

1 mg/kg PAM once every month for a total of 6 months for children with LCH may be a safe treatment.

In addition to having resolved the bone lesions, PAM therapy may have contributed to resolution of the skin and soft tissue lesion(s) of the children in the present study. No previous study has suggested that PAM is efficacious for LCH lesions in sites other than bone. PAM suppresses the activation and longevity of osteoclast-like MGCs, which are known to be present in LCH lesions of the skin and other sites as well as in LCH bone lesions [7]. However, it should be noted that none of the children in the present study had RO involvement at the commencement of PAM therapy. In addition, 2 children from our total cohort of 21 children with LCH who are not described in this report had RO involvement at the commencement of BPs therapy and experienced disease progression despite having received BPs and chemotherapy at disease onset. Children with a first reactivation had a statistically significantly better PFS, which may indicate that patients who experience more than one reactivation might have differing biological characteristics to those who experience no or only one reactivation. These findings suggest that BP therapy may be more effective for LCH children with a first reactivation involving a non-RO site.

In the present study, 9 of the 16 children received MC in addition to PAM. MC is a non-steroidal anti-inflammatory (NSAID) agent and a selective inhibitor of cyclooxygenase-2 (COX-2). NSAIDs block the arachidonic acid-prostaglandin pathway by inhibiting COX. It has been shown that COX and prostaglandins are over-expressed by LCH lesions and that prostaglandin E2 can induce bone resorption [27,28]. Several reports have described the effectiveness of NSAIDs in the treatment of LCH bone lesions [29–32]. The combined administration of MC and PAM did not affect PFS, however, and two children in the present study experienced disease reactivation shortly after the cessation of MC. MC might therefore have acted in an additive or synergistic manner with PAM in the children in the present study.

In conclusion, the intravenous administration of 1 mg/kg PAM once every month for 6 months may be an effective and safe treatment for children with LCH bone lesions. Although the long-term effects of BPs on LCH are unknown, it is possible that by preventing further disease reactivation in bone, PAM therapy may decrease or prevent late effects such as DI without the use of cytoreductive agents. However, a weakness of this study is its retrospective approach. Another weakness was that radiographs were not evaluated by independent radiologists but instead were evaluated by radiologists at each institution. A prospective study of children with reactivated LCH and no RO involvement is warranted to confirm these results, and this should be conducted with careful attention to the development of adverse effects.

ACKNOWLEDGMENTS

The authors thank all of the physicians who participated in this study. This work was supported by a Grant for Clinical Cancer Research and Research on Measures for Intractable Diseases from the Japanese Ministry of Health, Labour, and Welfare.

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ORIGINAL ARTICLE

Clinical features and outcome of X-linked lymphoproliferative syndrome type 1 (SAP deficiency) in Japan identified by the combination of flow cytometric assay and genetic analysis

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To cite this article: Kanegane H, Yang Xi, Zhao M, Yamato K, Inoue M, Hamamoto K, Kobayashi C, Hosono A, Ito Y, Nakazawa Y, Terui K, Kogawa K, Ishii E, Sumazaki R, Miyawaki T. Clinical features and outcome of X-linked lymphoproliferative syndrome type 1 (SAP deficiency) in Japan identified by the combination of flow cytometric assay and genetic analysis. *Pediatric Allergy Immunology* 2012; **00**.

Keywords

flow cytometry; genetic analysis; hematopoietic stem cell transplantation; SLAM-associated protein; X-linked lymphoproliferative syndrome

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Accepted for publication 18 January 2012

DOI:10.1111/j.1399-3038.2012.01282.x

Abstract

Objective: X-linked lymphoproliferative syndrome (XLP) type 1 is a rare immunodeficiency, which is caused by mutations in *SH2D1A* gene. The prognosis of XLP is very poor, and hematopoietic stem cell transplantation (HSCT) is the only curative therapy. We characterized the clinical features and outcome of Japanese patients with XLP-1.

Methods: We used a combination of flow cytometric analysis and genetic analysis to identify XLP-1 and reviewed the patient characteristics and survival with HSCT.

Results: We identified 33 patients from 21 families with XLP-1 in Japan. Twenty-one of the patients (65%) who did not undergo a transplant died of the disease and complications. Twelve patients underwent HSCT, and 11 of these (92%) survived.

Conclusion: We described the clinical characteristics and outcomes of Japanese patients with XLP-1, and HSCT was the only curative therapy for XLP-1. The rapid and accurate diagnosis of XLP with the combination of flow cytometric assay and genetic analysis is important.

X-linked lymphoproliferative syndrome (XLP) is a rare inherited immunodeficiency estimated to affect approximately one in one million males, although it may be under-diagnosed (1). XLP is characterized by extreme vulnerability to Epstein–Barr virus (EBV) infection, and the major clinical phenotypes of XLP include fulminant infectious mononucleosis (FIM) or EBV-associated hemophagocytic lymphohistiocytosis (HLH) (60%), lymphoproliferative disorder (30%),

and dysgammaglobulinemia (30%) (2). In addition, XLP is associated with a variety of other clinical manifestations including vasculitis, aplastic anemia, and pulmonary lymphoid granulomatosis. Patients with XLP often develop more than one phenotype over time.

The responsible gene was first identified as *SH2D1A*/*SLAM-associated protein (SAP)* located in the region of Xq25 (3–5). However, some of the presumed patients with

XLP do not harbor *SH2D1A* mutations, although they are clinically and even histologically similar to XLP patients with *SH2D1A* mutations. A second causative gene that encodes X-linked inhibitor of apoptosis protein (XIAP), namely *XIAP* or *BIRC4* gene, has been identified (6). Patients with XLP-2 (*XIAP* deficiency) sometimes present with splenomegaly and hemorrhagic colitis, but no lymphoma. The *SH2D1A* and *XIAP* genes are close together at Xq25, but the molecular pathogenesis and clinical features of these diseases seem to be distinct (7, 8).

The vast majority of patients with XLP die in childhood; the survival rate is very poor, even with treatment (2). Hematopoietic stem cell transplantation (HSCT) is the only curative therapy for XLP (9, 10). Therefore, rapid definitive diagnosis and immediate treatment are extremely significant for better prognosis and survival of patients with XLP. We previously established the anti-SAP monoclonal antibody (mAb) and applied it to flow cytometric diagnosis of patients with XLP-1 (11). We performed a nationwide survey for XLP-1 with the flow cytometric assay and genetic analysis and identified a total of 33 patients from 21 families with XLP-1 in Japan (11–15). In this study, we elucidated the clinical and genetic characteristics of these patients. Twelve patients with XLP-1 underwent HSCT, and 11 of these (92%) survived. We also describe the outcomes of HSCT in Japan.

Materials and methods

Study subjects

The subjects in this study were largely male patients with FIM or EBV-HLH treated until the end of 2011. In addition, a few male patients with lymphoma or hypogammaglobulinemia with unknown genetic origin were suspected of having XLP. After written informed consent was obtained, 5–10 ml of venous blood was collected into heparin-containing syringes and delivered to the laboratory. Patients and families provided informed consent for genetic analyses in accordance with the 1975 Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the University of Toyama. Several patients were described in our previous reports (11–15).

Flow cytometric analysis of SAP

Flow cytometric analysis of SAP was performed as previously described (11, 12). The peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation and immediately fixed in 1% paraformaldehyde for 30 min at room temperature and then permeabilized in 0.5% saponin for 15 min on ice. To test the expression of SAP in lymphocytes, these cells were incubated with 2 µg/ml anti-SAP mAb, termed KST-3 (rat IgG1) or irrelevant rat IgG1, for 20 min on ice and further stained with a 1:1000 dilution of FITC-labeled goat anti-rat IgG antibody (Zymed, South San Francisco, CA, USA) or Alexa Fluor 488-conjugated goat anti-rat IgG antibody (Molecular

Probes, Eugene, OR, USA) for 20 min on ice. To evaluate SAP expression in CD8⁺ T and NK cells, PBMC were stained with phycoerythrin (PE)-conjugated anti-CD8 and anti-CD56 mAbs (DAKO Japan, Kyoto, Japan), respectively, before cellular fixation and permeabilization. The stained cells were analyzed using a flow cytometer (EPICS XL-MCL; Beckman Coulter KK, Tokyo, Japan).

SH2D1A mutation detection

The *SH2D1A* mutations were detected by direct sequencing as described previously (5, 14). Genomic DNA was purified from PBMC with a QIAamp Blood Kit (Qiagen, Hilden, Germany) and amplified using primers encompassing each exon-intron boundary of the *SH2D1A* genes. The sequencing reaction was carried out using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with an automated ABI PRISM 310 DNA sequencer (Applied Biosystems).

Results

SAP expression in patients with XLP-1

Fresh blood cells were available in 19 patients with XLP-1. All the examined patients demonstrated markedly deficient SAP expression in lymphocytes, especially in CD8⁺ T cells and NK cells (Fig. 1 and Table 1).

SH2D1A mutations

All the mutations including unpublished data are summarized with the clinical data (Table 1). There were three gross deletions (the whole gene and two exons 3 and 4), four nonsense mutations (all Arg55stop), eight missense mutations (Ala3Ser, Tyr7Cys, two His8Asp, Gly27Ser, Asp33Tyr, Ser34Gly and Gly49Val), two small deletions (584delA and 1021delAA), two small insertions (312insG and 545insA), and two splicing anomalies (416C>T and IVS2+1G>A). The substitution of 416C with T revealed an aberrantly spliced cDNA with deletion of the last 22 bases of exon 1, and IVS2+1G>A resulted in skipping of exon 2.

Clinical characteristics of Japanese patients with XLP-1

Eighteen of the 33 patients (55%) had FIM or EBV-HLH, 12 patients (36%) had hypogammaglobulinemia, seven patients (21%) had malignant lymphoma or lymphoproliferative disease, and two patients (P4.2 and P7.2) had lymphocytic vasculitis. One patient (P7.1) had aplastic anemia. Twenty-seven patients (82%) were associated with EBV infection at the disease onset. Two patients (P16.1 and P19.3) presented with non-EBV-HLH. Interestingly, malignant lymphoma and lymphocytic vasculitis in P4.2 were not associated with EBV infection, but the patient later developed EBV-HLH at the age of 14 yr and died of HLH. Two patients (P17.2 and P21.1) had encephalitis; and P17.2 developed acute disseminated encephalomyelitis caused by human

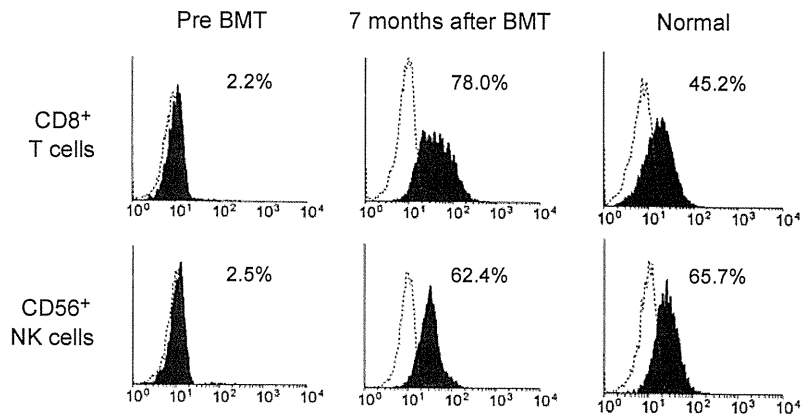


Figure 1 The SAP expression in CD8⁺ T cells and NK cells from the patient (P16.1) and a normal adult donor. Dotted lines and shaded areas indicate staining by the control antibody and anti-SAP mAb (KST-3), respectively. A flow cytometric analysis demonstrated that deficient SAP expression in CD8⁺ T cells and NK cells from the patient increased after he had undergone hematopoietic stem cell transplantation.

herpes virus 6 infection and P21.1 developed EBV encephalitis. Approximately 70% of the patients (23 of 33) were diagnosed by the time they were 5 yr of age, but two patients (P13.1 and P20.1) were diagnosed in adulthood. Eleven families (52%) had X-linked family histories. Ten patients (30%) presented with more than one clinical manifestation over time. Ten sibling cases were observed in this study, and seven families manifested different phenotypes. Fifteen patients (45%) were treated with intravenous immunoglobulin replacement therapy. In this study, the mortality rate was 21 of 32 patients (66%), and all the living patients were post-transplanted. Clinical characteristics of this study are summarized in comparison with those of previous study (Table 2).

Hematopoietic stem cell transplantation for patients with XLP-1

Twelve patients with XLP underwent HSCT in Japan (Table 3), and one patient (P9.2) died of *Pseudomonas* sepsis and multiple organ failure 14 days after HSCT. Two patients (P1.2 and P7.2) were transplanted from matched sibling donors, but the other patients were transplanted from matched or one-locus-mismatched unrelated donors, or mismatched familial donors. Various types of conditioning regimen were performed. Five patients (P1.2, P7.2, P9.1, P10.1, and P14.1) underwent HSCT following myeloablative conditioning, but the other patients did so following reduced intensity conditioning (RIC). Acute graft versus host disease (GVHD) was observed in 6 of 11 patients (Grade I, two patients; Grade II, three patients; Grade III, one patient). Chronic GVHD was observed in five patients, among whom 4 (P1.2, P7.2, P10.1, and P18.1) had extensive types and one (P14.1) had a limited type. Eleven patients (92%) have survived and had complete chimerism with a median follow-up of 7 yr and 9 months. A flow cytometric assay could be conducted to evaluate SAP expression in CD8⁺ T cells and NK cells after HSCT in five patients (P7.2, P10.2, P16.1, P17.2, and P18.1). All the patients demonstrated an increase in SAP

expression in CD8⁺ T cells and NK cells after undergoing HSCT (Fig. 1).

Discussion

X-linked lymphoproliferative syndrome is a rare but life-threatening disease. A large cohort showed that most patients with XLP died by the age of 40 yr and more than 70% of the patients died before the age of 10 yr (2). Early diagnosis in non-familial cases may be difficult because XLP is heterogeneous in its clinical presentation. The ability to screen rapidly and make an accurate diagnosis of patients with XLP facilitates the initiation of life-saving treatment and preparation for HSCT. In a previous study, we generated an anti-SAP mAb, termed KST-3, which was applied to the flow cytometric evaluation of SAP deficiency (XLP-1) (11). All the patients evaluated in this study showed deficient SAP expression, although some patients with missense mutations might demonstrate normal expression of SAP, as shown in Western blotting (16).

Various types of *SH2D1A* mutation have been identified in Japan (11–15). The *SH2D1A*base (<http://bioinf.uta.fi/SH2D1Abase>) discloses that 133 unrelated patients were identified to have *SH2D1A* mutations. Missense and nonsense mutations appear in one-quarter each, and other types of mutation appear in half of the patients in this database. In the present study, Arg55stop mutations were most frequently found, in keeping with the *SH2D1A*base. No genotype and phenotype correlation was evident in this study, as well as in previous studies (1, 17).

Large cohort studies have shown that the major clinical phenotypes of XLP include FIM (60%), dysgammaglobulinemia (30%), and malignant lymphoma (30%) (1, 2). Aplastic anemia, lymphoid granulomatosis, and systemic vasculitis are minor clinical presentations at frequencies of approximately 3%. Although the present study included a limited number of patients with XLP-1, the distribution of the clinical manifestations seems to be similar to that in previous large studies

Table 1 Clinical and genetic data of patients with X-linked lymphoproliferative syndrome

Patient ID	Age at diagnosis	Family history	Clinical presentation	Epstein-Barr virus status	IVIg	Outcome	Cause of death	Age at death or presence	<i>SH2D1A</i> mutation	SAP expression
1.1	12 yr	+	Hypo- γ , LPD	+	+	Dead	GVHD	12 yr	NE	NE
1.2	7 yr	+	Hypo- γ , LPD	+	+	Alive*		21 yr	Asp33Tyr	NE
2.1	3 yr	-	FIM	+	-	Dead	FIM	3 yr	Arg55stop	NE
3.1	2 yr	+	FIM	+	-	Dead	FIM	2 yr	Arg55stop	NE
3.2	2 yr	+	FIM	+	-	Dead	FIM	2 yr	Arg55stop	NE
4.1	2 yr	+	FIM	+	-	Dead	FIM	2 yr	416C>T, fs	NE
4.2	4 yr	+	ML, vasculitis, HLH	-	-	Dead	HLH (MOF)	14 yr	416C>T, fs	Deficient
5.1	1 yr	+	FIM	+	+	Dead	FIM	1 yr	del of whole gene	NE
6.1	1 yr	-	FIM	+	-	Dead	FIM	1 yr	Gly27Ser	NE
7.1	1 yr	+	Hypo- γ , aplastic anemia	+	+	Dead	Sepsis	1 yr	NE	NE
7.2	3 yr	+	Hypo- γ , vasculitis	-	+	Alive*		30 yr	His8Asp	Deficient
8.1	1 yr	-	FIM	+	+	Dead	FIM	1 yr	584delA, fs	NE
9.1	6 yr	+	Hypo- γ	+	+	Alive*		18 yr	Arg55stop	Deficient
9.2	6 months	+	FIM	+	+	Dead*	Sepsis	6 yr	Arg55stop	Deficient
10.1	4 yr	+	ML	+	-	Alive*		15 yr	Gly49Val	Deficient
10.2	0 months	+	Healthy	-	-	Alive*		4 yr	Gly49Val	Deficient
11.1	1 yr	+	FIM	+	+	Dead	FIM (MOF)	1 yr	del of exons 3, 4	NE
11.2	2 yr	+	FIM	+	+	Dead	FIM (MOF)	2 yr	del of exons 3, 4	Deficient
11.3	0 month	+	Healthy	-	+	Alive*		9 yr	del of exons 3, 4	Deficient
12.1	12 yr	+	Hypo- γ , ML	+	-	Dead	ML	12 yr	Ser34Gly	Deficient
12.2	10 yr	+	Hypo- γ	+	-	Unknown	Unknown	Unknown	Ser34Gly	Deficient
13.1	23 yr	-	FIM	+	-	Dead	FIM	23 yr	Tyr7Cys	Deficient
14.1	8 yr	-	Hypo- γ , ML	+	-	Alive*		16 yr	Arg55stop	Deficient
15.1	2 yr	-	FIM	+	-	Dead	FIM	2 yr	His8Asp	NE
16.1	10 yr	-	Hypo- γ , HLH	-	+	Alive*		17 yr	545insA, fs	Deficient
17.1	2 yr	+	FIM	+	-	Dead	FIM	2 yr	IVS2+1G>A	Deficient
17.2	2 yr	+	ADEM	-	-	Alive*		8 yr	IVS2+1G>A	Deficient
18.1	6 yr	-	Hypo- γ	+	+	Alive*		12 yr	312insG, fs	Deficient
19.1	10 months	+	Hypo- γ	+	+	Dead	DIC	10 months	NE	NE
19.2	1 yr	+	FIM	+	-	Dead		1 yr	NE	NE
19.3	3 yr	+	Hypo- γ , HLH, ML	+	+	Alive*		18 yr	del of exons 3, 4	Deficient
20.1	41 yr	-	FIM	+	-	Dead	FIM	42 yr	Ala3Ser	Deficient
21.1	3 yr	-	Encephalitis, LPD	+	-	Dead	Encephalitis	3 yr	538insA, fs	Deficient

Hypo- γ , hypogammaglobulinemia; LPD, lymphoproliferative disease; GVHD, graft versus host disease; FIM; fulminant infectious mononucleosis; HLH, hemophagocytic lymphohistiocytosis; MOF, multiple organ failure; ML, malignant lymphoma; ADEM, acute disseminated encephalomyelitis; DIC, disseminated intravascular coagulation; NE, not examined; fs, frameshift; del, deletion; ins, insertion.

P17.1 and 17.2 are monozygotic twins. Asterisk indicates the patients who underwent hematopoietic stem cell transplantation. P1.2, P2.1, P3.1, P3.2, P4.1, P5.1, P6.1, P7.2, P8.1, and P10.1 were described by Sumazaki et al. (14) P5.1 was described by Honda et al. (13) P9.1, P9.2, P11.1, P11.2, P11.3, P12.1, and P12.2 were described by Shinozaki et al. (11) P13.1 was described by Hoshino et al. (15) P16.1, P17.1, P17.2, P18.1, P19.3, and P20.1 were described by Zhao et al. (12). [Correction added on 10 April 2012, after first online publication: the *SH2D1A* mutation of P21.1 has been corrected.]

(Table 2) (2, 17). Lymphoid granulomatosis was not found in Japanese patients, but two patients have presented with systemic vasculitis (18). The vasculitis in these patients mainly affected the brain and was associated with encephalopathy. The mortality was different among clinical phenotypes, and the mortality of each phenotype in our study decreased from that in the XLP registry (2). However, in a recent worldwide study, the mortality associated with HLH decreased to 65%, lymphoproliferative disease to 8%, and dysgammaglobulinemia to 5% (16).

Hematopoietic stem cell transplantation is the only curative treatment for XLP-1. Twenty-one patients with XLP-1

did not undergo HSCT, and these patients died of the disease and complications. The outcome of one patient (P12.2) was unknown. Twelve patients underwent HSCT in Japan, and 11 patients survived. Most of the transplants were performed in different institutions, but the outcomes are similar to previously published data (9, 10, 17). This study revealed that unrelated donors could be used as donors as well as sibling donors. Although various types of conditioning regimen were performed, more than half included RIC regimen, and the result of RIC regimen is similar to that of myeloablative regimen. The RIC regimen should be performed for patients with XLP-1 to avoid regimen-related toxicity or morbidity (17). In

Table 2 Clinical phenotypes of patients with X-linked lymphoproliferative syndrome

Phenotype	Present study (33 cases)		Seemayer (272 cases) (2)		Booth (91 cases) (17)	
	Incidence	Mortality	Incidence	Mortality	Incidence	Mortality
FIM or HLH	18 (55%)	16/18 (89%)	157 (58%)	127/132 (96%)	35.2%	65.6%
ML or LPD	7 (21%)	3/7 (43%)	82 (30%)	46/71 (65%)	24.2%	9.0%
Hypogammaglobulinemia	12 (36%)	4/11 (36%)	84 (31%)	34/75 (45%)	50.5%	13.0%

FIM, fulminant infectious mononucleosis; HLH, hemophagocytic lymphohistiocytosis.

Table 3 Characteristics of HSCTs

Patient ID	Age at HSCT	Donor	Sources	Conditioning regimen	GVHD prophylaxis	Acute GVHD	Chronic GVHD	Outcome
1.2	7 yr	MSD (6/6)	PBSC	TBI/CY	CsA/sMTX	Grade I	Extensive	Alive (14 yr 8 months)
7.2	24 yr	MSD (6/6)	BM	BU/CY/ATG	CsA/sMTX	Grade II	Extensive	Alive (6 yr 6 months)
9.1	8 yr	MUD (6/6)	BM	BU/VP/CY	FK/sMTX	None	None	Alive (10 yr 6 months)
9.2	6 yr	mMFD (3/6)	BM	TBI 6Gy/BU 4 mg/kg	MMF/sMTX/mPSL	NE	NE	Dead (14 days)
10.1	4 yr	mMUD (5/6)	BM	BU/CY/AraC	FK/sMTX	Grade II	Extensive	Alive (11 yr 2 months)
10.2	1 yr	MUD (6/6)	BM	BU/TAI 3Gy/Flu/CY/ATG	FK/sMTX	None	None	Alive (3 yr 3 months)
11.3	8 months	mMUD (5/6)	PBSC	Flu/Mel/ATG/TAI 6Gy	FK/sMTX/mPSL	Grade II	None	Alive (9 yr 2 months)
14.1	10 yr	MUD (6/6)	BM	BU/CY	CsA/sMTX	Grade III	Limited	Alive (8 yr 2 months)
16.1	11 yr	mMUD (5/6)	BM	BU/TAI 3Gy/Flu/CY/ATG	FK/sMTX	None	None	Alive (5 yr 6 months)
17.2	3 yr	mMFD (4/6)	BM	Flu/Mel/TBI 3 Gy	FK/sMTX	Grade I	None	Alive (8 yr 10 months)
18.1	7 yr	MUD (6/6)	BM	Flu/Mel/TBI 3 Gy	FK/sMTX	None	Extensive	Alive (4 yr 7 months)
19.3	15 yr	MUD (6/6)	BM	Flu/Mel/TBI 3 Gy	FK/sMTX	None	None	Alive (3 yr 7 months)

MSD, matched sibling donor; MUD, matched unrelated donor; mMFD, mismatched familial donor; mMUD, mismatched unrelated donor; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; CY, cyclophosphamide; BU, busulfan; ATG, anti-thymoglobulin; VP, etoposide; Gy, gray; AraC, cytosine arabinoside; TAI, total abdominal irradiation; Flu, fludarabine; Mel, melphalan; GVHD, graft versus host disease; CsA, cyclosporine A; sMTX, short methotrexate; FK, tacrolimus; MMF, mycophenolate mofetil; mPSL, methylprednisolone; NE, not evaluated; HSCT, hematopoietic stem cell transplantation.

this study, two patients (P10.2 and P11.3) were diagnosed because of a family history and presented no clinical features of XLP. Their parents wanted them to undergo HSCT because of the poor prognosis of the disease. Although the decision to transplant a relatively well child has been more challenging, these patients underwent transplant and were free from chronic GVHD.

In conclusion, this study verified the clinical usefulness of a flow cytometric assessment of SAP to search for XLP-1 (SAP deficiency). Flow cytometric analysis of XIAP is also useful to detect patients with XLP-2 (7, 19, 20). A male with any of the clinical phenotypes of XLP with or without EBV infection should be initially examined with a flow cytometric assay to evaluate both SAP and XIAP (21). We also identified nine Japanese patients with XIAP deficiency with a combination of flow cytometry and genetic analysis (22). Needless to say, a mutation analysis is the gold standard for confirming a definite diagnosis. The outcome of patients with

XLP-1 seemed to be poor in Japan, and HSCT is the only curative treatment for patients with XLP-1.

Acknowledgments

This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and a grant from the Ministry of Health, Labour, and Welfare of Japan. The authors would like to thank Ms. Chikako Sakai and Mr. Hitoshi Moriuchi for their excellent technical assistance and Dr. Rebecca A. Marsh for critical discussion. We would also like to thank many doctors for providing blood samples and medical records of the patients: Sadao Suga, Akihiro Yachie, Takeshi Shichijo, Tadashi Matsubayashi, Takeshi Taketani, Hiroyuki Moriuchi, Tatsuo Kondo, Takumi Hoshio, Yo Umeda, Mariko Fujimatsu, Junichi Kiyasu, and Takeo Mukai.

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