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	*SH2D1A* 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

	Present study (33 cases)		Seemayer (272 cases) (2)		Booth (91 cases) (17)	
Phenotype	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 methotraxate; 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.

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Allogeneic hematopoietic cell transplantation for XIAP deficiency: an international survey reveals poor outcomes

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Allogeneic hematopoietic cell transplantation for XIAP deficiency: an international survey reveals poor outcomes

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Key Points

- High mortality rates are observed in patients with XIAP deficiency treated with myeloablative conditioning regimens for hematopoietic cell transplantation.

There have been no studies on patient outcome after allogeneic hematopoietic cell transplantation (HCT) in patients with X-linked inhibitor of apoptosis (XIAP) deficiency. To estimate the success of HCT, we conducted an international survey of transplantation outcomes. Data were reported for 19 patients. Seven patients received busulfan-containing myeloablative conditioning (MAC) regimens. Eleven patients underwent reduced intensity conditioning (RIC) regimens predominantly consisting of alemtuzumab, fludarabine, and melphalan. One patient received an intermediate-intensity regimen. Survival was poor in the MAC group, with only 1 patient surviving (14%). Most deaths were from transplantation-related toxicities, including venoocclusive disease and pulmonary hemorrhage. Of the 11 patients who received RIC, 6 are currently surviving at a median of 570 days after HCT (55%). Preparative regimen and HLH activity affected outcomes, and of RIC patients reported to be in remission from HLH, survival is 86% ($P = .03$). We conclude that MAC regimens should not be used for patients with XIAP deficiency. It is possible that the loss of XIAP and its antiapoptotic functions contributes to the high incidence of toxicities observed with MAC regimens. RIC regimens should be pursued with caution and, if possible, efforts should be made to ensure HLH remission before HCT in these patients. (*Blood.* 2013;121(6):877-883)

Introduction

Deficiency of X-linked inhibitor of apoptosis (XIAP) is associated with X-linked lymphoproliferative disease (XLP) and familial hemophagocytic lymphohistiocytosis (FHLH) phenotypes. Traditionally, patients with inherited immune deficiencies that cause HLH have been treated with allogeneic hematopoietic cell transplantation (HCT) because of the life-threatening nature of HLH. There is extensive experience with transplantation in patients with FHLH. Over the past 10 years, survival has generally approximated 60% with myeloablative conditioning (MAC) regimens.[1-7] More recently, improvements have been made with reduced-intensity conditioning (RIC) protocols, and current survival rates are as high as 80%.[8-11] There is less experience with transplantation in patients with XLP because of SLAM-associated protein (SAP) deficiency, but survival is generally accepted to be greater than 70% regardless of the intensity of the conditioning protocol.[12-14]

To date, little has been published concerning the outcomes of HCT for patients with XIAP deficiency. XIAP deficiency was first discovered in 2006,[15] and is associated with XLP, FHLH, and colitis phenotypes.[15-18] Patients with XIAP deficiency are unique compared with patients with the other genetic forms of HLH because, as the name suggests, XIAP is an inhibitor of apoptosis that is widely expressed outside of the immune system.[19] Thymocytes from XIAP-deficient mice have been shown to have normal apoptotic responses to a variety of apoptotic stimuli,[20] but hepatocytes are more sensitive to death induced by treatment with cross-linked Fas ligand.[21] XIAP-deficient mouse embryonic fibroblasts are also more sensitive to death after infection with MHV-68.[22] In addition, there is increasing experience with the use of XIAP inhibitors in conjunction with traditional cancer treatment. In this setting, XIAP inhibitors generally increase the susceptibility

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Table 1. Patient characteristics

Patient no	Age at initial presentation	EBV HLH before HCT	Non-EBV HLH before HCT	HLH not in full remission before HCT	Colitis before HCT	Other	XIAP mutation	Protein expression
1	3 mo	–	+	–	–		1443_1449 delins 24 (P482fsX508)	NE*
2	2 mo	–	+	+	–		1443_1449 delins 24 (P482fsX508)	NE
3	2 mo	–	+	–	–		563 G → A (G188E)	Reduced
4	Asymptomatic (symptomatic brother)	–	–	–	–		563 G → A (G188E)	Reduced
5	15 mo	–	–	–	+	Recurrent enterocutaneous fistulas; multiple episodes of polymicrobial sepsis	608G → A (C203Y)	Reduced
6	9 mo	–	+	–	–		E99KfsX129	Absent
7	9 y	+	–	–	–		497G → T, R166I	NE
8	7 mo	–	+	+	–		1141C → T (R381X)	Reduced
9	Infancy	–	+	–	–		1481 T → A (I494N)	NE
10	4 mo	–	+	+	–		1445 C → G (P482R)	Reduced
11	1 y	+	–	+	–	Repeated infections: pneumonia, otitis media, history of paracentesis, mastoidectomy	1189 delA (I397fsX414)	Absent
12	1 y	+	–	+	–		387_390del (D130fsX140)	Not reported
13	3 mo	–	+	–	–		Gross Deletion Exons 1-5	Absent
14	1 y	–	–	–	+	Recurrent fevers; pneumococcal sepsis.	758 C → G (S253X)	Absent
15	3 y	–	+	–	–	Ventricular septal defect	356_359del (N119fs384)	NE
16	7 y	+	+	–	–		1141C → T (R381X)	Reduced
17	8 y	+	–	+	–		310 C → T (Q104X)	Absent
18	Infancy	–	+	–	–	Liver failure in infancy required liver transplantation; nodular lung disease; positive CMV and fungal elements	Gross deletion exon 6	Truncated (robust detection of a smaller molecular weight protein by Western blot)
19	17 y	+	–	–	–		894_898 del 5 (K299fsX307)	Absent

*Not examined.

of cancer cells to undergo apoptosis.[23,24] Because of the importance of XIAP in preventing apoptosis, patients with XIAP deficiency may be at increased risk of treatment-related toxicities because of increased sensitivity to chemotherapeutic agents.

To investigate whether deficiency of XIAP adversely affects the survival of patients undergoing allogeneic HCT, we conducted an international survey to collect information regarding the transplantation outcomes of patients confirmed to have XIAP deficiency.

Methods

Data collection

Approval for this retrospective study was granted by the Cincinnati Children's Hospital Institutional Review Board. A spreadsheet questionnaire was sent to physicians who provided treatment for patients with XIAP deficiency who underwent allogeneic HCT. Physicians were identified through contact with our center, our review of the literature regarding XIAP deficiency, or a request made to all members of the Histiocyte Society.

Patients

Only patients with a confirmed *XIAP/BIRC4* (baculoviral inhibitor of apoptosis repeat containing protein 4) mutation or with a sibling with a confirmed mutation were included in this study (Table 1), which was conducted in accordance with the Declaration of Helsinki. Supplemental lymphocyte protein analysis was performed in some patients using either Western blot or intracellular flow cytometric analysis.[15-17,25]

Transplantation procedures

Patients received transplantation at centers in the United States (n = 12), Europe (n = 6), and Japan (n = 1) between the years 2001-2011. Transplantation procedures were carried out per institutional standard practices. Conditioning regimens and graft characteristics are listed in Table 2. Conditioning regimens were classified as MAC if they contained an alkylating agent (busulfan) or total body irradiation (TBI) at a dose that would not allow autologous BM recovery.[26] Conditioning regimens were classified as RIC if they did not meet the definition of MAC regimen.[26] If there was uncertainty regarding the intensity of the regimen (n = 1, patient 8), it was classified as an intermediate-intensity regimen. Neutrophil engraftment was considered to be the day the neutrophil count reached 0.5×10^9/L. Engraftment studies were done using either XY FISH for sex-mismatched donors or variable number of tandem repeat analysis for same-sex donors. Mixed chimerism was defined as having 5% or more host-derived cells in the whole blood on more than 1 occasion. Acute and chronic GVHD were assessed by standard criteria.[27,28] Patients received GVHD prophylaxis per institutional standard practices. Other routine transplantation care, such as antimicrobial prophylaxis, IV Ig replacement, and fluid and nutrition supplementation when needed, were also provided per institutional standard practices.

Table 2. Transplantation procedures

Patient no	Age at HCT, y	Type of conditioning	Conditioning regimen	Graft HLA match*	Graft source	Relationship
1	0.42	MAC	Bu, Mel, ATG	5/6	Cord	Unrelated
2	0.58	MAC	Bu, Cy, ATG, Etop	6/6	Cord	Unrelated
3	1	MAC	Bu, Cy, ATG	7/8	BM	Unrelated
4	4	MAC	Bu, Cy, ATG	10/10	BM	Unrelated
5	5	MAC	Bu, Flu, ATG	6/6	Cord	Unrelated
6	10	MAC	Bu, Cy, ATG	6/6	BM	Unrelated
7	14	MAC	Bu, Cy, ATG, Etop	7/8	PBSCs	Unrelated
8	1	Intermediate	TBI (6 Gy), Flu, Cy, Mel (80 mg/m^2)	7/8	Cord	Unrelated
9	0.40	RIC	Alem, Flu, Mel	8/8	BM	Unrelated
10	0.98	RIC	Alem, Flu, Mel	9/10	BM	Unrelated
11	2	RIC	Alem, Flu, Mel	9/10	BM	Unrelated
12	3	RIC	Alem, Flu, Mel	9/10	Cord	Unrelated
13	3	RIC	Alem, Flu, Mel	8/8	BM	Unrelated
14	3	RIC	Alem, Flu, Mel	10/10	BM	Unrelated
15	4	RIC	Alem, Flu, Mel	8/8	PBSCs	Maternal
16	7	RIC	Alem, Flu, Treo, Thio	10/10	PBSCs	Unrelated
17	9	RIC	Alem, Flu, Mel	7/8	BM	Unrelated
18	11	RIC	Alem, Flu, Mel	8/8	BM	Unrelated
19	19	RIC	Alem, Flu, Mel	10/10	BM	Sibling

Bu indicates busulfan; Mel, melphalan; ATG, antithymocyte globulin; Cy, cyclophosphamide; Etop, etoposide; Flu, fludarabine; Alem, alemtuzumab; Treo, treosulfan; Thio, thiotepa; and PBSCs, peripheral blood stem cells.

*Six to 10 alleles (HLA-A, HLA-B, HLA-C, HLA-DRB1, or HLA-DQB1).

Statistical analysis

Survival was analyzed using Kaplan-Meier curves created with XLSTAT 2011 software (Addinsoft). Comparison of survival curves was done using the log-rank test. For multivariate analysis of survival time and the impact of preparative regimen (MAC vs RIC), donor match, (full match vs mismatch), and HLH activity (remission vs nonremission), Cox proportional hazard regression model analysis was used. The patient who received the intermediate-intensity regimen was excluded from these analyses. Statistical significance was considered as $P < .05$.

Results

Patients

Nineteen patients with XIAP deficiency underwent allogeneic HCT between 2001 and 2011 at a median age of 3 years (range, 0.4-19). Patient characteristics before HCT and *XIAP/BIRC4* mutations are listed in Table 1. Approximately one-third of patients had developed EBV-related HLH before HCT, and approximately two-thirds of patients had developed non-EBV HLH before HCT. Six of these patients were reported to have either active HLH or HLH in partial remission just before HCT. Two patients with colitis were diagnosed and treated as having Crohn disease before the diagnosis of XIAP deficiency.

Transplantation procedures

Graft characteristics and conditioning regimens are shown in Table 2. Seven patients received a MAC protocol.[26] Most patients received busulfan, cyclophosphamide, and antithymocyte globulin with or without etoposide (n = 5). The remaining 2 patients received busulfan with either fludarabine or melphalan and antithymocyte globulin. Eleven patients received a RIC protocol.[26] Ten RIC patients received alemtuzumab, fludarabine, and melphalan, and 1 patient received alemtuzumab, fludarabine, treosulfan, and thiotepa. The remaining patient (patient 8) received an intermediate protocol consisting of TBI (6 Gy), fludarabine, cyclophosphamide, and melphalan (80 mg/m^2).

Eleven patients received fully matched related (n = 2) or unrelated (n = 9) grafts based on typing of 6-10 HLA antigens (HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1). Eight patients received a single allele mismatched graft. The stem cell source was BM in 11 patients, cord blood in 5 patients, and peripheral blood stem cells in 3 patients.

Engraftment

All patients engrafted with a median of 15 days (range, 8-22) except for patient 11, who died before engraftment on day +13.

Toxicities

There was a high incidence of conditioning-related toxicities among MAC patients (Table 3). There were 3 cases of hepatic venoocclusive disease (VOD), which contributed to deaths on days +17, +50, and +144 in patients 6, 2, and 1, respectively. Two of these patients also developed pulmonary hemorrhage. One patient (patient 3) developed pulmonary hypertension of uncertain etiology with pulmonary hemorrhage after transplantation and died on day +170. This patient had received MAC after having previously undergone HCT twice with RIC.

There were no cases of hepatic VOD or pulmonary hemorrhage in patients who received RIC. However, 1 patient (patient 11) developed multiorgan failure and cardiac toxicity with asystole and died at day +13. A second patient (patient 15) suffered an unexpected death related to idiopathic pneumonitis and respiratory failure at day +125.

Patient 8, who received the intermediate preparative regimen (consisting of TBI, fludarabine, cyclophosphamide, and melphalan), suffered posttransplantation cytokine storm syndrome with acute respiratory distress syndrome and died on day +22.

GVHD

Three patients developed acute GVHD of grade 2 or greater (Table 3). One additional patient developed acute GVHD after receiving a donor lymphocyte infusion that was administered as an intervention for declining donor contribution to hematopoiesis.

Table 3. Toxicities and complications

Patient no	VOD	Pulmonary hemorrhage	Acute VHD	Pneumonitis or ARDS	Confirmed bacteremia/sepsis	Fungal infection	Viremia with EBV, CMV, adenovirus, or HHV6	BK virus hemorrhagic cystitis
1	+	+	–	NR	NR	NR	NR	NR
2	+	–	–	NR	NR	NR	NR	NR
3	–	+ (shown by autopsy, not clinically)	II	–	+ (*S marcescens*)	–	+ (EBV, adenovirus)	–
4	–	+ (related to fungal septic thrombosis of the pulmonary veins and pulmonary artery)	III	–	–	+ (fungal septic thrombosis of the pulmonary veins and pulmonary artery)	+ (EBV, adenovirus)	+
5	–	–	I	–	+ (*K oxytoca, Enterococcus* sp, *P aeruginosa*)	–	+ (CMV, adenovirus, HHV6)	–
6	+	+	–	–	–	–	–	–
7	–	–	III	+	–	–	–	–
8	–	–	–	+	–	–	–	–
9	–	–	–	–	–	–	+ (adenovirus)	–
10	–	–	– (+ after DLI)	–	+ (*K oxytoca, S maltophilia, P aeruginosa*)	–	–	–
11	–	–	–	+	–	–	–	–
12	–	–	–	–	–	–	–	+
13	–	–	–	–	–	–	+ (adenovirus)	–
14	–	–	–	–	–	–	+ (EBV, CMV)	–
15	–	–	–	+	–	–	–	–
16	–	–	–	–	–	–	+ (adenovirus)	–
17	–	–	I	–	+ (*S aureus*)	–	+ (adenovirus)	–
18	–	–	–	–	+ (*S aureus*)	–	+ (CMV)	–
19	–	–	–	–	–	–	–	–

ARDS indicates acute respiratory distress syndrome; NR, not reported; and DLI, donor lymphocyte infusion.

Two patients developed chronic GVHD (limited, n = 1, and extensive, n = 1).

Infections

Most patients experienced an infectious complication of HCT (Table 3). Common viral complications included EBV viremia (n = 3, all patients received rituximab), CMV viremia (n = 3, all patients received CMV-directed therapy), and adenovirus viremia (n = 7, 4 patients received adenovirus-directed therapy). Other reported viral complications included human herpesvirus 6 (HHV6) viremia and encephalitis (n = 1), varicella zoster (n = 1), and BK virus hemorrhagic cystitis (n = 2).

Reported bacterial infections included pneumonias, bacteremias and episodes of sepsis (n = 5) related to *Serratia marcescens*, *Klebsiella oxytoca*, *Stenotrophomonas maltophilia*, *Enterococcus* sp, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. One patient developed fatal fungal septic thrombosis of the pulmonary veins and pulmonary artery.

Donor contribution to hematopoiesis

Six patients were reported to develop mixed donor and recipient chimerism (< 95% donor cells detected in peripheral blood) at a median of 37 days after HCT. All of these patients had received RIC. Patient 12 was reported to lose the graft by 35 days after HCT. For the remaining 5 patients (patients 9, 10, 13, 18, and 19), the lowest observed donor contributions to hematopoiesis ranged from 13.8%-92%. Three patients received a stem cell boost and/or donor lymphocyte infusion(s). At the time of last follow-up at a median of 867 days after HCT (range, 139-1706), all 5 patients possessed greater than 90% donor contribution to hematopoiesis and remained free of disease.

Survival and outcome

Only 1 of the 7 patients who received MAC is currently surviving, 414 days after HCT (Table 4). The other 6 patients died at a median of 97 days after HCT (range, 17-247) from toxicities and complications including VOD, pulmonary hemorrhage, pulmonary hypertension, GVHD, sepsis, multiorgan failure, and fungal septic thrombosis of pulmonary veins and pulmonary artery with pulmonary hemorrhagic necrosis.

Patient 8, who received an intermediate-conditioning regimen, also died, on day +22, of posttransplantation cytokine storm syndrome with acute respiratory distress syndrome.

Of the patients who received RIC, 6 of 11 are currently alive and well at a median of 570 days after HCT (55%). All but 1 survivor were given a Lansky or Karnofsky score of 100 at the time of last follow-up. Patients 10, 11, 12, 15, and 17 died at a median of 140 days after HCT (range, 13-416). Reported causes of death were heterogeneous and included pneumonitis with respiratory failure, cardiac toxicity with asystole and multiorgan failure, encephalitis, and ongoing CNS HLH (with loss of graft), sepsis, and pneumonia with respiratory failure (Table 4).

The 1-year probabilities of survival for MAC and RIC patients are 14% and 57%, respectively (Figure 1A), with long-term probabilities of survival of 14% and 43%, respectively (Figure 1B).

Influences on survival

We examined the significance of multiple factors known to influence transplantation outcomes including preparative regimen (MAC vs RIC),[11] donor match,[29] and HLH disease status at the time of transplantation.[2-4] HLH disease status at the time of transplantation was based on the judgment of the treating/contributing physician who reported HLH to be in remission, in partial

Table 4. Patient outcomes

Patient no	Follow-up, d	Outcome	Cause of death
1	144	Died	VOD and pulmonary hemorrhage
2	50	Died	VOD and MOF
3	170	Died	Pulmonary hypertension
4	247	Died	Fungal septic thrombosis of pulmonary veins and pulmonary artery with pulmonary hemorrhagic necrosis
5	414	Alive and well; limited skin GVHD	
6	17	Died	Pulmonary hemorrhage, VOD
7	50	Died	GVHD, MOF
8	22	Died	ARDS, posttransplantation cytokine storm syndrome
9	1765	Alive and well	
10	285	Died	Drug-resistant *P aeruginosa* sepsis
11	13	Died	Cardiac toxicity, MOF, asystole
12	140	Died	Encephalitis, HLH with CNS involvement
13	1057	Alive and well	
14	149	Alive and well	
15	125	Died	Pneumonitis and respiratory failure
16	273	Alive and well	
17	416	Died	Pneumonia and respiratory failure; chronic extensive GVHD
18	867	Alive and well	
19	139	Alive and well	

MOF indicates multiorgan failure; and ARDS, acute respiratory distress syndrome.

remission, or active. The patient who received the intermediate-intensity regimen (patient 8) was excluded from the analysis. Although there are a limited number of patients in our series, it is notable that of the surviving patients (n = 7), all were reported to be in remission of HLH at the time of HCT. Of the deceased patients (n = 12), half were reported to be in partial remission or have active disease at the time of HCT. It is also notable that of the 7 surviving patients, all but 1 received grafts from HLA-matched donors, whereas of the 12 deceased patients, only 3 received grafts from HLA-matched donors. Multivariate analysis suggested that MAC regimens and HLH that was not in remission conveyed statistically significant negative influences on survival (Figure 1C and Table 5). Match was significant in univariate analysis (data not shown), but was not significant once controlled for conditioning regimen and HLH remission status. Survival for patients receiving RIC who were reported to be in remission from HLH is 86% (P = .03; Figure 1C).

Because XIAP functions as an inhibitor of apoptosis and is widely expressed, we also sought to determine whether residual protein expression may offer some protective benefit for survival after allogeneic HCT. Twelve patients were reported to have had analysis of XIAP protein expression. Of 5 patients with no detectable XIAP, 2 are alive and well (40%). Of 7 patients with detectable decreased or truncated protein expression, 3 are alive and well (43%). We conclude that in this limited cohort, the presence of detectable XIAP does not appear to confer a survival advantage.

Discussion

Deficiency of XIAP is a newly recognized disorder, and the results of the present study survey reveal that transplantation outcomes overall appear poor compared with the outcomes typically expected of patients with XLP and FHLH. There was a high incidence of conditioning-related toxicity, which may be related to the lack of ubiquitously expressed XIAP and the resultant loss of its antiapoptotic and other functions. In particular, only 1 patient treated with MAC is currently surviving (14%). This is in sharp contrast to the typical survival rates in other forms of HLH, which are generally greater than 50%.[1-7,11-13] There was a preponderance of hepatic VOD and pulmonary hemorrhage in MAC patients. Although VOD has been reported in patients with HLH who undergo MAC regimens, it appears that the 50% incidence of VOD in this series is high compared with previous reports of 20%-30%.[3,4] However, because of the small number of patients included in the present study, it is difficult to conclude definitively that XIAP deficiency predisposes patients to an increased risk of liver and pulmonary toxicity. In addition, a high proportion of MAC patients received grafts from HLA-mismatched donors or had HLH that was not in remission at the time of transplantation, which may have contributed to the poor outcomes. Regardless, based on the poor survival outcomes, MAC protocols should be cautioned against and avoided in patients with XIAP deficiency.

With regard to the RIC cohort, the overall survival of just over half of patients appears to be decreased compared with the relatively high survival rates expected for HLH patients undergoing RIC HCT, which are typically greater than 80%.[10,11] However, the causes of death among the patients with XIAP deficiency were heterogeneous and we found no clear evidence to suggest that the deaths were related to deficiency of XIAP. The survival of RIC patients reported to be in remission from HLH was 86%, and the impact of HLH status was significant. This suggests that RIC transplantation outcomes for patients with XIAP deficiency who are in remission from HLH may be equivalent to that of other forms of XLP and FHLH. Infectious complications were common after HCT in both MAC and RIC patients. These complications do not appear to be increased compared with reports of transplantation outcomes for patients with HLH.[9,11]

Given our findings, the question of whether to pursue allogeneic RIC HCT is somewhat difficult to answer and is further complicated by the limited amount of information regarding outcomes of patients with XIAP deficiency not treated with transplantation. In the largest published series to date (N = 30), approximately 40% of patients with XIAP deficiency died at a mean age of 16 years predominantly because of HLH, colitis, or complications of allogeneic HCT.[30] Overall, the small numbers of patients make it difficult to draw a firm conclusion regarding recommendations for

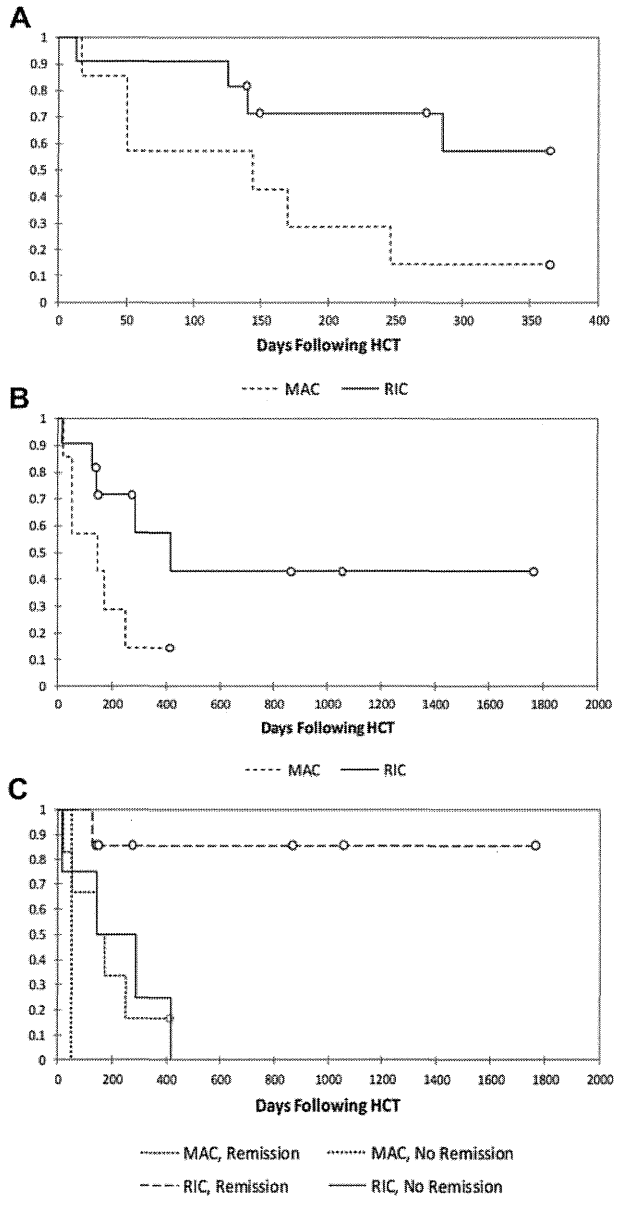

Figure 1. Kaplan-Meier survival analyses. Shown are analyses of 1-year survival (A), long-term survival (B), and survival stratified by reported HLH status at the time of transplantation (C; $P = .035$) in patients treated with MAC or RIC regimens.

RIC HCT for patients with XIAP deficiency. At this time, based on the available information, it is the our opinion that RIC protocols should be pursued with caution in young patients with XIAP deficiency who have a compelling clinical history and for whom a good stem cell donor is available. Preferably, patients should have no active lymphoproliferative disease or HLH and aggressive efforts should be made to ensure remission of HLH. The outcomes of all patients with XIAP deficiency should be monitored to further support evidence-based decisions regarding optimal treatment strategies.

Table 5. Cox proportional hazard regression model analysis

Variable	*P*	HR	HR 95% CI	
A				
Conditioning (MAC vs RIC)	.0251	7.524	1.287	44.000
Match (match vs mismatch)	.2744*	0.471	0.122	1.816
HLH activity (not in remission vs remission)	.0806	4.322	0.837	22.330
B				
Conditioning (MAC vs RIC)	.0181	6.348	1.371	29.394
HLH activity (not in remission vs remission)	.0218	5.301	1.275	22.046

In part A of the table, multivariate analysis included preparative regimen, match, and HLH activity; in part B, the effects of preparative regimen and HLH activity were analyzed with removal of the nonsignificant match effect.

HR indicates hazard ratio; and CI, confidence interval.

*The effect of match was statistically significant in univariate analysis.

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Authorship

Contribution: R.A.M. and K.R. designed the study, collected and analyzed the patient data, and wrote the manuscript; P.K., K.L., I.M., A.F., S.L., P.S., V.B., K.H., H.K., S.M., D.A.M., D.D., J.C., D.N.D., P.J.A., P.V., A.R.K., M.B.J., and J.J.B. collected the patient data and edited the manuscript; D.L. and M.K. performed the statistical analyses; and A.H.F. designed and oversaw the study and edited the manuscript.

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Pediatr Blood Cancer

Wiskott–Aldrich Syndrome Presenting With a Clinical Picture Mimicking Juvenile Myelomonocytic Leukaemia

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Background. Wiskott–Aldrich syndrome (WAS) is a rare X-linked immunodeficiency caused by defects of the WAS protein (*WASP*) gene. Patients with WAS typically demonstrate micro-thrombocytopenia. ***Procedures.*** The report describes seven male infants with WAS that initially presented with leukocytosis, monocytosis, and myeloid and erythroid precursors in the peripheral blood (PB) and dysplasia in the bone marrow (BM), which was initially indistinguishable from juvenile myelomonocytic leukaemia (JMML). ***Results.*** The median age of affected patients was 1 month (range, 1–4 months). Splenomegaly was absent in four of these patients, which was unusual for JMML. A mutation analysis of genes in the RAS-signalling pathway did not support a diagnosis of JMML. Non-haematological features, such as eczema (n = 7) and bloody stools (n = 6), ultimately led to the diagnosis of WAS at a median age of 4 months (range, 3–8 months), which was confirmed by absent (n = 6) or reduced (n = 1) WASP expression in lymphocytes by flow cytometry (FCM) and a *WASP* gene mutation. Interestingly, mean platelet volume (MPV) was normal in three of five patients and six of seven patients demonstrated occasional giant platelets, which was not compatible with WAS. ***Conclusions.*** These data suggest that WAS should be considered in male infants presenting with JMML-like features if no molecular markers of JMML can be detected. Pediatr Blood Cancer

Key words: children; juvenile myelomonocytic leukaemia; Wiskott–Aldrich syndrome

INTRODUCTION

Wiskott–Aldrich syndrome (WAS) is a rare X-linked recessive disorder, characterized by micro-thrombocytopenia, eczematous skin disease, and recurrent infections. The incidence of WAS is 1–10 in 1 million male new-borns. Affected patients have a predisposition to autoimmune diseases and lymphoid malignancies [1,2]. The responsible gene is *WASP*, which encodes the 502 amino acid WASP protein [3]. WASP is expressed selectively in hematopoietic cells and is involved in cell signalling and cytoskeleton reorganization [3]. Specific types of defects in WASP are often but not invariably associated with the severity of disease and clinical phenotype. Lack of WASP expression causes the most severe phenotype (i.e., classic WAS), whereas inactivating *WASP* missense mutations allow residual protein expression and can cause less severe X-linked thrombocytopenia (XLT) [4,5]. Gain-of-function mutations generate X-linked neutropenia (XLN) [6,7].

Juvenile myelomonocytic leukaemia (JMML) is a rare disease in children that occurs with an estimated incidence of 1–2 cases per million [8]. JMML has characteristics of both myelodysplastic syndrome (MDS) and myeloproliferative disorders (MPD) and is categorized in the MDS/MPD category in the World Health Organization (WHO) classification [9–11]. Clinical and haematological manifestations of JMML include hepatosplenomegaly, skin rash, lymphadenopathy, leukoerythroblastosis, monocytosis, and thrombocytopenia. Recent studies show that deregulated activation of the RAS/MAPK signalling pathway plays a central role in the pathogenesis of JMML. Gene mutations in either the *RAS*, *PTPN11*, *NF1*, or *CBL* genes involved in this pathway are detected in about 80% of JMML patients [12–18].

Micro-thrombocytopenia is the key haematological finding in patients with WAS. However, myelopoiesis and erythropoiesis are usually not affected, despite the fact that WASP is expressed in various hematopoietic cells [19]. The present report describes seven cases of male infants with classical WAS who demonstrated haematological abnormalities mimicking JMML. Importantly, patients can present with JMML-like features before the full clinical manifestations of WAS become apparent. Moreover,

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normal mean platelet volume (MPV) and the presence of the giant platelets complicated the diagnostic evaluation in some of our patients.

PATIENTS AND METHODS

Patients

In 2007, we described a case of a male patient (patient #1) with WAS who demonstrated JMML-like clinical features [20]. Briefly, thrombocytopenia was detected shortly after birth. He suffered from bloody diarrhoea from the age of 9 days. At the age of 42 days, leukocytosis with myeloid/erythroid precursors and monocytosis was detected. Bone marrow (BM) aspirates showed hypercellularity with significant predominance of myelopoiesis and dysplastic features. The morphological features were compatible with JMML. Subsequently, the white blood cell (WBC) count increased to 52.0×10^9/L with the appearance of peripheral blasts (3%) and persistent fever. Intravenous administration of various antibiotics had no effect on fever and leukocytosis. Oral 6-mercaptopurine (6-MP) was administered, which resulted in disappearance of leukocytosis. Positive results of cytomegalovirus (CMV)-IgM/IgG and a low level pp65 CMV-antigen (Ag) cells were transitionally noted without CMV-related symptoms. Intravenous administration of ganciclovir (GCV) led to the elimination of CMV-Ag but not to any improvement of JMML-like features. At the age of 7 months, mild atopic dermatitis-like eczema was recognized, which finally led to the clinical and molecular diagnosis of WAS.

The MDS committee of the Japanese Society of Paediatric Hematology/Oncology (JSPHO) study coordinating center of the European Working Group of MDS in Childhood (EWOG-MDS) perform the morphological review of peripheral blood (PB) and BM smears and laboratory examinations for the diagnosis of JMML in Japan and Germany, respectively. By January 2011, WAS was diagnosed in six Japanese males (including patient #1) and one German male who were initially referred with a suspected diagnosis of JMML. Patient #4 was recently reported [21]. Approval for the study was obtained from the institutional review board of Nagoya University, Nagoya, Japan, and University of Freiburg, Freiburg, Germany. Informed consent was provided by parents according to the Declaration of Helsinki.

Diagnostic Tests for Wiskott–Aldrich Syndrome

Intracellular WASP expression in lymphocytes was analysed by flow cytometry (FCM) by the standard method described previously [4,22]. DNA purification and sequencing of genomic DNA, RNA isolation, reverse transcription-polymerase chain reaction, and sequencing of cDNA for the mutational analysis of *WASP* gene was performed as reported previously [23].

Diagnostic Tests for Juvenile Myelomonocytic Leukemia

Mutational screening for *PTPN11*, *NRAS*, and *KRAS* genes was performed in six patients, as previously reported [24–27]. In patients #6 and #7, the c-CBL gene, which has been recently found in about 10% of JMML patients, was also screened as described previously [16,18]. None of the patients had clinical signs of neurofibromatosis type 1 (NF1). *In vitro* colony assay for granulocyte–macrophage colony stimulating factor (GM-CSF) hypersensitivity assay was performed as a supportive diagnostic tool for JMML as previously reported [28,29].

RESULTS

Clinical Characteristics and Laboratory Findings

The clinical characteristics of these patients are summarized in Table I. Thrombocytopenia and bloody diarrhoea were observed soon after birth in all patients except for patient #6. JMML-like clinical manifestations occurred within the first few months of life. Eczema developed between 0 and 3 months after birth in all patients. Splenomegaly was seen in three of seven patients and massive splenomegaly was present in two patients. At the presentation of JMML-like features, episodes of recurrent infections, which suggest an immunodeficiency, were not observed in any patients. However, in three patients, recurrent bacterial, or viral infections (cases #5, #6, and #7) were documented during the clinical course.

The laboratory findings at the presentation of JMML-like disease are summarized in Table II. The WBC count was increased in all patients except for in patient #7. Monocytosis and myeloid/erythroid precursors were seen in PB in all patients. All patients had anaemia. The MPV before platelet transfusions ranged between 6.9 and 7.9 fl (normal, 7.2–11.7 fl) in the five patients that were evaluated. Hb F levels were normal in three patients examined. The platelet morphology demonstrated anisocytosis in all patients. Occasional giant platelets, which are defined as platelets bigger than red cells, were observed in six patients. These features were unusual for WAS. Full BM with significant predominance of myelopoiesis and a marked left shift of the myeloid lineage was seen in all patients. The number of megakaryocytes was normal or increased. Dysplasia in megakaryopoiesis, myelopoiesis, and erythropoiesis was observed in seven, four, and four patients, respectively. The common dysplasia in the megakaryopoiesis included hypolobulations of nuclei and small megakaryocytes with single or double round nuclei. In the myelopoiesis, nuclear abnormalities such as double nuclei, ring nuclei, or pseudo-Pelger–Huet anomaly nuclei were often seen. The dysplasia of erythropoiesis was mild, if observed, and included nuclear lobulation and double nuclei. The karyotype was normal in all patients. The serum levels of immunoglobulin were variable (Table II). Evaluation of T cell function revealed normal responses to phytohemagglutinin and concanavalin A in the four patients that were examined. The numbers of peripheral T and B cells and the CD4/8 ratio were normal in four patients. Patient #7 demonstrated B-lymphocytopenia and an elevated CD4/8 ratio.

Diagnostic Tests for Juvenile Myelomonocytic Leukemia

Molecular analysis of *PTPN11*, *N-RAS*, and *K-RAS* genes (n = 7) and the c-*CBL* gene (n = 2) documented no mutations in any of the examined patients. *In vitro* GM-CSF hypersensitivity was performed in all patients but patient #1 and was positive only in patient #4.

Diagnostic Tests for Wiskott–Aldrich Syndrome

FCM analysis showed absent (n = 6) or reduced (n = 1) WASP expression in the lymphocytes, which led to the confirmation of a diagnosis of WAS (Table III). Mutations of *WASP* genes

TABLE I. Clinical Features of the Patients

Patient	1	2	3	4	5	6	7
Age at the detection of thrombocytopenia	At birth	At birth	At birth	At birth	1 month	4 months	2 months
Age at the onset of JMML like haematological features	1 month	3 months	1 month	1 month	1 month	4 months	2 months
Age at the onset of eczema	1 month	3 months	Soon after birth	3 months	1 month	3 months	2 months
Age at the onset of bloody diarrhoea	At birth	20 days	At birth	1 week	1 month	No	1 month
Hepatomegaly/splenomegaly (cm under the costal margin)	Yes (3)/no	Yes (3)/yes#	No/no	No/no	No/no	Yes (5)/yes (7.5)	Yes (6)/yes (6)
Infectious episodes before the diagnosis of WAS	CMV antigenemia	No episode	No episode	No episode	Fever of unknown origin	Otitis media	Adenovirus and Rotavirus in stool
Infectious episodes between the diagnosis of WAS and HSCT	No episode	No episode	No episode	No episode	Bacterial and RSV pneumonia Rotavirus gastroenteritis	Otitis media Anal abscess	CMV pneumonia
HSCT (age)	10 months	10 months	17 months	4 months	18 months	13 months	7 months
Donor/stem cell source	U-CBT	MSD-BMT	U-CBT	MSD-BMT	1 antigen MMUD-BMT	MUD-BMT	MUD-BMT
Survival (age at the time of the last follow-up)	Alive (6 years 5 months)	Alive (5 years 4 months)	Alive (4 years 8 months)	Alive (12 months)	Alive (1 year 9 months)	Alive (1 year 6 months)	Alive (1 year 7 months)

JMML, juvenile myelomonocytic leukaemia; WAS, Wiskott–Aldrich syndrome; RSV, respiratory syncytial virus; CMV, cytomegalovirus; # splenomegaly was noted only by ultrasound; HSCT, hematopoietic stem cell transplantation; U-CBT, unrelated cord blood transplantation; MSD-BMT, bone marrow transplantation from an HLA matched sibling donor; MUD-BMT, BMT from an HLA matched unrelated donor; MMUD-BMT, BMT from an HLA-mismatched unrelated donor.

TABLE II. Laboratory Findings Accompanying the Juvenile Myelomonocytic Leukaemia-Like Haematological Features

Patient	1	2	3	4	5	6	7
Peripheral blood							
WBC count ($\times 10^9$/L)	35.5–50.0	12.0–18.0	13.5–22.1	15.0	35.0–50.0	6.0–12.0	7.5
Monocyte count ($\times 10^9$/L)	8.9	1.0–1.5	8	2.3	1.1	1.0–1.5	1.3
Blasts (%)	3	2	2	4	2	0	1
Immature myeloid/erythroid cells	Yes/Yes	Yes/Yes	Yes/Yes	Yes/Yes	Yes/Yes	Yes/Yes	Yes/Yes
Eosinophils (%)	3	12	4	7	2	5	2
Platelet count ($\times 10^9$/L)	44	40–90	31	24	53	11	26
MPV (fl)[a]	7.0	7.4	NE	6.9	7.5	NE	7.9
Platelet anisocytosis/giant platelets	Yes/Yes	Yes/Yes	Yes/Yes	Yes/No	Yes/Yes	Yes/Yes	Yes/Yes
Hb (g/dl)	8.9	8.0	9.2	6.1	11.6	9.5	8.0
Bone marrow							
Cellularity	Full[b]	Full	Full	Full	Full	Full	Full
M/E ratio	33	4	7	5.4	11	2	2
Blasts (%)	3.5	0.5	1	0	2	3.5	2
Karyotype	46,XY	46,XY	46,XY	46, XY	46, XY	46,XY	46,XY
Immunological examination							
Age at examination (months)	8	5	2	2	10	4	2/3/5
IgG (mg/dl)	2,554	468	638	102	792	3,780	1,170/2,120/2,070
IgM (mg/dl)	156	64	37	<5	33	353	122/244/156
IgA (mg/dl)	49	52	38	39	129	124	25/45.4/58.2
IgE (mg/dl)	494	368	89	8	16	1,330 (10 months)	258/693/7,995
LBT (PHA, ConA)	Normal	Normal	NE	NE	NE	Normal	Normal
CD4/8 ratio	Normal	Normal	NE	Normal	NE	Normal	Increased (7.0/22.2/1.1)

WBC, white blood cell; MPV, mean platelet volume; M/E myeloid-/erythroid-cells; LBT, lymphoblastic test; PHA, phytohemagglutinin; conA, concanavalin A; NE, not evaluated. [a]Normal range (7.2–11.7 fl). [b]The cellularity was high (full bone marrow), which was normal for infants.

varied between patients. In patient #1, sequencing of *WASP* cDNA identified five nucleotides (CCGGG) inserted at position c.387 in exon 4, causing a frameshift at codon 140 that gave rise to a premature stop signal at codon 262, as reported previously [20]. Patients #2 and #3 had previously known nonsense mutations in exon 1 and exon 4, which led to the absence of WASP expression and a moderate to severe clinical phenotype of WAS [4,30–32]. Patient #4 had a known deletion in intron 8, which cause a frameshift and absence of WASP expression [4,5]. Patient #5 had a known splice anomaly in intron 6, which reduced expression of WASP and led to a clinical phenotype of either XLP or WAS [4,32]. Patient #6 had known deletion in exon 1, which was associated with a classic WAS phenotype [33]. Patient #7 had a nonsense mutation in exon 1, which has not been previously described.

Clinical Course of Patients

Patient #1 received 6-MP to control leukocytosis. In other patients, the JMML-like features were stable until allogeneic hematopoietic stem cell transplantation (HSCT), which was performed at the age of 4–18 months. All patients are alive after HSCT at the time of the last follow-up (Table I). Graft failure was observed in patient #7, and a second HSCT is currently planned for this patient.

DISCUSSION

Although WASP is expressed ubiquitously in hematopoietic cells and although *in vitro* results suggest that WASP is involved in the proliferation and differentiation of all hematopoietic progenitors, overt defects are restricted to micro-thrombocytopenia and immune-dysfunction in classical WAS. We previously described a case of a male presenting with a clinical picture of JMML, in whom WAS was ultimately diagnosed (patient #1) [20]. These haematological abnormalities had not been previously reported in patients with WAS. Since then, we have encountered six additional patients with WAS who presented with similar clinical characteristics. Morphological features were not distinguishable from JMML. Moreover, normal MPV and the presence

TABLE III. Results of the Diagnostic Tests for Wiskott–Aldrich Syndrome

Patient	1	2	3	4	5	6	7
Age at examinations	8 months	4 months	4 months	3 months	8 months	4 months	3 months
WASP protein expression	Absence	Absence	Absence	Absence	Reduced	Absence	Absence
WASP mutation	Exon 4	Exon 1	Exon 4	Intron 8	Intron 6	Exon1	Exon 1
	c.387–421 ins 5nt	c.37C>T	c.424C>T	c.777+1_+4 delGTGA	c.559+5G>A	c.31delG	c.C55>T
Mutation type	Insertion	Nonsense	Nonsense	Deletion	Splice anomaly	Deletion	Nonsense
Predicted protein change	Frameshift stop aa 262	R13X	Q142X	Frameshift stop aa 246	Frameshift stop aa 190	Frameshift stop aa 37	Q19X

of giant platelets in three and six patients, respectively, initially argued against a diagnosis of WAS, because micro-thrombocytes are known as a key diagnostic feature of WAS and XLP. The JMML-like features developed shortly after birth in all patients, before the full clinical picture of WAS become apparent. In our patients with JMML-like features, signs of immune defects were not present. Without recent advances in molecular diagnostic tests for WAS and JMML, it might otherwise be impossible to establish a diagnosis of WAS in these patients. Absent or reduced WASP expression by FCM–WASP and detection of *WASP* mutation ultimately led to a diagnosis of WAS. The mutations were distributed in different exons and introns, and there was no clustering. Thrombocytopenia since birth and some of the observed clinical features (e.g., atopic dermatitis-like eczema, persistent bloody stool, lack of splenomegaly) were unusual for JMML but were compatible with WAS.

The deregulated RAS signalling pathway plays a central role in the pathogenesis of JMML, and mutational analyses of *PTPN11*, *RAS*, and *c-CBL* genes located in the RAS signalling pathway have become important diagnostic tests. Mutations of one of these genes and a clinical diagnosis of NF1 can be found in more than 80% of patients with JMML. However, in up to 20% of patients without any molecular markers, a diagnosis of JMML relies on unspecific clinical and laboratory observations. We suggest that WAS should be considered within the differential diagnosis in male infants with clinical features of JMML if no mutations of the RAS signalling pathway can be detected. Importantly, clinicians should not exclude a diagnosis of WAS if the MPV is normal or if giant platelets are present. Rarely, patients with WAS can present with normal or large platelets [34,35].

The pathogenesis of JMML-like feature in these patients is unknown. There is no evidence that WASP is related to the RAS signalling pathway. The activation of this pathway does not seem to be a major cause of JMML-like features in our patients, because GM-CSF hypersensitivity was demonstrated only in one of six patients examined. Patients with WAS have an increased risk of viral infections. CMV, Epstein–Barr virus (EBV) and human herpes virus-6 (HHV-6) infections can mimic JMML in infants [36,37]. However, extensive screening failed to detect viral infections at the time, at which these patients presented with JMML-like features, except for patient #1, in whom CMV antigen was detected.

Leukocyte adhesion deficiency (LAD)-1 is a rare immunodeficiency caused by a mutation in the beta-2 integrin gene. The firm adhesion of leukocyte to the blood vessel wall is defective in LAD-1, which results in leukocytosis, mimicking JMML [38]. A defect of leukocyte adhesion due to abnormal integrin beta clustering has been described in the context of WAS [39]. A mechanism similar to that seen in LAD1 may be present in WAS with JMML-like features.

A recent report showed that WASP localizes to not only the cytoplasm but also to the nucleus and has a role in the transcriptional regulation at the chromatin level in lymphocytes [40]. Active *WASP* mutations, which cluster within the GTP-ase binding domain of WASP (L270P, S272P, and I294T), cause XLN and myelodysplasia [6,7]. Further, increased apoptosis associated with increased genomic instability in myeloid cells and lymphocytes has been described in the context of active *WASP* mutations [41,42]. Further research may identify new roles of WASP in transcriptional regulation and genomic stability in haematopoiesis, which may explain the JMML-like features, seen in WAS patients.

In conclusion, WAS should be considered in the differential diagnosis in male infants presenting with JMML-like features if no molecular markers of JMML can be demonstrated. A normal MPV and the presence of giant platelets do not exclude a diagnosis of WAS. Clinical information, such as bloody stool and eczema, may be helpful in pursuing a diagnosis of WAS in an infant with JMML like features.

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Somatic mosaicism for oncogenic *NRAS* mutations in juvenile myelomonocytic leukemia

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Brief report

Somatic mosaicism for oncogenic *NRAS* mutations in juvenile myelomonocytic leukemia

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Juvenile myelomonocytic leukemia (JMML) is a rare pediatric myeloid neoplasm characterized by excessive proliferation of myelomonocytic cells. Somatic mutations in genes involved in GM-CSF signal transduction, such as *NRAS*, *KRAS*, *PTPN11*, *NF1*, and *CBL*, have been identified in more than 70% of children with JMML. In the present study, we report 2 patients with somatic mosaicism for oncogenic *NRAS* mutations (G12D and G12S) associated with the development of JMML. The mutated allele frequencies quantified by pyrosequencing were various and ranged from 3%-50% in BM and other somatic cells (ie, buccal smear cells, hair bulbs, or nails). Both patients experienced spontaneous improvement of clinical symptoms and leukocytosis due to JMML without hematopoietic stem cell transplantation. These patients are the first reported to have somatic mosaicism for oncogenic *NRAS* mutations. The clinical course of these patients suggests that *NRAS* mosaicism may be associated with a mild disease phenotype in JMML. (*Blood*. 2012;120(7):1485-1488)

Introduction

Juvenile myelomonocytic leukemia (JMML) is a rare myeloid neoplasm characterized by excessive proliferation of myelomonocytic cells. Somatic mutations in genes involved in GM-CSF signal transduction, such as *NRAS*, *KRAS*, *PTPN11*, *NF1*, and *CBL*, have been identified in more than 70% of children with JMML.[1-3] The term "somatic mosaicism" is defined as the presence of multiple populations of cells with distinct genotypes in one person whose developmental lineages trace back to a single fertilized egg.[4] Somatic mosaicism of various genes, including some oncogenes, has been implicated in many diseases. For example, somatic mosaicism for *HRAS* mutations is found in patients with Costello syndrome.[5-7] Whereas germline mutations in causative genes (ie, *PTPN11*, *NRAS*, *NF1*, and *CBL*) are found in JMML patients,[3,8-11] the presence of somatic mosaicism for these genes has never been reported. In the present study, we describe 2 cases of JMML in which the patients display somatic mosaicism for oncogenic *NRAS* mutations (G12D and G12S).

Study design

Written informed consent for sample collection was obtained from the patients' parents in accordance with the Declaration of Helsinki, and molecular analysis of the mutational status was approved by the ethics committee of the Nagoya University Graduate School of Medicine (Nagoya, Japan).

Patient 1. A 10-month-old boy had hepatosplenomegaly and leukocytosis (72.1×10^9/L) with monocytosis (13.3×10^9/L; Table 1). The patient's BM contained 7% blasts with myeloid hyperplasia. Cytogenetic analysis revealed a normal karyotype and colony assay of BM mononuclear cells (BM-MNCs) showed spontaneous colony formation but GM-CSF hypersensitivity assay was not tested. The diagnostic criteria for JMML, as developed by the European Working Group on Myelodysplastic Syndrome in Childhood, was fulfilled,[12] and the patient was treated with IFN-α and 6-mercaptopurine. His clinical and laboratory findings gradually resolved without hematopoietic stem cell transplantation. However, 11 years after the diagnosis of JMML, the patient developed thrombocytopenia (7.6×10^9/L) and BM findings showed trilineage dysplasia with low blast count compatible with refractory anemia. The patient did not have any physiologic abnormalities, such as facial deformity, and there was no family history of malignancy or congenital abnormalities.

Patient 2. A 10-month-old boy had anemia, hepatosplenomegaly, and leukocytosis (31.8×10^9/L) with monocytosis (6.4×10^9/L; Table 1). The patient's BM exhibited myeloid hyperplasia and granulocytic dysplasia with 5% blasts. Cytogenetic

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Table 1. Patient characteristics

	Patient 1	Patient 2
Age, mo	10	10
Sex	Male	Male
Liver, cm	12	5
Spleen, cm	8	10
WBCs, $\times 10^9$/L	72.1	31.8
Monocytes, %	18.5	20
Blasts, %	4	2
Hb, g/dL	8.9	5.4
Platelets, $\times 10^9$/L	59	100
HbF, %	2.1	1.7
BM blasts, %	7	5
Karyotype	46,XY [20/20]	46,XY [20/20]
Monosomy 7 (FISH)	Negative	Negative
Spontaneous colony formation	Positive	Positive
Gene mutation	*NRAS*, G12D 35G > A	*NRAS*, G12S 34G > A
Treatment	IFN-α-2b, 6-MP	None
Observation period, mo	231	103
Outcome	Alive	Alive
Fraction of mutant alleles, % (pyrosequencing)		
Nail (whole)	24	12.5 (average)
Nail (left hand)	ND	26
Nail (right hand)	ND	13
Nail (left foot)	ND	8
Nail (right foot)	ND	3
Buccal smear cells	43	21
Hair bulbs	5	ND
Family studies		
Father	Wild-type	Wild-type
Mother	Wild-type	Wild-type
Sibling	ND	Wild-type

Hb indicates hemoglobin; 6-MP, 6-mercaptopurine; and ND, not done.

analysis revealed a normal karyotype. Colony assay of BM-MNCs showed spontaneous colony formation and GM-CSF hypersensitivity. Although the diagnostic criteria for JMML were fulfilled,[12] the patient's clinical symptoms and leukocytosis improved spontaneously within a few months without cytotoxic therapy or hematopoietic stem cell transplantation. The patient has remained healthy and has experienced no hematologic or physiologic abnormalities. The most recent follow-up examination was conducted when the patient was 8 years of age.

Detailed methods for experiments are described in supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Results and discussion

DNA sequencing for JMML-associated genes (ie, *NRAS*, *KRAS*, *PTPN11*, and *CBL*) was performed (Figure 1 and Table 1). In Patient 1, the *NRAS* G12D mutation was identified in BM-MNCs at the time of diagnosis of both JMML and MDS. We identified the same G12D mutation in DNA derived from buccal smear cells and nails of both hands; however, the sequence profile of the nails showed a low signal for the mutant allele compared with signal of blood cells. In Patient 2, the *NRAS* G12S mutation was identified in DNA from BM-MNCs, buccal smear cells, and nails of the left hand. However, the sequence profiles of buccal smear cells and nails of the left hand showed a low signal for the mutant variant. No mutation was detected in DNA from the PB-MNCs of the patient's parents or sibling.

We used pyrosequencing to quantify the fraction of mutated alleles in DNA samples from different somatic tissues (Figure 1 and Table 1). The frequency of mutated alleles varied by tissue type as follows. For Patient 1: BM-MNCs, 50%; nails, 24%; buccal smear cells, 43%; and hair bulbs, 5%. For Patient 2: buccal smear cells, 21%; nails of left hand, 26%; nails of right hand, 13%; nails of left foot, 8%; and nails of right foot, 3%. We cloned the PCR product of *NRAS* exon 2 from the nails of Patient 1 and picked up 15 clones. The clones were sequenced. Four of the 15 clones (27%) contained the mutant allele, which is consistent with the results of pyrosequencing analysis (24% mutant allele). Because the confirmed detection level by pyrosequencing technique was above 5%, results with a low percentage (< 5%) of mutant allele (ie, hair bulbs in Patient 1) should be interpreted with caution.[13,14]

We diagnosed 2 JMML patients as having somatic mosaicism of *NRAS* mutations: G12D for Patient 1 and G12S for Patient 2. The diagnoses were based on negative familial studies and mutational allele quantification analyses that showed diversity in the chimeric mutational status of different somatic tissues. Although DNA from buccal smear cells might be contaminated with WBCs, we also identified mutations in DNA from the nail tissue, which is known to be a good biologic material without contamination from hematopoietic cells, in both patients. These data suggest that a portion of the *NRAS*-mutated somatic cells were derived from one cell that acquired the mutation at a very early developmental stage. Although both somatic and germline mutations of RAS pathway genes (ie, *PTPN11*, *NRAS*, *NF1*, and *CBL*) are found in some JMML patients,[3,8-11] somatic mosaicism for these genes has never been reported. To the best of our knowledge, the present study is