

**Table IV** Immune-related diseases in PID patients*(a) Immune-related diseases with each PID*

Primary immunodeficiency	Total	<i>n</i>	Immune-related disease
I. Combined T and B cell immunodeficiencies	75	2	(2.6%)
MHC class II deficiency (suspected)	1	1	ITP with AIHA 1 <sup>a</sup>
CD4 deficiency	1	1	Hashimoto disease 1
II. Predominantly antibody deficiencies	378	24	(6.3%)
Common variable immunodeficiency disorders	93	16	ITP 3, RA 2, AIHA 2, Hashimoto's disease 2, IBD 2, SLE 1, MG 1, ADEM 1, Autoimmune hepatitis 1, Uveitis 1
Hyper-IgM syndrome	32	3	JIA 1, SLE (complicated with C1q deficiency) 1, IBD 1
Selective IgA deficiency	28	3	SLE 1, SLE with Kikuchi disease 1, RA 1
IgG subclass deficiency	50	2	ITP with AIHA 1, ITP with MS 1
III. Other well-defined immunodeficiency syndromes	165	5	(3.0%)
Wiskott–Aldrich syndrome	57	3	AIHA 2, Kawasaki disease 1
DiGeorge syndrome	33	2	AIHA 1, ITP 1
IV. Diseases of immune dysregulation	38	10	(26.3%)
X-linked lymphoproliferative syndrome	5	1	IBD 1
Autoimmune lymphoproliferative syndrome	6	4	ITP 3, Graves' disease with IBD 1
APECED	5	1	T1DM with Hashimoto's disease and Vogt–Koyanagi–Harada disease 1
IPEX syndrome	6	4	T1DM 1, T1DM with ITP, AIN and IBD 1, Autoimmune enteritis 1, AIHA with Autoimmune enteritis and Hashimoto's disease 1
V. Congenital defects of phagocyte number, function, or both	153	25	(16.3%)
Chronic granulomatous disease	87	25	IBD 20, ITP 2, JIA 1, MCTD 1, Kawasaki disease 1
VI. Defects in innate immunity	12	5	(41.7%)
NEMO deficiency	7	4	IBD 3, IBD with JIA 1
WHIM syndrome	3	1	T1DM 1
VII. Autoinflammatory disorders	74	3	(4.0%)
Familial Mediterranean fever	36	2	SLE 1, Kawasaki disease 1
TNF receptor associated periodic syndrome	9	1	SLE 1
VIII. Complement deficiencies	23	3	(13.0%)
C4 deficiency	1	1	SLE with RA 1
C6 deficiency	1	1	IBD 1
C9 deficiency	11	1	Kawasaki disease 1
IX. Undetermined	5	1	(20%)
Nakajo syndrome	1	1	SLE 1
Total	923	78	(8.5 %)

*(b) Immune-related manifestations associated with PID*

Immune-related diseases	<i>n</i>
IBD (including autoimmune enteritis)	33
ITP	13
AIHA	8
SLE	8
RA/JIA	6
Hashimoto's disease/Graves' disease	5
Kawasaki disease	4
T1DM	4
Uveitis (including Vogt–Koyanagi–Harada disease)	2
ADEM/MS	2
Others	5

*n* Number of PID patients who had immune-related disorders, *ADEM* acute disseminated encephalomyelitis, *AIHA* autoimmune hemolytic anemia, *AIN* autoimmune neutropenia, *APECED* autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, *IBD* inflammatory bowel disease, *IPEX* immunodysregulation, polyendocrinopathy, enteropathy X-linked, *ITP* immune thrombocytopenic purpura, *JIA* juvenile idiopathic arthritis, *MCTD* mixed connective tissue disease, *MG* myasthenia gravis, *MS* multiple sclerosis, *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus, *T1DM* type 1 diabetes mellitus, *WHIM* warts, hypogammaglobulinemia, infections, and myelokathexis

<sup>a</sup> The number of patients

observed in urban areas [5, 7, 16]. This may be because many PID patients were treated or followed by PID specialists distributed nationwide in Japan; this is assumed by the location of hospitals with which they were affiliated.

The distribution ratios of BTK deficiency (14.7%) and CGD (11.9%) in Japan were higher than those in a previous report from Europe (5.87% and 4.33%, respectively), while those of CIDs and other well-defined immunodeficiency syndromes were comparable [17]. The prevalence of BTK deficiency was previously reported to be 1/900,000–1,400,000 in a European cohort study [18]. In contrast, this value was estimated to be 1/300,000 in Japan in our study. BTK deficiency appears to be common in Japan, although this may be partially because more patients, including those showing atypical clinical manifestations, were diagnosed more accurately by the recently established genetic diagnostic network in Japan [19]. This is supported by the highest proportion of Japanese patients in the international mutation database for X-linked agammaglobulinemia (BTKbase) [20]. The reason for the low number of registered CGD patients in Europe in a recent report (1/620,000) [17] is unknown; the prevalence of CGD was 1 in 250,000 in a previous European survey [21], which was similar to our results (1 in 380,000 in this study and 1 in 280,000 in our previous study [22]). The percentage of BTK deficiency and CGD would be lower if more adult cases were registered because the prevalence of these disorders is low in adults. CVID was the most commonly reported PID (20.7%) in Europe, and the onset of symptoms was observed most commonly in the third decade of life in these patients [17, 23]. In this study, CVID constituted 11.0% (136 cases) of PID cases, and only 29 cases were reported from internal medicine departments (Table II). A lower number of registered CVID patients may have led to a lower number of reported patients with antibody deficiency and a lower prevalence of PID, although it is still possible that CVID is not as common in Japan as in European countries. There was no significant difference in the distribution rate of SIgAD between Japanese and Europeans, although SIgAD is rare in Japanese (1/18,500) compared with Caucasians (1/330–2,200) according to seroepidemiologic studies [24]. This may be because most SIgAD patients lack clinical manifestations. The distribution ratio of auto-inflammatory disorders in Japan (9%) was much higher than that in Europe (1.02%) [17] (Table II). Considering the disease type of the auto-inflammatory disorders was not specified in 22 cases (20%), it is possible that many other patients with auto-inflammatory disorders remain undiagnosed in Japan as well as in other countries.

The percentage of men (69.7%) with PID is higher in Japan than in Europe (60.8%) or Kuwait (61.8%), but is equivalent to that in Taiwan (70.2%) [6, 13, 17]. The higher

ratio of men, particularly in younger generation (<15 years), appears to be due to the larger number of X-linked PID patients (BTK deficiency, X-CGD,  $\gamma$ c deficiency, etc.) in this study compared to that in Europe or Kuwait. Adolescents or adults ( $\geq 15$  years) constituted 42.8% of the patients in this study, which is equivalent to the number in the European study ( $\geq 16$  years: 46.6%), while those >16 years constituted only 10.9% in the previous survey [3, 17]. In this study, it was found that CVID and SIgAD are common in adults (Table II) and that antibody deficiencies are more common with increasing age (Fig. 2b). A reason for the increased number of adult PID patients may be long-term survival of PID patients due to improved treatments such as immunoglobulin replacement therapy. In addition, an increased likelihood of patients being diagnosed by internists as having late-onset PID, e.g., CVID and SIgAD, may have contributed to these values [17, 25, 26]. Therefore, it is important for internists to be well-informed regarding PID. In contrast, CIDs are fatal during infancy without hematopoietic stem cell transplantation or gene therapy. Because hematopoietic stem cell transplantation has been widely performed in Japan since the 1990s, surviving patients with CID are limited to the younger generation, similar to French patients (Fig. 2b) [5, 27, 28].

It has been reported that PID patients are at increased risk of developing malignant diseases, in particular, non-Hodgkin lymphoma, leukemia, and stomach cancer [29]. Although lymphoma and leukemia were relatively common, stomach cancer was not observed in our study. In the previous survey in Japan, eight of nine PID patients with malignant disorders (including one gastric cancer patient) died [3]. It is possible that some PID patients with malignant disorders were not registered because they were deceased. PID is also associated with immune-related diseases because of a defect in the mechanisms to control self-reactive B and T cells. The frequency of immune-related manifestations varied among individual PID patients, as reported previously [30, 31]. Four PID patients who had developed Kawasaki disease, one patient with WHIM syndrome and type 1 diabetes mellitus, and one patient with TRAPS and SLE in our study may provide new pathophysiological insights of these diseases and the association between PID and autoimmune diseases.

## Conclusions

We report the prevalence and clinical characteristics of PIDs in Japan. Although the advances in diagnostic technologies and treatments have improved the prognoses of PID, many patients continue to experience severe complications such as malignancy and immune-related diseases as well as infections. To improve the quality of life of PID patients, it is necessary to pay attention to

complications and treat them appropriately. Web-based PID databases and consultation systems have been created in Japan (Primary Immunodeficiency Database in Japan [4] and Resource of Asian Primary Immunodeficiency Diseases in Asian countries [32]) to reveal precise information regarding PID and to promote cooperation between doctors and researchers [19].

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**Conflict of Interest** There is no actual or potential conflict of interest in relation to the study.

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## Outcome of unrelated umbilical cord blood transplantation in 88 patients with primary immunodeficiency in Japan

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### Summary

We report the results of umbilical cord blood transplantation (UCBT) performed in 88 patients with primary immunodeficiency (PID) between 1998 and 2008 in Japan; severe combined immunodeficiency (SCID,  $n = 40$ ), Wiskott–Aldrich syndrome (WAS,  $n = 23$ ), chronic granulomatous disease ( $n = 7$ ), severe congenital neutropaenia (SCN,  $n = 5$ ) and other immunodeficiencies ( $n = 13$ ). Five-year overall survival (5-year OS) for all patients was 69% [95% confidence interval (CI), 57–78%], and was 71% and 82% for SCID and WAS, respectively. The main cause of death before day 100 was infection (17/19), while that after day 100 was graft-versus-host disease (GVHD) (5/7). Using multivariate analyses, pre-transplant infection, no conditioning,  $\geq 2$  human leucocyte antigen (HLA) mismatches or diagnosis other than SCID, SCN or WAS were all associated with poor prognosis. Reduced-intensity conditioning was associated with decreased overall mortality compared with myeloablative therapy. The cumulative incidence of grade 2–4 acute GVHD at day 100 was 28% (95% CI, 19–38%), and that of chronic GVHD at day 180 was 13% (95% CI, 7–23%). We conclude that UCBT should be considered for PID patients without an HLA-matched sibling. The control of pre-transplant infection and selection of HLA-matched donors will lead to a better outcome.

**Keywords:** primary immunodeficiency, severe combined immunodeficiency, Wiskott–Aldrich syndrome, cord blood transplantation, reduced-intensity conditioning.

Allogeneic haematopoietic stem cell transplantation (HSCT) has been successfully used as a curative therapy for most severe forms of primary immunodeficiency (PID) (Zeidler *et al*, 2000; Antoine *et al*, 2003; Sakata *et al*, 2004; Rao *et al*, 2005; Kobayashi *et al*, 2006; Mazzolari *et al*, 2007; Dvorak & Cowan, 2008; Griffith *et al*, 2008; Cuvelier *et al*, 2009). Stem cell transplantation from a human leucocyte antigen (HLA)-identical family donor provides better prognosis than bone marrow transplantation from an unrelated donor (Antoine *et al*, 2003). Survival with this type of transplantation from a matched unrelated donor has improved significantly over the years in patients with severe combined immunodeficiency (SCID), whereas no improvement in survival has been observed with this transplantation in non-SCID patients (Antoine *et al*, 2003). The optimal stem cell source for PID patients with no HLA-identical sibling remains to be determined (Dvorak & Cowan, 2008; Griffith *et al*, 2008; Cuvelier *et al*, 2009).

Umbilical cord blood grafts from unrelated donors have been successfully used, primarily in children and subsequently in adults (Kurtzberg *et al*, 1996; Wagner *et al*, 1996; Gluckman *et al*, 1997; Rubinstein *et al*, 1998; Rocha *et al*, 2000, 2004; Laughlin *et al*, 2004). Theoretically, unrelated cord blood transplantation (UCBT) has the following distinct advantages in PID patients: (i) the cord blood product is rapidly accessible in most cases; (ii) the incidence and severity of graft-versus-host disease (GVHD) is not excessive, even in mismatched transplantation and (iii) the risk of latent viral transmission is low. The disadvantages of UCBT include slower haematopoietic/immunological reconstitution and graft failure, which have been observed with UCBT for malignant disorders, and naivety of lymphocytes to pathogens (Brown *et al*, 2008; Griffith *et al*, 2008; Szabolcs *et al*, 2008). Rapid immune reconstitution is particularly important in PID patients with ongoing infection who undergo UCBT.

The limited data available show that UCBT can be a curative measure in patients with SCID, Wiskott–Aldrich syndrome (WAS), chronic granulomatous disease (CGD) and severe congenital neutropaenia (SCN) (Knutsen & Wall, 2000; Bhattacharya *et al*, 2003, 2005; Fagioli *et al*, 2003; Knutsen *et al*, 2003; Kobayashi *et al*, 2006). Most of the available data have come from a single centre, and thus, detailed information on the outcome and problems associated with UCBT in PID patients is still lacking. In this study, we report the results of UCBT performed in 88 PID patients between 1998 and 2008 in Japan.

## Methods

### Collection of data

All UCBTs carried out for PIDs through the Japan Cord Blood Bank Network (JCBBN) between August 1998 and January 2008 was enrolled in this study. Eighty-eight patients with PID underwent UCBT during this period. All data were provided

by JCBBN, which collects recipients' clinical information at day 100 after transplantation. Recipients' data on survival, disease status and long-term complications are renewed annually by administering follow-up questionnaires. Latest data acquisition was performed in November 2009. The present study was approved by the institutional ethical and data management committees of JCBBN.

### Patients

A summary of patients enrolled in this study is shown in Table I. Forty patients had SCID (45%) and 48 (55%) had non-SCID. Patients with familial haemophagocytic syndrome were not included in this study. The median age at the time of transplantation was 10 months (range, 0–248 months).

### Procedures

Cryopreserved, unrelated cord blood cells were used as the source of haematopoietic stem cells. The type of conditioning used and median cell dose infused are shown in Table I.

In most cases, HLA matching was performed by both serological and DNA typing for HLA-A, HLA-B and HLA-DRB1. In this study, HLA mismatch was defined according to serological or low-resolution molecular typing for HLA-A and HLA-B and high-resolution molecular typing for HLA-DRB1. Of the UCB donors, 29 (33%) were HLA fully compatible. Of the mismatched donors, 40 (45%) were 1-antigen mismatched, 15 (17%) were 2-antigen mismatched and four (5%) were 3-antigen mismatched (Table I). In 48 patients in whom high-resolution genotypical typing was performed for HLA-A, HLA-B and HLA-DRB1, 11 were fully matched, 13 were 1-antigen mismatched, 16 were 2-antigen mismatched, five were 3-antigen mismatched and three were 4-antigen mismatched.

Immunosuppressive prophylaxis against GVHD after UCBT consisted of ciclosporin A (CyA)- and tacrolimus-based regimens in 48 and 35 patients, respectively. Five patients were not administered any immunosuppressive drug after UCBT.

Various techniques including karyotyping, HLA typing and fluorescence *in situ* hybridization for the XY chromosome and variable number of tandem repeats were used to confirm the engraftment of donor cells.

### Definitions

Neutrophil recovery was defined by an absolute neutrophil count of at least  $0.5 \times 10^9/l$  for three consecutive days. Platelet recovery was defined by a count of at least  $20 \times 10^9/l$ , unsupported by transfusion for 7 d. Reticulocyte recovery was defined by a count of at least 20%.

Patients without conditioning or with only anti-thymocyte globulin (ATG) were categorized as receiving no conditioning. Patients administered busulfan (BU)/cyclophosphamide (CY)  $\pm$  total body irradiation (TBI) or total lymphoid irradiation

Table I. Age at the time of transplantation, type of conditioning and HLA disparity.

	Patients (N)	Median age at transplantation (months) (range)	Median cell dose ( $\times 10^7/\text{kg}$ ) (range)	Second or third transplantation (N)	Conditioning			HLA disparity			
					No (N)	RIC (N)	MAT (N)	0 (N)	1 (N)	2 (N)	3 (N)
Total	88	9 (0–248)	8.60 (1.89–31.1)	8	14	31	43	29	40	15	4
SCID	40	6.5 (0–27)	11.4 (4.55–31.1)	1	12	18	10	17	15	5	3
WAS	23	14 (4–84)	6.49 (2.89–13.6)	1	0	2	21	7	10	6	0
CGD	7	63 (31–248)	6.00 (1.89–12.3)	5	1	4	2	2	4	1	0
SCN	5	10 (4–124)	5.99 (4.16–9.19)	0	0	1	4	1	4	0	0
Others	13	37 (6–194)	8.11 (3.01–19.8)	1	1	6	6	2	7	3	1

RIC, reduced-intensity conditioning; MAT, myeloablative therapy. Definition of conditioning regimens are described in *Methods* section. 'Others' include four CD40L deficiency, two common variable immunodeficiency and one of each of the following disorders: Major histocompatibility complex (MHC) class II deficiency, DiGeorge syndrome, X-linked lymphoproliferative disorder, NEMO (NF- $\kappa$ -B essential modulator) deficiency, IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked) syndrome, Idiopathic CD4 lymphopenia and Blau syndrome.

(TLI), BU/CY + ATG  $\pm$  TLI, BU/CY + fludarabine (Flu) or CY/etoposide/high-dose cytarabine were categorized as receiving myeloablative therapies (MATs). CY dose ranged from 120 to 240 mg/kg (median, 200 mg/kg) in patients receiving MAT.

TBI < 4 Gy was classified as 'low-dose TBI'. Patients administered Flu/melphalan (L-PAM)  $\pm$  low-dose TBI or TLI, Flu/BU  $\pm$  TLI or Flu/CY (50–60 mg/kg)  $\pm$  low-dose TBI/TLI, Flu + low-dose TBI or Flu + ATG were categorized as receiving reduced-intensity conditioning (RIC). L-PAM dose was  $\leq 140$  mg/m<sup>2</sup> in patients receiving RIC.

GVHD was graded according to the standard criteria (Przepiora *et al*, 1995).

### Statistical analyses

The probability of survival was estimated by the product-limit method, and the log-rank test was used for group comparisons. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of neutrophil, platelet and reticulocyte recovery and that of acute and chronic GVHD. Death before recovery was the competing event for haematological recovery, and death without GVHD was the competing event for GVHD. Gray's test was used for group comparisons of cumulative incidence (Gray, 1988; Gooley *et al*, 1999). The Cox regression model was used to analyse data for the identification of prognostic factors. Factors found to be significant ( $P < 0.05$ ) or marginally significant ( $P < 0.1$ ) in univariate analysis were included in multivariate analysis. The variables considered were patient age at the time of transplantation, diagnosis, duration from diagnosis to transplantation, second or third transplantation, HLA disparity, presence of infection at the time of transplantation, conditioning regimen and cell dose infused. Variables with >2 categories were included in the final model using dichotomized dummy variables when at least one of the categories showed significant effect on survival. Continuous variables were dichotomized for the prognostic factor analyses. Variables were dichotomized as follows; patient age greater or

<12 months at transplantation, dichotomized at a median nucleic cell dose of  $<8.2 \times 10^7/\text{kg}$  vs.  $\geq 8.2 \times 10^7/\text{kg}$  and CD34 cell dose of  $<2.1 \times 10^5/\text{kg}$  and  $\geq 2.1 \times 10^5/\text{kg}$ , shorter than or equal to or longer than 180 d for time to transplant. All  $P$ -values were two-sided.

## Results

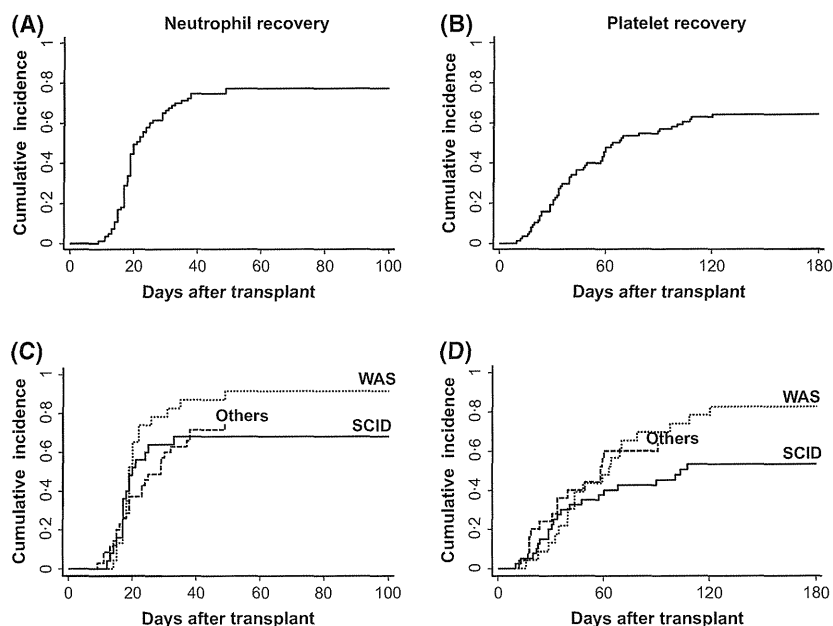
### Engraftment

Sixty-seven patients (76%) achieved stable engraftment. The cumulative incidence of neutrophil, platelet and reticulocyte recovery at day 100 after transplantation was 77% [95% confidence interval (CI), 66–85%], 56% (95% CI, 45–65%) and 64% (95% CI, 53–73%) respectively (Fig 1A, B; data not shown). The median time for neutrophil, platelet and reticulocyte recovery was 19 d (range, 9–104 d), 40 d (range, 10–122 d) and 27 d (range, 12–98 d), respectively. The cumulative incidences of neutrophil recovery were not statistically different among the disease groups (SCID, 74%; WAS, 91% and others, 68% at day 100 after transplantation) (Fig 1C), although incidence was low in CGD patients ( $N = 7$ , 43%).

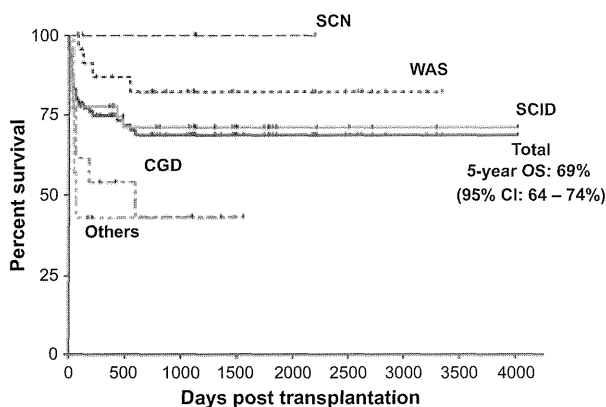
The time required for neutrophil recovery was similar in all disease groups, while that required for platelet recovery varied to some extent among the different disease groups. Platelet engraftment was slightly delayed in WAS patients, but the time required for engraftment in these patients was not significantly different from that required in other patients (Fig 1D).

Forty-three, 31 and 14 patients received MAT, RIC and no conditioning, respectively. No difference was observed in the incidence of neutrophil recovery between the MAT and RIC groups (84% vs. 87% at day 100). Similarly, no difference was observed in platelet recovery between these two groups (data not shown).

The cell dose infused ranged from 1.89 to  $31.1 \times 10^7/\text{kg}$ , with a median of  $8.60 \times 10^7/\text{kg}$ . No correlation was observed between the cell dose infused and engraftment.



**Fig 1.** Cumulative incidence of neutrophil and platelet recovery after UCBT. (A) The cumulative incidence of neutrophil recovery 77% (95% CI, 66–85%). (B) The cumulative incidence of platelet recovery 56% (95% CI, 45–65%). The cumulative incidence of neutrophil (C) and platelet (D) recovery according to disease category is shown.



**Fig 2.** Kaplan–Meier estimates of overall survival after umbilical cord transplantation.

Five of 21 patients with engraftment failure received a second transplantation. Two WAS patients achieved successful engraftment in the second transplantation, while one SCID and two CGD patients did not survive the second transplantation. Only two of the remaining 16 patients who rejected the UCB graft remained alive at the latest data analysis.

*Survival and causes of death*

Of the 88 PID patients who underwent UCBT, 62 remained alive at the latest follow-up. Five-year OS for all patients was 69% (95% CI, 57–78%) (Fig 2), while that for SCID and WAS patients was 71% and 82%, respectively. All five SCN patients

remained alive, although one patient had rejected the graft on day 79 after UCBT. Three of seven CGD patients survived UCBT; this low survival rate may be due to the fact that UCBT was selected in five patients after the first or second failed bone marrow transplantation (BMT). Seven of 14 patients categorized as ‘other diseases’ remained alive at the latest follow-up.

Table II summarizes the survival and causes of death after UCBT. Of the 26 patients who died, 19 had died within day 100 (17 from infection) and seven (SCID, six and congenital CD4 lymphopenia, one) had died within day 28 after UCBT.

Causes of early death ( $\leq 28$  d) were cytomegalovirus (CMV) disease (three patients), *Pneumocystis pneumonia* (one patient), interstitial pneumonia (one patient), bacterial infection (one patient) and veno-occlusive disease (VOD) (one patient). All those who died of CMV disease had CMV pneumonia before transplantation.

The cause of death between days 28 and 100 in the remaining 12 patients was bacterial infection (seven had concomitant fungal infection, one also had VOD and one had CMV disease), CMV disease (two patients), fungal infection (one patient), multiple organ failure (one patient) and VOD (one patient). Four of seven CGD patients died of bacterial or fungal infection without engraftment. Although detailed data on bacterial/fungal infections at the time of transplantation were not collected, all the CGD patients were administered both antimicrobial and antifungal agents at the time of transplantation.

The causes of death after day 100 were GVHD (five patients), Epstein–Barr virus (EBV)-associated post-transplant lymphoproliferative disorder (EBV-PTLD, one patient) and

Table II. Survival and causes of death.

	Cases (N)	Alive (N)	Death (day)			Infection at CBT (N)	Cause of death (<day 100)			Cause of death (≥day 100)	
			<28 (N)	<100 (N)	≥100 (N)		Bac/Fung infection (N)	Viral infection (N)	Others (N)	GVHD (N)	Others (N)
Total	88	62	7	19	7	18	10	7	VOD 3 MOF1	5	PTLD 1 AI 1
SCID	40	29	6	9	2	11	2	6	1 (VOD)	1	1 (AI)
WAS	23	19	0	1	3	1	1	0	0	3	0
CGD	7	3	0	4	0	5	4	0	1 (VOD)	0	0
SCN	5	5	0	0	0	0	0	0	0	0	0
Others	13	6	1	5	2	1	3	1	1 (VOD) 1 (MOF)	1	1 (PTLD)

Bac/Fung infection, bacterial and/or fungal infection. VOD, veno-occlusive disease; MOF, multiple organ failure; AI, adrenal insufficiency; PTLD, post-transplant lymphoproliferative disorder. Cause of death total does not equal the number of deceased patients because one patient died of VOD and bacterial infection.

adrenal insufficiency (one patient). None of the other patients died of infection after day 100.

### GVHD

All but five patients in the present study received either CyA- or tacrolimus-based immunosuppressant prophylaxis for GVHD. The cumulative incidence of grade 2–4 acute GVHD at day 100 was 28% (95% CI, 19–38%), and that of grade 3–4 GVHD was 8% (95% CI, 4–15%) (Fig 3A, D).

The incidence of grade 2–4 GVHD was higher in patients who underwent 2- or 3-antigen-mismatched UCBT compared with those who underwent HLA-matched or HLA-1-antigen-mismatched UCBT, but it was not statistically significant ( $P = 0.071$ ) (Fig 3B). On the other hand, no difference was observed in the incidence of grade 3–4 GVHD between <2-antigen-mismatched and >2-antigen-mismatched transplants (Fig 3E), although grade 3–4 GVHD was not observed by high-resolution DNA typing in patients who underwent genotypically HLA-matched transplantation.

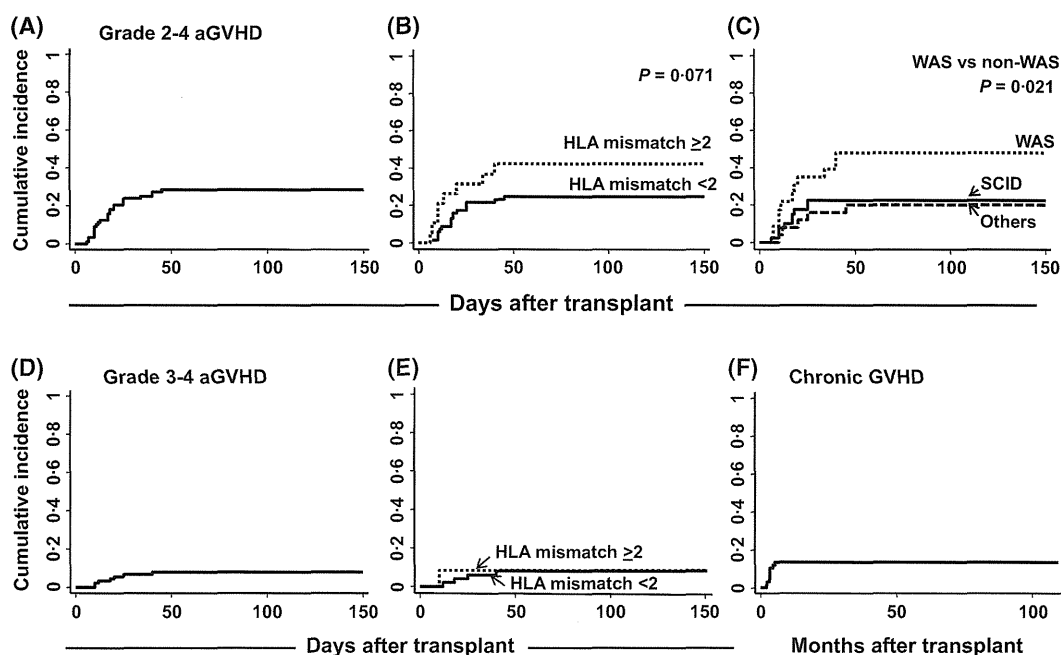


Fig 3. Cumulative probability of acute and chronic GVHD after UCBT. The cumulative incidence of grade 2–4 acute GVHD (aGVHD) at day 100 was 28% (95% CI, 19–38%) (A). The incidence was higher in transplantation mismatched for  $\leq 2$  antigens (B) and in that for WAS patients (C). The cumulative incidence of grade 3–4 acute GVHD at day 100 was 8% (95% CI, 4–15%) (D) and the incidence was not different between patients undergoing transplantation for  $\geq 2$ -antigen mismatched transplant and those undergoing <2-antigen mismatched transplant (E). The cumulative incidence of chronic GVHD at day 180 was 13% (95% CI, 7–23%) (F).



When focusing on differences among the disease groups (Fig 3C), a significantly higher incidence of grade 2–4 GVHD was observed in WAS patients than in non-WAS patients,  $P = 0.021$ . In addition, three of five WAS patients who developed grade 3–4 GVHD died of either GVHD (two patients) or VOD (one patient).

Chronic GVHD was observed in nine patients, and its cumulative incidence at day 180 was 13% (95% CI, 7–23%) (Fig 3F).

### Infections

Twenty-eight patients (SCID, 11; WAS, eight; CGD, three and other diseases, six) developed bacterial infection after UCBT. Sixteen of the 28 patients remained alive at the time of data collection.

Fungal infection mainly caused by *Aspergillus* species was observed in eight patients (CGD, three; SCID, two; WAS, two and X-linked hyperIgM syndrome, one). Three of the eight patients died of bacterial infection, bacterial/fungal infection or GVHD.

Twenty patients (SCID, eight; WAS, four; CGD, two; SCN, two and others, four) developed CMV disease after UCBT. CMV was detected before conditioning in all eight SCID patients of which four patients died of CMV disease after transplantation. Twelve of the 20 patients remained alive at the time of analysis.

Other notable virus-related complications were respiratory syncytial virus bronchiolitis accompanied by chronic GVHD in one SCID patient and EBV-PTLD in one patient with Blau syndrome; both infections led to a fatal outcome. One WAS patient had severe haemorrhagic colitis caused by Coxsackie virus B infection, which was treated successfully by infusion of expanded CD4 T cells prepared from the infusion residual of donor cord blood (Tomizawa *et al*, 2005). Another WAS patient had persistent norovirus infection. Interstitial pneumonia not due to CMV or *Pneumocystis* was noted in three patients of which one patient had parainfluenza/rhinovirus infection, while the causative agent for infection in the remaining two patients was not identifiable.

### Risk factors for overall mortality

Lastly, we analysed the factors contributing to overall survival. Using univariate analyses, the following were found to be significant contributory factors to a poor prognosis: HLA mismatch of  $\geq 2$  antigens, time to transplant  $> 180$  d, second or third transplantation, ongoing infection at the time of transplantation, no conditioning for UCBT and diagnosis other than SCID, SCN or WAS (Table III). The dose of transfused nucleated cells or CD34-positive cells did not affect the 5-year OS.

Using multivariate regression analyses, the following were found to be significant contributory factors to patient death: infection at the time of transplantation, no conditioning, HLA

Table III. Univariate analyses of factors that contributed to 5-year OS.

Factors	Hazard ratio	95% CI	P-value
Age: $\geq 12$ months	1.73	(0.78–3.83)	0.175
Diagnosis			
WAS and SCN	1.00		
SCID	2.34	(0.75–7.36)	0.145
Other diseases	5.39	(1.70–17.0)	0.004*
Nucleic cell dose: $\geq 8.2 \times 10^7$ /kg	1.51	(0.69–3.29)	0.299
CD34 cell dose: $\geq 2.1 \times 10^5$ /kg	0.86	(0.36–2.08)	0.744
HLA disparity			
6/6 matched	1.00		
5/6 matched	1.68	(0.58–4.83)	0.337
4/6 matched	3.78	(1.23–11.60)	0.020*
3/6 matched	3.24	(0.63–16.74)	0.160
4/6 or 3/6 matched	2.64	(1.20–5.83)	0.016*
Time to transplant: $\geq 180$ d	1.89	(0.85–4.17)	0.117
Infection at transplant	6.24	(2.61–14.9)	$< 0.0001^*$
Second or third transplantation	3.37	(1.26–9.02)	0.016*
Conditioning			
MAT	1.00		
RIC	0.41	(0.13–1.23)	0.111
No conditioning	2.89	(1.21–6.93)	0.017*

\*Significant contributory factors to the poor prognosis.

mismatch of  $> 2$  antigens and diagnosis other than SCID, SCN or WAS (Table IV). RIC was determined to be the favourable factor for patient survival ( $P = 0.01$ ) (Fig 4 and Table IV).

### Discussion

This paper reports the outcome of UCBT for 88 PID patients, the largest cohort of PIDs to receive UCBT to date. The overall survival rate for PID patients undergoing UCBT was comparable to that previously reported for 46 Japanese PID patients undergoing BMT from either HLA-identical siblings or unrelated donors (Sakata *et al*, 2004), and also to that reported by the European Society of Immunodeficiency and other stem cell transplantation centres for PID patients receiving BMT from HLA-matched related donors, HLA-mismatched related donors or unrelated donors (Antoine *et al*, 2003; Rao *et al*, 2005; Dvorak & Cowan, 2008). The time for haematopoietic recovery was comparable to or better than the median recovery time observed in a large cohort of UCBT in children with haematopoietic disorders (Thomson *et al*, 2000; Michel *et al*, 2003) and in adults with leukaemia (Laughlin *et al*, 2004; Atsuta *et al*, 2009). The incidence of grade 2–4 GVHD (28%) in UCBT was lower compared with that reported in unrelated donor BMT in PID patients in Japan (47%) (Sakata *et al*, 2004), with that reported in BMT in 90 SCID patients (34%) (Neven *et al*, 2009) and with that observed in the studies of UCBT for childhood haematological malignancies (Thomson *et al*, 2000; Michel *et al*, 2003; Sawczyn *et al*, 2005). The incidence of chronic GVHD (13%) after UCBT was slightly

Table IV. Multivariate analyses of factors that contributed to 5-year OS.

Factors	HR	95% CI	P-value
<b>Diagnosis</b>			
WAS and SCN	1.00		
SCID	1.71	(0.39–7.38)	0.475
Other diseases	7.50	(2.06–27.19)	0.002*
<b>HLA disparity</b>			
6/6 matched	1.00		
5/6 matched	1.53	(0.50–4.66)	0.454
4/6 matched	5.64	(1.66–19.14)	0.006*
3/6 matched	1.04	(0.68–23.96)	0.124
4/6 or 3/6 matched	3.87	(1.63–9.19)	0.002*
<b>Infection at transplant</b>			
4.61		(1.74–12.16)	0.002*
<b>Conditioning</b>			
MAT	1.00		
RIC	0.20	(0.06–0.69)	0.011†
No conditioning	4.87	(1.79–13.3)	0.002*

\*Significant contributory factors to an unfavourable prognosis.

†Significant contributory factors to a favourable prognosis.

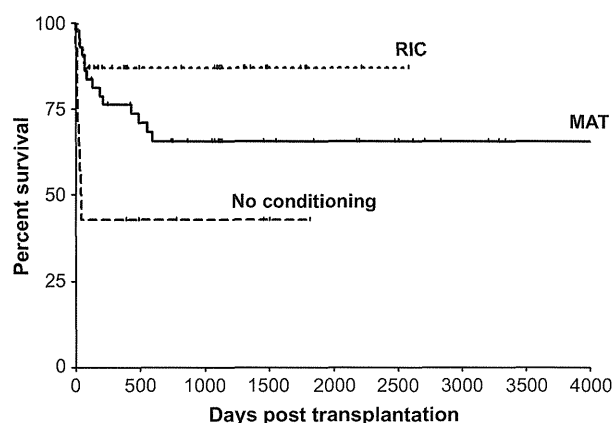


Fig 4. Kaplan–Meier estimates of overall survival after umbilical cord transplantation. Comparison of overall survival between reduced intensity conditioning (RIC), myeloablative therapy (MAT), and no conditioning is shown. For 5-year OS, MAT versus RIC,  $P = 0.111$ , MAT versus no conditioning,  $P = 0.017$  in univariate analysis.

lower than that after URBMT in PID patients in Japan (20%) (Sakata *et al*, 2004), and was lower compared to that in UCBT studies for childhood leukaemia (Michel *et al*, 2003; Sawczyn *et al*, 2005). Thus, UCBT in PID patients in the present study was associated with a good survival rate, good engraftment rate, rapid haematological recovery and a lower incidence of acute and chronic GVHD.

Given that the 5-year OS for SCID patients (71%) was better than that for SCID patients receiving bone marrow from HLA-mismatched related donors in both Japan (5-year OS, 36%, Imai, Morio, Kamachi, Kumaki, Ariga, Nonoyama, Miyawaki, and Hara, unpublished observations) and Europe (5-year OS, 52%, Antoine *et al*, 2003), UCBT would be particularly

beneficial for patients requiring rapid access to donor units yet lacking a matched related donor.

The present study found that several key risk factors were associated with overall mortality. First, infection was the major cause of mortality during the first 100 d after UCBT in PID patients, and the frequency was much higher than that observed in other disorders following UCBT (Rocha & Gluckman, 2006; Kurtzberg *et al*, 2008, Szabolcs *et al*, 2008). As predicted and reported in previous studies (Antoine *et al*, 2003; Cuvelier *et al*, 2009), infection at the time of transplantation was associated with poor survival ( $P < 0.0001$ ), suggesting that the control of pre-existing infection at the time of UCBT is critically important.

Eight of 11 SCID patients who had active infection, mainly CMV pneumonia, died before day 50, while 26 of 28 patients without infection at the time of UCBT remained alive at the time of data collection. UCBT without conditioning was selected for 12 patients, of which seven had CMV infection and one had *Pneumocystis* pneumonia at the time of transplantation. Six out of the seven patients died of CMV infection; and one patient with *Pneumocystis* pneumonia did not survive UCBT.

UCBT in WAS patients achieved a good 5-year OS, as reported in a previous study of 15 cases (Kobayashi *et al*, 2006). One of the key factors would have been the time from diagnosis to transplantation. In our WAS patients, UCBT was performed at a median age of 14 months (range, 4–84 months), when most patients were thrombocytopenic, but did not yet have uncontrolled infection or autoimmunity.

Four CGD patients died of bacterial or fungal infection without engraftment. Although these patients were not categorized as those with active infection at the time of transplantation, they required intravenous administration of antimicrobial and antifungal agents before and after transplantation.

Second, HLA disparity was a risk factor associated with overall mortality. Lower survival was observed in UCB recipients transplanted with a  $\geq 2$  antigen-mismatched graft compared with those transplanted with a  $< 2$  antigen-mismatched graft [Hazard Ratio (HR) = 3.87,  $P = 0.002$ ]. Although no difference was observed in 5-year OS between recipients of HLA-matched and those of HLA 1-antigen mismatched UCBT in the present study, we would need data from a larger number of patients with information on more extensive and sensitive HLA typing to discuss the impact of fully matched HLA on transplant outcome.

Finally, non-SCID/SCN/WAS patients showed a significantly lower survival rate (HR = 5.40,  $P < 0.0001$  by multivariate analyses). Although a previous large-scale study showed that results of HSCT according to disease did not show obvious disease-specific findings (Antoine *et al*, 2003), it is not yet known if UCBT is suitable for all types of PIDs. This may indicate donor source other than UCB is preferable for certain types of PID. Although the success of UCBT noted for X-linked hyperIgM syndrome, bare lymphocyte syndrome and

X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency (Tono *et al*, 2007) is encouraging, optimization of transplantation procedures and determination of suitable timing for UCBT may be necessary for this group of patients. Alternatively, this may simply indicate an expansion of transplantation to less favourable clinical conditions or to less favourable transplantation conditions. Studies on a larger cohort are necessary for drawing any conclusion on whether diagnosis is significant overall.

Recent studies suggest improved survival after BMT for PID with the RIC regimen; however, to date, comparison of CBT using RIC *versus* MAT has not been made. In our study, 87% of patients on the RIC regimen and 66% on the MAT regimen remained alive at the latest follow-up. Multivariate analyses revealed that the RIC regimen is associated with a higher 5-year OS than the MAT regimen (HR = 0.20,  $P = 0.011$ ). Although it is premature to conclude that RIC provides an equal or superior outcome to MAT for all PID patients, non-myeloablative treatment may be beneficial at least for certain types of PID. RIC was selected preferentially in SCID and CGD patients, with good survival rates: 17 of 18 SCID patients and three of four CGD patients remain alive. As a result of this, we are in the process of initiating a clinical trial of UCBT with RIC in SCID patients. On the other hand, only two of 23 WAS patients received RIC. Our previous data showed that a conditioning regimen other than BU/CY or BU/CY/ATG was the only independent factor associated with failure in HSCT for WAS patients (Kobayashi *et al*, 2006). However, whether this holds true for UCBT in younger WAS patients should be determined.

Notably, although the outcome of UCBT for WAS in this cohort was excellent compared with that from previously reported HSCT results using different donor sources (Kobayashi *et al*, 2006; Friedrich *et al*, 2009), UCBT in WAS patients was associated with a high rate of grade 2–4 acute GVHD (11 of 23 patients) and a post-transplant infectious episode (13 of 23 patients). Eight patients experienced bacteraemia/sepsis and six suffered a viral infection (CMV pneumonia, four; Coxsackie virus enterocolitis, one and persistent norovirus infection, one). The high rate of serious infections and GVHD in WAS patients after transplantation warrants further study in search of preventive measures that might include RIC for severe, transplantation-related toxicities.

Long-term follow-up of the clinical and immunological status is necessary when considering the lifespan of PID patients. Recent studies on the long-term outcome after HSCT

for SCID revealed the presence of relatively late complications, such as chronic GVHD, autoimmune events, severe or recurrent infections, chronic human papilloma virus infection, nutritional problems and late rejection in 50% of patients (Mazzolari *et al*, 2007; Neven *et al*, 2009). Similarly, long-term follow-up of HSCT in WAS patients revealed that 20% of patients developed chronic GVHD-independent autoimmunity (Ozsahin *et al*, 2008). One possible measure that might be taken to avoid the chronic problems associated with CBT would be to select a HLA-matched UCB unit, as HLA disparity was a risk factor for both overall survival and the development of GVHD in our study. The advantage of RIC over MAT in preventing late complications needs careful assessment, together with data on mortality, engraftment and early post-transplant complications.

Finally, the issue of SCID patients who died before or without receiving SCT, most likely due to uncontrolled infection, still remains unresolved. This suggests that the early diagnosis of SCID and prevention of opportunistic infection within a protected environment and the administration of appropriate prophylactic drugs is critically important for the improvement of survival in SCID patients in general. To that end, neonatal screening with the employment of T cell receptor excision circles should be beneficial for an improved outcome in SCID patients (McGhee *et al*, 2005; Morinishi *et al*, 2009).

We report the results of UCBT for 88 PID patients in Japan. Despite the limitations of a retrospective, non-randomized study, our study suggests that unrelated umbilical cord blood can be considered as a promising stem cell source for children with congenital immunodeficiency when a HLA-matched related donor is not available.

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# Clinical and Host Genetic Characteristics of Mendelian Susceptibility to Mycobacterial Diseases in Japan

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## Abstract

**Purpose** The aim of this study is to investigate clinical characteristics and genetic backgrounds of Mendelian susceptibility to mycobacterial diseases (MSMD) in Japan.

**Methods** Forty-six patients diagnosed as having MSMD were enrolled in this study. All patients were analyzed for the *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* gene mutations known to be associated with MSMD.

**Results** Six patients and one patient were diagnosed as having partial interferon- $\gamma$  receptor 1 deficiency and nuclear factor- $\kappa$ B-essential modulator deficiency, respectively. Six of the seven patients had recurrent disseminated

mycobacterial infections, while 93% of the patients without these mutations had only one episode of infection.

**Conclusions** The patients with a genetic mutation were more susceptible to developing recurrent disseminated mycobacterial infections. Recurrent disseminated mycobacterial infections occurred in a small number of patients even without these mutations, suggesting the presence of as yet undetermined genetic factors underlying the development and progression of this disease.

**Keywords** Disseminated mycobacterial infection · IFN- $\gamma$ R1 deficiency · NEMO deficiency · flow cytometric analysis

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## Introduction

Although the outcome of mycobacterial infection is influenced by many factors, including the virulence of the pathogen and the environment of the host, it has been demonstrated that host genetic factors play important roles in the defense against mycobacteria [1]. Mendelian susceptibility to mycobacterial diseases (MSMD, MIM 209950) is a rare primary immunodeficiency syndrome characterized by a predisposition to develop infections caused by weakly virulent mycobacteria, such as *Mycobacterium bovis* bacille Calmette-Guerin (BCG) and environmental non-tuberculous mycobacteria (NTM) [2–4]. These patients are vulnerable to systemic salmonellosis and infections with *Mycobacterium tuberculosis*, the virulent mycobacterial species, to a lesser extent [5, 6]. Diseases caused by other intracellular pathogens, such as *Nocardia*, *Listeria*, *Paracoccidioides*, *Histoplasma*, and *Leishmania*, and some viruses, such as human herpes virus-8, have only rarely been reported, mostly in single patients [7–12].

To date, interferon (IFN)- $\gamma$  receptor 1 (*IFNGR1*) [13–15], IFN- $\gamma$  receptor 2 (*IFNGR2*) [16], interleukin (IL)-12 p40 subunit (*IL12B*) [17], IL-12 receptor  $\beta$  subunit (*IL12RB1*) [18–20], signal transducer and activator of transcription-1 (*STAT1*) [21], and nuclear factor- $\kappa$ B-essential modulator (*NEMO*) [22] mutations were identified as the causes of this primary immunodeficiency. On the other hand, no genetic etiology has yet been reported to be identified for about half of all patients with MSMD [3]. In addition, there have been no precise reports on the clinical characteristics and genetic backgrounds of MSMD in Asian countries, including Japan, which has a high prevalence of tuberculosis.

In this study, we analyzed patients who had a recurrent or disseminated infection with intracellular pathogens to clarify the clinical manifestations and host genetic backgrounds of MSMD in Japan.

## Materials and Methods

### Subjects

We studied 46 patients (30 males and 16 females) diagnosed as having MSMD because of recurrent infections, or blood-borne infections such as osteomyelitis/arthritis, and multiple infections at different anatomic sites by intracellular bacteria including BCG, NTM, *Salmonella* species, *Listeria monocytogenes*, or *M. tuberculosis* in 34 hospitals in Japan from 1999 to 2009. There was no consanguinity in these families. The clinical information on each patient was collected using a standardized case report form. Informed consent was obtained from the parents of the subjects before the study. This study was approved by the Ethics Committee of Kyushu University.

### Flow Cytometric Analysis

Two-color flow cytometric analysis was performed to investigate IFN- $\gamma$  receptor 1 (IFN- $\gamma$ R1) expression levels on the patients' monocytes by using an EPICS XL instrument (Beckman Coulter, Miami, FL, USA). Peripheral blood mononuclear cells (PBMCs) were stained with mouse anti-IFN- $\gamma$ R1 monoclonal antibody (MAb) (Genzyme, Cambridge, MA, USA), followed by rat phycoerythrin anti-mouse immunoglobulin antibody (BD Bioscience Pharmingen, San Diego, CA, USA). Cells were washed twice and stained with a phycoerythrin 5.1 (PC5)-anti-CD14 MAb (Beckman Coulter). IFN- $\gamma$ R1 expression was analyzed on monocytes determined by their side scatter and CD14 positivity.

### Genomic DNA and cDNA Sequence Analysis

The *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* genes were analyzed for coding exons and flanking intronic

sequences. These genes were amplified by polymerase chain reaction (PCR) after whole genome amplification with a GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Little Chalfont, UK). The PCR products were treated with an Exo-SAP-IT kit (GE Healthcare, Amersham, UK) and then were analyzed by direct sequencing with an ABI 3130 DNA sequencer (Perkin-Elmer, Foster City, CA, USA). Detected mutations were confirmed by sequencing the PCR product using cDNA as a template.

### Statistical Analysis

Comparisons of the proportions were analyzed by the  $\chi^2$  test. The Mann–Whitney *U* test was used to compare differences between quantitative variables. A *P* value less than 0.05 was considered to be statistically significant.

## Results

The median age of the patients was 8 years (range, 6 months–41 years), and the median age at the onset of infection was 1 year and 4 months (range, 4 months–6 years). The male to female ratio was 1.9:1. Only one patient had not received a BCG vaccination. There were 59 episodes of disseminated mycobacterial infections in the 46 patients. Nine (19%) of 46 patients had two or more episodes of these infections. Two of the patients had three episodes, and one had four episodes of these infections. In all episodes, BCG was the most common pathogen (82.6%, Table I). The *Mycobacterium avium* complex (MAC) was isolated during eight episodes of these infections. *M. tuberculosis* was also confirmed in two episodes of infection. No severe *Salmonella* species, *L. monocytogenes*, or viral infections were observed.

The common clinical manifestations were osteomyelitis/arthritis, lymphadenitis, and subcutaneous abscess/dermatitis (Table I and Fig. 1a). Only one patient was diagnosed as having arthritis, and the lesion spread to the adjacent bone. Two patients showed hepatosplenomegaly during the BCG infection, and two patients with the MAC infection developed pulmonary abscess. Among the BCG infections, the median intervals of time between BCG vaccination and the development of primary BCG infection were 3 (1–10 months), 4 (2–36 months), and 11 months (5–46 months) for the subcutaneous abscess/dermatitis, lymphadenitis, and osteomyelitis/arthritis, respectively (Fig. 1b).

We performed the genetic analysis on these patients for the *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* genes. Six patients (five families) and one patient had mutations in the *IFNGR1* and *NEMO* genes, respectively (Table II). Five of the seven patients who had a mutation in the *IFNGR1* gene were the patients that we

**Table I** The clinical manifestations of the patients with MSMD

	Patients with genetic mutation, n (%)	Patients without a genetic mutation, n (%)	Total n (%)
<b>Causative pathogen<sup>a</sup></b>			
BCG	3 (42.9)	35 (89.7)	38 (82.6)
<i>M. avium</i> complex	1 (14.3)	3 (10.2)	4 (8.7)
BCG+ <i>M. avium</i> complex	2 (28.5)	0 (0)	2 (4.3)
<i>M. avium</i> complex+ <i>M. tuberculosis</i>	1 (14.3)	1 (2.6)	2 (4.3)
<b>Sites of infection<sup>b</sup></b>			
Osteomyelitis/arthritis	7 (43.8)	24 (55.8)	31 (52.5)
Lymphadenitis	8 (50.0)	8 (18.6)	16 (27.1)
Dermatitis/subcutaneous	3 (18.8)	11 (25.6)	14 (23.7)
Pulmonary abscess	0 (0)	2 (4.7)	2 (3.4)

The total number exceeds 59 because some patients had multiple lesions at the same time

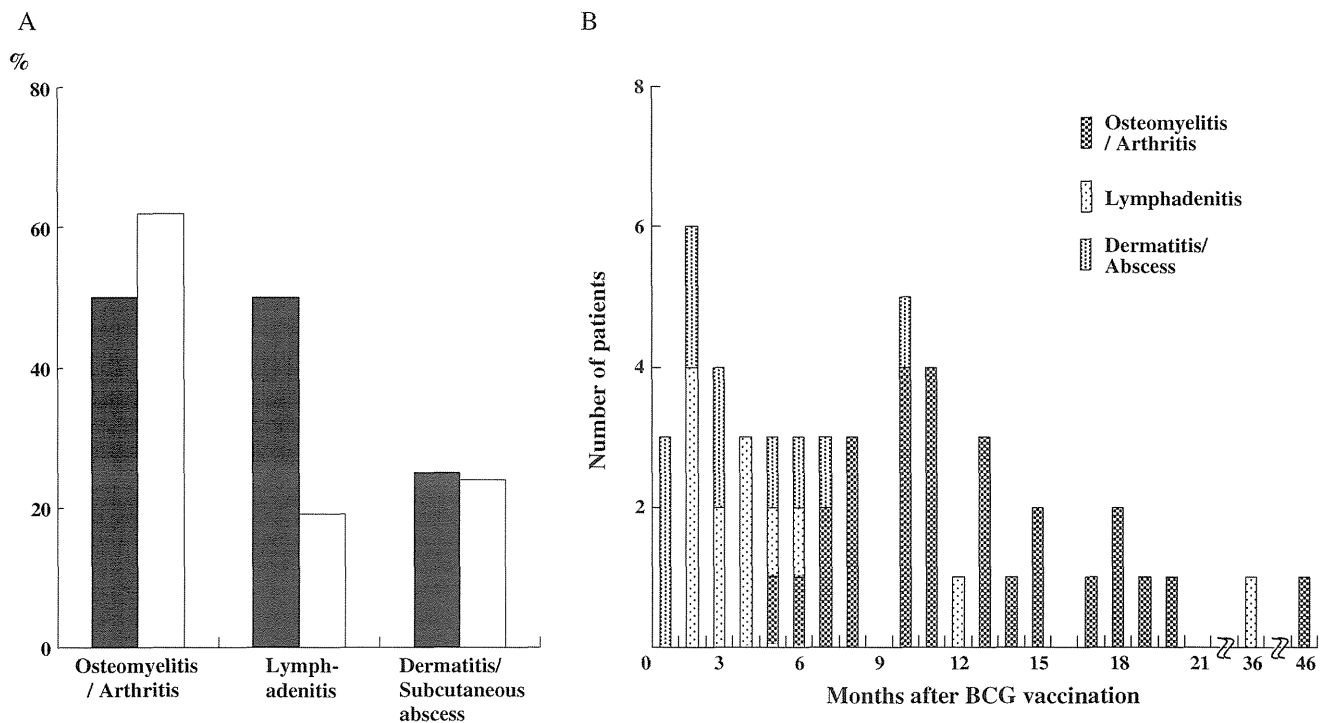
<sup>a</sup>n=7 for patients with a genetic mutation and n=39 for patients without a genetic mutation

<sup>b</sup>n=16 for patients with a genetic mutation and n=43 for patients without a genetic mutation

reported previously [14, 15], and the other two patients were newly identified. All of the IFN- $\gamma$ R1-deficient patients were heterozygotes, and the mutation was in the transmembrane domain in one patient (774del4: patient 5) and in the intracellular domain in five patients (811del4: patient 1, 818del4: patients 2–4, and 832 G>T, E278X: patient 6), which led to the expression of a truncated protein with a dominant negative effect on the IFN- $\gamma$ R1 signaling (Table II and Fig. 2a). The IFN- $\gamma$ R1 expression

levels were significantly increased in all six patients with IFN- $\gamma$ R1 deficiency (Fig. 2b). Patient 7 had a missense mutation in *NEMO* (943 G>C, E315Q). The CD14-positive cells from this patient produced a lower level of TNF in response to LPS stimulation (data not shown), which was consistent with the defect in NF- $\kappa$ B signaling.

The proportions of the patients with recurrent mycobacterial infection or multiple osteomyelitis/arthritis were



**Fig. 1** The clinical features of the patients with BCG infection. The distribution of the sites of infections (a) and the intervals between BCG vaccination and the first onset of BCG infection (b) are shown.

The black bar and the white bar represent the proportion of the patients with and without genetic mutations, respectively

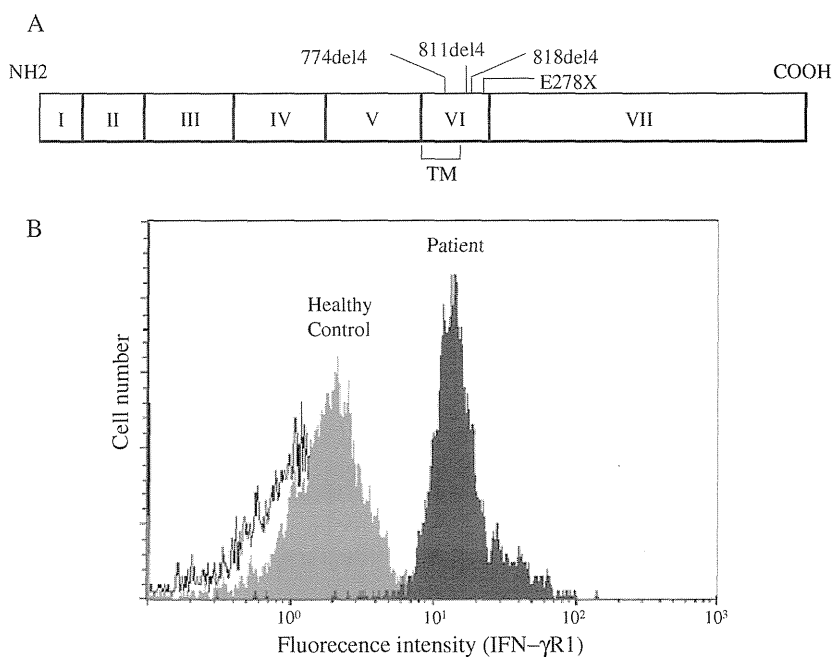


**Table II** Characteristics of the patients with a genetic mutation

Patient no.	Sex	Age	Age of onset	Episodes of infections prior to detection of the genetic mutation	Genetic mutation	
1 <sup>a</sup> [14]	F	1 year 7 months	10 months	BCG lymphadenitis and dermatitis Multiple BCG osteomyelitis	<i>IFNGR1</i> 811del4	
2 <sup>a</sup> [14]	M	1 year 9 months	8 months	BCG lymphadenitis, hepatomegaly Multiple BCG osteomyelitis	<i>IFNGR1</i> 818del4	
3 <sup>a</sup> [14]	M	2 years	2 years	Multiple BCG osteomyelitis	<i>IFNGR1</i> 818del4	
4 <sup>a</sup> [14]	M	41 years	3 years	<i>M. tuberculosis</i> lymphadenitis (twice) Multiple MAC osteomyelitis	<i>IFNGR1</i> 818del4	
5 <sup>a</sup> [15]	F	12 years	6 months	BCG lymphadenitis Multiple MAN osteomyelitis	<i>IFNGR1</i> 774del4	
6	M	19 years	4 months	BCG lymphadenitis and dermatitis Multiple BCG osteomyelitis MAC subcutaneous abscess Multiple MAC osteomyelitis	<i>IFNGR1</i> E278X	
Patient 4 is the father of patient 2 <i>MAC Mycobacterium avium</i> complex <sup>a</sup> These patients were reported previously	7	M	10 years	10 months	<i>M. tuberculosis</i> lymphadenitis Multiple MAC lymphadenitis Sepsis, bacterial pneumonia (four times)	<i>NEMO</i> E315Q

significantly higher in those with the genetic mutations (Table III). There were no significant differences in the age at the onset of mycobacterial infection, or in the interval of time between BCG vaccination and the first onset of BCG infection between the patients with and without genetic mutations. One patient diagnosed with BCG dermatitis died of persistent diarrhea of unknown etiology, while the others are still alive.

**Fig. 2** *IFNGR1* gene mutations and the analysis of IFN- $\gamma$ R1 expression on monocytes. The sites of *IFNGR1* gene mutations in the six IFN- $\gamma$ R1-deficient patients (a) and the increased IFN- $\gamma$ R1 expression level on monocytes in patient 2 are shown (b)



## Discussion

In the present study, we investigated the clinical characteristics and the genetic backgrounds of the patients diagnosed as having MSMD in Japan. We observed that the patients with the genetic mutation were susceptible to developing recurrent mycobacterial infections and multiple osteomyelitis/arthritis, and IFN- $\gamma$ R1 deficiency was the most

**Table III** Comparison of the patients with and without a genetic mutation

	Patients with a genetic mutation ( <i>n</i> =7)	Patients without a genetic mutation ( <i>n</i> =39)
Age of onset (months)	10 (4–36)	14 (4–75)
Male to female ratio	2.5:1	1.8:1
Familial history ( <i>n</i> )	2	0
Median interval between BCG vaccination and the first onset of BCG infection (months)	9.5 (7–15, <i>n</i> =4)	10 (1–46, <i>n</i> =35)
Recurrent cases (%)	85.7*	7.7
Patients with multiple osteomyelitis/arthritis (%)	100* ( <i>n</i> =6)	4.2 ( <i>n</i> =24)

\**p*<0.0001

frequent genetic defect identified in these patients. The prevalence of MSMD is estimated to be at least 0.59 cases per million births, and the disease does not seem to be confined to any ethnic group or geographic region, according to a national retrospective study of idiopathic disseminated BCG infection in France [23, 24]. This is the first epidemiological study associated with MSMD in Japan which showed the difference in the clinical manifestation and the genetic background between Japan and Western countries.

The *IFNGR1* mutations identified in this study were in exon IV, within the transmembrane domain, or the intracellular domain of the *IFNGR1* gene (Fig. 2a), which led to a truncated protein lacking signaling motifs [25]. The truncated protein also lacks the recycling motif, which leads to the overexpression of the mutant protein (Fig. 2b) [25]. These mutations are located in important hot spots in the patients diagnosed with dominant partial IFN- $\gamma$ R1 deficiency [13], and the flow cytometric analysis of IFN- $\gamma$ R1 expression levels may be a useful method for the screening for this disease [15]. The *NEMO* mutation found in patient 7 was in exon VIII within the leucine zipper domain of the *NEMO* gene. A previous study reported that a mutation in this region disrupted a common salt bridge in the leucine zipper domain and impaired T-cell-dependent IL-12 production [22].

The patients with the genetic mutations were susceptible to recurrent mycobacterial infections and multiple osteomyelitis/arthritis as described previously [3], but no fatal mycobacterial infection was observed in this study. Unlike complete IFN- $\gamma$ R1 and IFN- $\gamma$ R2 deficiencies, which often cause fatal mycobacterial infections [13, 16], the patients with dominant partial IFN- $\gamma$ R1 and *NEMO* deficiencies have been reported to have a relatively mild disease and a better prognosis [13, 22]. These factors might have contributed to the good outcome of the patients in this study. In addition, the low virulence of BCG might contribute to the characteristics of BCG infection in Japan, because the BCG Tokyo 172 strain that is used in Japan for vaccination is the least virulent BCG substrain.

The *IL12RB1* mutation has been reported to be the most common cause of MSMD [4]. However, none of the patients in this study was diagnosed as having an IL-12

receptor  $\beta$ 1 deficiency. In Japan, this disease was reported in only one patient with disseminated lymphadenitis caused by *M. avium* complex [18]. It has been suggested that most complete IL-12 receptor  $\beta$ 1-deficient individuals may be asymptomatic, and only those that also have a second mutation in another gene may be more prone to infections [26, 27]. These symptomatic IL-12 receptor  $\beta$ 1-deficient patients are mainly found in families with consanguineous parents [19, 27]. Consanguineous marriages are uncommon in Japan, and there were no consanguineous families in this study. This might be the reason why no IL-12 receptor  $\beta$ 1-deficient patients were observed. Alternatively, it is possible that the causative gene mutations associated with MSMD are different among races, because the number of patients with IL-12 receptor  $\beta$ 1 deficiency was also lower than those with IFN- $\gamma$ R1 deficiency in Taiwan [28].

Although another patient had multiple osteomyelitis, and three patients had recurrent disseminated mycobacterial infections in these studies, they did not have mutations in any of the six genes. It was previously reported that no genetic etiology has yet been identified in about half of patients with disseminated and recurrent mycobacterial infections [3, 4]. This suggests the presence of as yet undetermined genetic factors in the development of this disease.

In the present study, the number of patients with genetic mutations might be too small to conclusively indicate the differences in the clinical manifestations and the host genetic backgrounds of MSMD between Japan and Western countries. However, in terms of the genetic etiology and the prognosis, it remains possible that the features of the patients diagnosed as having MSMD in the present study are different from those in previous reports [3]. Further investigations of a large number of patients are therefore warranted to more precisely evaluate the clinical manifestations and the host genetic background of MSMD in Japan.

## Conclusions

We found that the patients diagnosed as having MSMD in Japan seem to have different genetic features, as well as

different clinical manifestations, compared with those in Western countries. A few patients with recurrent mycobacterial infections without mutations in the six known genes might suggest a contribution of other genetic, as well as environmental, factors in the susceptibility to recurrent infections.

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# Defective IL-10 signaling in hyper-IgE syndrome results in impaired generation of tolerogenic dendritic cells and induced regulatory T cells

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**Hyper-IgE syndrome (HIES) is a primary immunodeficiency characterized by recurrent staphylococcal infections and atopic dermatitis associated with elevated serum IgE levels. Although defective differentiation of IL-17–producing CD4<sup>+</sup> T cells (Th17) partly accounts for the susceptibility to staphylococcal skin abscesses and pneumonia, the pathogenesis of atopic manifestations in HIES still remains an enigma. In this study, we examined the differentiation and function of Th1, Th2, regulatory T cells (T<sub>reg</sub> cells), and dendritic cells (DCs) in HIES patients carrying either *STAT3* or *TYK2* mutations. Although the *in vitro* differentiation of Th1 and Th2 cells and the number and function of T<sub>reg</sub> cells in the peripheral blood were normal in HIES patients with *STAT3* mutations, primary and monocyte-derived DCs showed defective responses to IL-10 and thus failed to become tolerogenic. When treated with IL-10, patient DCs showed impaired up-regulation of inhibitory molecules on their surface, including PD-L1 and ILT-4, compared with control DCs. Moreover, IL-10–treated DCs from patients displayed impaired ability to induce the differentiation of naive CD4<sup>+</sup> T cells to FOXP3<sup>+</sup> induced T<sub>reg</sub> cells (iT<sub>reg</sub> cells). These results suggest that the defective generation of IL-10–induced tolerogenic DCs and iT<sub>reg</sub> cells may contribute to inflammatory changes in HIES.**

Hyper-IgE syndrome (HIES) is a rare complex primary immunodeficiency, characterized by atopic dermatitis, extremely high serum IgE levels, staphylococcal skin abscesses, and pneumonia associated with disproportionately mild inflammatory responses (Grimbacher et al., 2005; Minegishi, 2009). Treatments so far are symptomatic, including the prevention of bacterial and fungal infections and management of eczema. Previous studies suggested the benefit from bone marrow transplantation, Ig replacement, and IFN and G-CSF administration

(Grimbacher et al., 2005), but a general role for immune reconstitution and modulation in HIES is unproven. To improve the long-term quality of life of HIES patients, it is necessary to develop a new treatment strategy based on a better understanding of molecular mechanisms of this syndrome. We recently demonstrated that

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Abbreviations used: cDC, conventional DC; DN, dominant-negative; HIES, hyper-IgE syndrome; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; iT<sub>reg</sub> cell, induced T<sub>reg</sub> cell; MoDC, monocyte-derived DC; mRNA, messenger RNA; nT<sub>reg</sub> cell, natural T<sub>reg</sub> cell; pDC, plasmacytoid DC; Q-PCR, quantitative RT-PCR.

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