysfunction. Long-term full engraftment was maintained in only one patient (P6), in whom T-cell number reached 1×10^9/l until 6 months after HSCT with TCD. The patient is currently alive and well with normal platelet count, normal T/B-cell function and IVIG is not required for 14 years.

On the other hand, sustained engraftment was achieved in 21 out of 22 patients transplanted without TCD. Among 18 patients successfully transplanted without TCD, two (P13, P15) resulted in mixed chimerism. In P13, who received RIC, most neutrophils are of recipient origin, and T cells and most B cells are of donor origin. P15 achieved

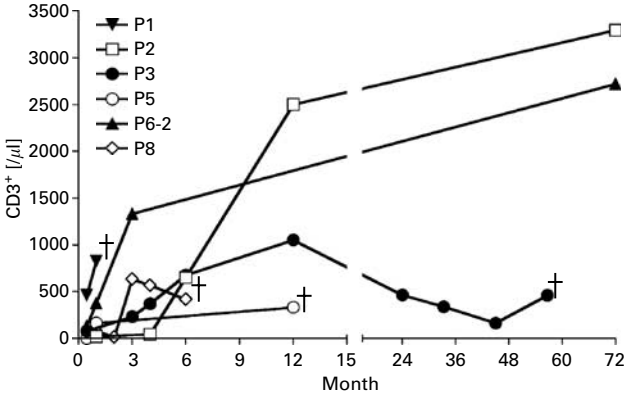

Figure 1 T-cell recovery of the patients transplanted with TCD. Numbers of $CD3^+$ cells after HSCT in six patients transplanted with TCD are represented. P2 received thymus transplantation 5 months after SCT. Crosses indicate the death of each patient.

stable mixed chimerism in which 90% of neutrophils are of recipient origin, most lymphocytes are of donor origin and platelet count is around 50×10^9/l without transfusion. None of these 18 long-term survivors, including two patients with mixed chimerism, require IVIG.

GVHD and other toxicity (Table 6)
Grade 2–4 acute GVHD developed in nine out of 27 evaluable patients. Three patients with grade 4 acute GVHD (P1, P20, P25) died. The other six patients with grade 2 acute GVHD were successfully treated with mPSL pulse therapy followed by PSL. Chronic GVHD occurred in six out of 24 evaluable patients, and resolved in all but one (P20) of these six patients.

Hepatic VOD occurred in four patients and one patient (P9) died of this complication. Thrombotic microangiopathy (TMA) occurred in four patients and one patient (P20) died of it.

Post transplant infections (Table 2 and 6)
Bacterial infections during the early post transplant period could be treated by systemic antibiotics, but the infections with prolonged use of immunosuppressive drugs for GVHD or with poor immunological reconstitution were difficult to treat and were fatal in some patients (P4, P5, P23).

Mycobacterium infection occurred in three SCID patients, including one tuberculosis (P2),[22] one BCG infection (P11) and one *Mycobacterium avium complex* infection (P14).[12] In all three patients, these infections were difficult to overcome, but finally resolved with long-term medication of multiple drug and with immunological reconstitution after HSCT.

No fungal or protozoal infections were seen in SCID and WAS patients. However, these infections were common in XHIM patients. In eight XHIM patients, three (P26–28) had PCP, two (P25, P27) had candidiasis, one (P25) had fatal aspergillosis and two (P23, P30) had disseminated *C. parvum* infection after HSCT. Although *C. parvum* was effectively eradicated by high-dose azithromycin in P30, it was not overcome in P23.

CMV disease was diagnosed in six patients and two (P2, P7) of these died of CMV disease despite the treatment with ganciclovir and foscarnet. EBV-LPD occurred in two patients (P3, P8), both of whom were transplanted with TCD and these two patients died of this complication.

Outcome (Table 6, Figure 2)
The mean and median follow-up periods of survivors were 51.6 and 85.6 months, respectively (range 3.3–168.0 months). Survival curves according to year of transplant and donor source are shown in Figure 2a and b, respectively. In Figure 2a, all transplants during 1984–1995 and those during 1996–2005 were performed with TCD and without TCD, respectively. In patients transplanted with TCD, only one (P6) of eight patients (12.5%) is alive. Five-year overall survival rate ($\pm$1 s.d.) of these patients is 25.0 ($\pm$15.3)% (Figure 2b). The causes of death were GVHD (P1), extensive CMV disease (P2, P7), EBV-LPD (P3, P8) and sepsis (P4, P5) (Table 6).

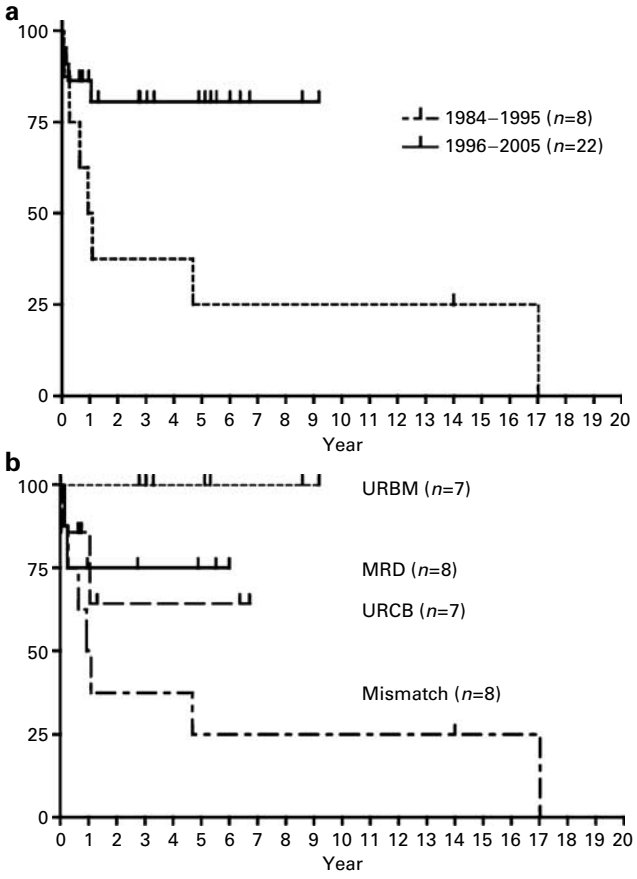

Figure 2 Survival curves according to year of transplant (**a**) and to donor source (**b**). All HSCTs during 1984–1995 were undergone with TCD and those during 1996–2005 were without TCD. In (**a**), 5-year overall survival rate during 1996–2005 (80.4$\pm$8.9%) was significantly higher than that during 1984–1995 (25.0$\pm$15.3%) ($P = 0.017$).

On the other hand, overall survival rate during 1996–2005 significantly improved. Eighteen out of 22 patients (81.8%) transplanted without TCD during 1996–2005 are alive. The 5-year overall survival rate of these patients is 80.4 ($\pm$8.9)% (Figure 2a, $P = 0.017$ compared with HSCT during 1984–1995, which is identical to HSCT with TCD). Of these, seven of seven transplanted from UR-BM (100%), five of seven from UR-CB (71.4%) and six of eight from MRD (75.0%) are alive and well (Figure 2b), and none of these survivors requires IVIG replacement therapy. VOD (P12) and TMA (P20) were the causes of death in two UR-CB patients. Two XHIM patients (P23, P25) transplanted from MRD died of infection. All of these four deceased patients had severe pretransplant complications.

Autoimmune diseases in two WAS patients resolved after HSCT. Although one (P20) was deceased by TMA, pre-existing severe arthritis in another (P16) resolved after HSCT and no medication is required. IgA nephropathy of this patient is stable and only microscopic hematuria exists after HSCT with no medication.

The mean and median days of post-HSCT in-patient hospitalization in 19 survivors were 172 and 248 days, respectively (range 63–429 days).

Discussion

In this study, we retrospectively analyzed the results of 31 HSCTs for 30 patients with PID, especially with three distinctive disorders: SCID, WAS and XHIM.

TCD

Until 1995, unrelated donor stem cell transplant was not available in Japan; thus, we performed HSCT with TCD from HLA-haploidentical relatives for patients without MRD. We used soybean lectin with E-rosetting in the first HSCT, but the patient (P1) had fatal GVHD in spite of using CsA. The other SCTs with TCD for seven patients (four SCID and three WAS) were undertaken using monoclonal antibodies and resulted in no or transient (grade 0 or 1) acute GVHD (Table 6). The reason for this minimal GVHD may be owing to the low dose of infused $CD3^+$ cells. Although the initial transplant courses were uneventful, all of these seven patients failed to develop durable T-cell function. One WAS patient was treated by the second BMT from the same donor and full T-cell reconstitution was achieved. However, the other six patients suffered from prolonged and fatal infections after 100 days, including two extensive CMV diseases, two EBV-LPD and two sepsis. As shown in Figure 1, the initial increase of T-cell number after HSCT may predict the following durable T-cell engraftment and function. One reason for these results may be the insufficient number of infused $CD34^+$ cells as suggested by others[23] (Table 5).

Although TCD-BMT was one of the commonly used transplantation modalities, our result on BMT with TCD was far from satisfactory, which led us to use unrelated donor stem cells that became available in 1990s.

Non-TCD

Our result of 22 HSCTs without TCD with conditioning including 14 unrelated donor transplants was encouraging (5-year overall survival: 80.4%). All 18 survivors are alive and well with complete chimerism except for two patients (P13, P15). All 18 survivors, including two patients with RIC, are free of IVIG.

GVHD

Severe (grade 3–4) acute GVHD was rare (two out of 22). In two patients with grade 4 GVHD, one was transplanted from MRD (P25) and the other was from HLA one locus-mismatched UR-CB (P20). In all the other HLA genotypically mismatched HSCT from UR-BM ($n=3$) and UR-CB ($n=5$), severe GVHD did not occur. Thus, HLA disparity seemed not to be correlated with severe GVHD.

Conditioning

In SCID, myeloablative conditioning (MAC) was not tolerated by one patient (P12) because of severe pre-existing organ dysfunctions. Most patients with SCID carry infections or organ dysfunctions at the time of HSCT; thus, RIC seems to be suitable as pointed out by others.[24] In our latest two patients with SCID (P13, P14), we performed HSCT with RIC. The clinical course of P13 was uneventful; T cells were fully engrafted and IVIG is not required. P14 experienced *M. avium* complex infection, which required prolonged multidrug antimycobacterial therapy, but finally resolved.

In XHIM, MAC was tolerated in five patients without organ dysfunction (P24, P26–29), but not in two patients with severe organ dysfunction (P23, P25). According to these results and to other reported results,[24,25] RIC seems to be feasible for patients with organ dysfunction. We underwent HSCT with RIC for an XHIM patient who had liver dysfunction and hepatic encephalopathy at the time of HSCT (P30). P30 did not experience hepatic failure, but suffered from severe involuntary movement associated with EBV reactivation, which gradually resolved with immunological reconstitution.

In WAS, the conditioning regimen consisted of BU + CY for HSCT from MRD (P16, P22) and adding ATG for HSCT from unrelated donor[26] (P17–19, P21) seemed to be tolerable and to be feasible for full engraftment. On the other hand, one UR-BMT with TBI, CY and AraC (P15) resulted in mixed chimera with relatively low platelet and prolonged need of IVIG. The role of ATG should be determined by prospective studies with much higher numbers of patients. RIC regimen remains to be assessed in WAS patients.

Cord blood transplantation

Four of five patients with SCID and one of two patients with WAS transplanted with UR-CB are alive and well with fast and durable engraftment with no need for IVIG. This result is encouraging, and thus if MRD is not available, we plan to continue to perform UR-CBT for SCID patients because of the availability when urgently required. In WAS patients without MRD, UR-BMT has been preferred[27] rather than haploidentical BMT with TCD, but successful UR-CBT was also reported recently.[28] Thus, UR-CBT may be a treatment of choice for patients with WAS, if urgent HSCT is necessary or if the patient lack a suitable UR-BM donor.

Pretransplant condition

In our results, pretransplant condition in PID was important and seemed to be correlated with the outcome. In HSCT without TCD, all four deceased patients (P12, P20, P23, P25) had severe pretransplant complications. Transplant at an early age seems to be important and may alter the outcome. In our three patients with SCID, BCG vaccination had already been performed before the diagnosis of SCID and BCG infection became overt and difficult to control after HSCT in one patient (P11). As BCG vaccination is performed before 6 months of age in Japan, early diagnosis by neonatal screening[29] and early HSCT seem to be important and necessary, especially in SCID patients.[30] Recurrence of PCP was observed in three XHIM patients in spite of the prophylactic use of cotrimoxazole, but it was controllable in all three cases. However, one of the recurrences of *C. parvum* infection in two XHIM patients was difficult to control, indicating the requirement of early HSCT for XHIM as pointed out by others.[25]

Conclusion

In summary, our results for HSCT with TCD for patients with PID were disappointing, but the result of HSCT without TCD including unrelated donors was excellent and promising. Early diagnosis and early HSCT may alter the outcome and may allow us to conduct a multicenter study because many various pre-existing problems in each patient limited us in using the same conditioning regimen. In patients with no suitable donor including cord blood, and in patients in whom urgent HSCT is necessary, HSCT from HLA-haploidentical relative by $CD34^+$ selection may be a treatment of choice. Multicenter studies are needed to determine the best procedures in HSCT for patients with these rare disorders.

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