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Autoimmune lymphoproliferative syndrome–like disease with somatic *KRAS* mutation

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Brief report

Autoimmune lymphoproliferative syndrome–like disease with somatic *KRAS* mutation

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Autoimmune lymphoproliferative syndrome (ALPS) is classically defined as a disease with defective FAS-mediated apoptosis (type I-III). Germline *NRAS* mutation was recently identified in type IV ALPS. We report 2 cases with ALPS-like disease with somatic *KRAS* mutation. Both cases were characterized by prominent autoimmune cytopenia and lymphoadenopathy/splenomegaly. These patients did not satisfy the diagnostic criteria for ALPS or juvenile myelomonocytic leukemia and are probably defined as a new disease entity of RAS-associated ALPS-like disease (RALD). (*Blood*. 2011;117(10):2887-2890)

Introduction

Autoimmune lymphoproliferative syndrome (ALPS) is a disease characterized by dysfunction of the FAS-mediated apoptotic pathway,[1,2] currently categorized as: type Ia, germline *TNFRSF6/FAS* mutation; type Ib, germline *FAS ligand* mutation; type Is, somatic *TNFRSF6/FAS* mutation; and type II, germline *Caspase 10* mutation. Patients exhibit lymphadenopathy, hepatosplenomegaly, and autoimmune diseases, such as immune cytopenia and hyper-γ-globulinemia. An additional subclassification has been proposed that includes types III and IV, whereby type III has been defined as that with no known mutation but with a defect in FAS-mediated apoptosis and type IV as one showing germline *NRAS* mutation.[3] Type IV is considered exceptional because the FAS-dependent apoptosis pathway is not involved in the pathogenesis, and this subclass is characterized by a resistance to interleukin-2 (IL-2) depletion-dependent apoptosis. Recent updated criteria and classification of ALPS suggested type IV ALPS as a RAS-associated leukoproliferative disease.[4]

Juvenile myelomonocytic leukemia (JMML) is a chronic leukemia in children. Patients show lymphadenopathy, hepatosplenomegaly, leukocytosis associated with monocytosis, anemia, thrombocytopenia, and occasional autoimmune phenotypes. Approximately 80% of patients with JMML have been shown to have a genetic abnormality in their leukemia cells, including mutations of *NF1*, *RAS* family,[5] *CBL*, or *PTPN11*. The hallmarks of the laboratory findings of JMML include spontaneous colony formation in bone marrow (BM) or peripheral blood mononuclear cells (MNCs) and hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF) of $CD34^+$ BM-MNCs.[6]

Germline RAS pathway mutations cause Costello (*HRAS*), Noonan (*PTPN11*, *KRAS*, and *SOS1*), and cardio-facio-cutaneous syndromes (*KRAS*, *BRAF*, *MEK1*, and *MEK2*). Patients with Costello and Noonan syndromes have an increased propensity to develop solid and hematopoietic tumors, respectively[7]; among these tumors, the incidence of JMML in patients with germline mutation of *NF1* or *PTPN11* is well known.

We present 2 cases with autoimmune cytopenia and remarkable lymphadenopathy and hepatosplenomegaly, both of which were identified as having a somatic KRAS G13D mutation without any clinical features of germline *RAS* mutation, such as cardio-facio-cutaneous or Noonan syndrome.

Methods

All studies were approved by the ethical board of Tokyo Medical and Dental University.

Case 1

A 9-month-old boy had enormous bilateral cervical lymphadenopathy and hepatosplenomegaly (supplemental Figure 1A-B, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Blood test revealed the presence of hemolytic anemia and autoimmune thrombocytopenia. Hyper-γ-globulinemia with various autoantibodies was also noted. ALPS and JMML were nominated as the diseases to be differentially diagnosed. Detailed clinical history and laboratory data are provided as Supplemental data. The patient did not satisfy the criteria for the diagnosis of ALPS or JMML as discussed in "Results and discussion."

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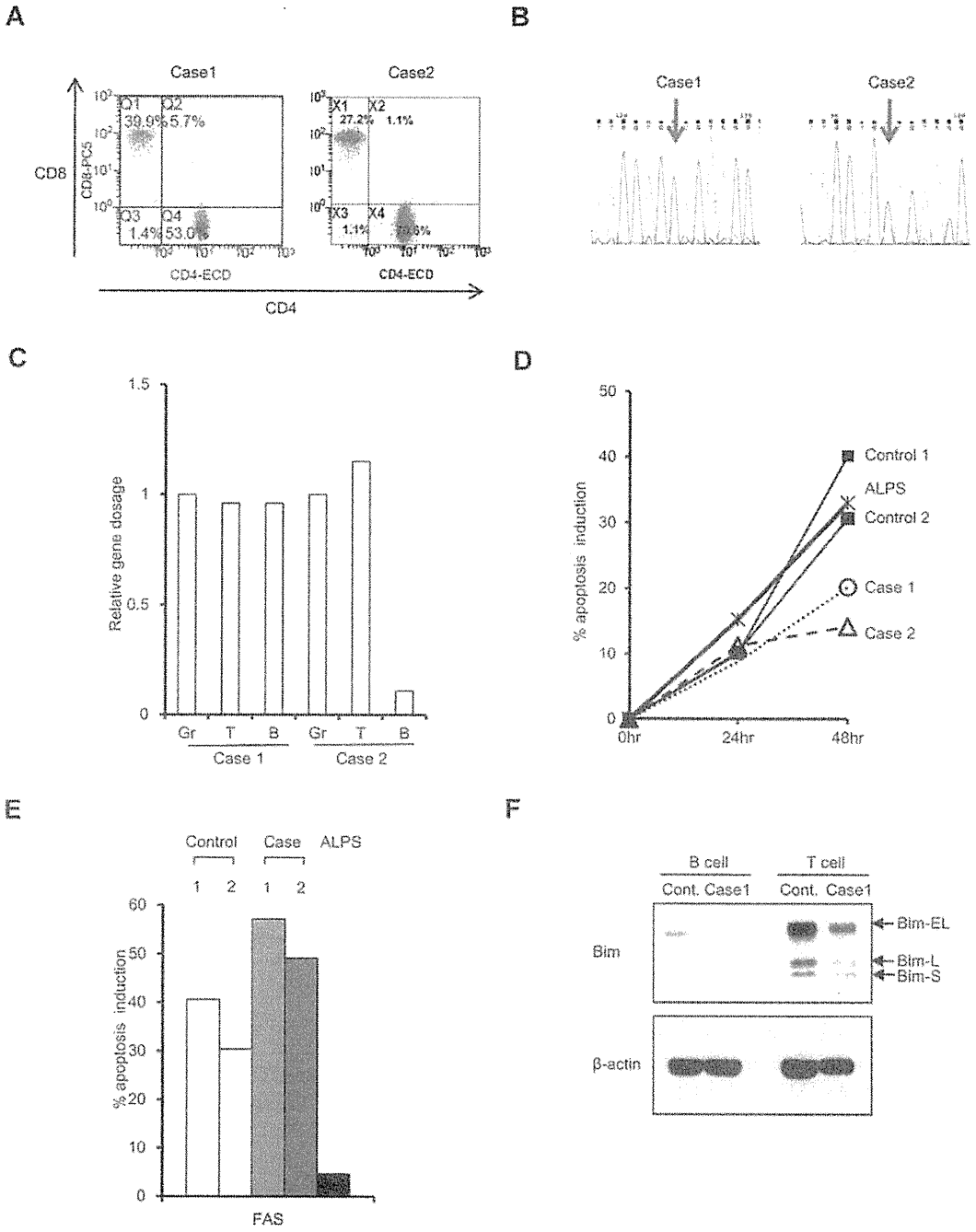

Figure 1. Molecular cell biologic assay of RALD. (A) Flow cytometric analysis of double-negative T cells. CD8 and CD4 double staining was performed in T-cell receptor-αβ-expressing cells. (B) Electropherogram showing KRAS G13D mutation in BM-MNCs in case 1 (left panel) and case 2 (right panel). (C) Gene dosage of mutated allele in granulocytes (Gr), T cells (T), and B cells (B). Relative gene dosage was estimated by a mutant allele-specific polymerase chain reaction method in cases 1 and 2 using albumin gene as internal control. (D) Apoptosis assay using activated T cells. Apoptosis percentage was measured by flow cytometry with annexin V staining 24 and 48 hours after IL-2 depletion. (E) Apoptosis percentage was measured 24 hours after addition of anti-FAS CH11 antibody (final 100 ng/mL). (F) Western blotting analysis of Bim expression.

Case 2

A 5-month-old girl had a fever and massive hepatosplenomegaly (supplemental Figure 1D). She was initially diagnosed with Evans syndrome based on the presence of hemolytic anemia and autoimmune thrombocytopenia with hyper-γ-globulinemia and autoantibodies. Spontaneous colony formation assay and GM-CSF hypersensitivity of BM-MNCs showed positivity. Then, tentative diagnosis of JMML was given, even though she showed no massive monocytosis or increased fetal hemoglobin. Detailed clinical history and laboratory data are provided in supplemental data.

Detailed methods for experiments are described in supplemental data.

Results and discussion

Case 1 showed a high likelihood of being a case of ALPS according to the symptoms and clinical data presented (supplemental Table 1), except for number of double-negative T cells, which was only 1.4% of T-cell receptor-αβ cells (Figure 1A). JMML was also nominated as a disease to be differentiated because remarkable hepatosplenomegaly with thrombocythemia and moderate monocytosis was

noted. However, no hypersensitivity to GM-CSF as determined by colony formation assay for BM-MNCs (data not shown) or phosphor-STAT5 staining (data not shown) was observed. DNA sequence for JMML-associated genes, such as *NRAS, KRAS, HRAS, PTPN11*, and *CBL*, was determined, and KRAS G13D mutation was identified (Figure 1B). The mutation was seen exclusively in the hematopoietic cell lineage, and no mutation was seen in the oral mucosa or nail-derived DNA. Granulocytes, monocytes, T cells, and B cells were all positive for KRAS G13D mutation (data not shown). The proportion of mutated cells in each hematopoietic lineage was quantitated by mutation allele-specific quantitative polymerase chain reaction methods, which revealed that mutated allele was almost equally present in granulocytes, T cells, and B cells (Figure 1C). $CD34^+$ hematopoietic stem cells (HSCs) were also positive for KRAS G13D mutation, and 60% of colony-forming units-granulocyte macrophage (CFU-GM) developed from isolated CD34 cells carried the KRAS G13D mutation (data not shown). These observations suggest that the mutation occurred at the HSCs level, and HSC consists of wild-type and mutant HSCs.

NRAS-mutated type IV ALPS was previously characterized by apoptosis resistance of T cells in IL-2 depletion.[3] Then, activated T cells were subjected to an apoptosis assay by FAS stimulation or IL-2 depletion. Remarkable resistance to IL-2 depletion, but not to FAS-dependent apoptosis (Figure 1D-E), was seen. This was in contrast to T cells from FAS-mutated ALPS type Ia, which showed remarkable resistance to FAS-dependent apoptosis and normal apoptosis induction by IL-2 withdrawal (Figure 1D-E). Western blotting analysis of activated T cells or Epstein-Barr virus-transformed B cells showed reduced expression of Bim (Figure 1F).

In case 2, autoimmune phenotype and hepatosplenomegaly were remarkable, as shown in Supplemental data. The patient was initially diagnosed as Evans syndrome based on the presence of hemolytic anemia and autoimmune thrombocytopenia. Double-negative T cells were 1.1% of T-cell receptor-$\alpha\beta$ cells in the peripheral blood, which did not meet with the criteria of ALPS. Although spontaneous colony formation was shown in peripheral blood- and BM-MNCs, and GM-CSF hypersensitivity was demonstrated in BM-MNCs derived $CD34^+$ cell (supplemental Table 2), she showed no massive monocytosis or increased fetal hemoglobin. Thus, the diagnosis was less likely to be ALPS or JMML. DNA sequencing of JMML-related genes, such as *NRAS, KRAS, HRAS, PTPN11*, and *CBL*, identified somatic, but not germline, KRAS G13D mutation (Figure 1B). KRAS G13D mutation was detected in granulocytes and T cells. Mutation was not identified in B cells by conventional DNA sequencing (data not shown). Mutant allele-specific quantitative polymerase chain reaction revealed that mutated allele was almost equally present in granulocytes and T cells, but barely in B cells (Figure 1C). Activated T cells showed resistance to IL-2 depletion but not to FAS-dependent apoptosis (Figure 1D-E).

Both of our cases were characterized by strong autoimmunity, immune cytopenia, and lymphadenopathy or hepatosplenomegaly with partial similarity with ALPS or JMML. However, they did not meet with the well-defined diagnostic criteria of ALPS[2] or JMML.[6] It is interesting that case 2 presented GM-CSF hypersensitivity, which is one of the hallmarks of JMML. Given the strict clinical and laboratory criteria of JMML and ALPS, our 2 cases should be defined as a new disease entity, such as RAS-associated ALPS-like disease (RALD). Recently defined NRAS-mutated ALPS type IV may also be included in a similar disease entity.

There are several cases of JMML reported simultaneously having clinical and laboratory findings compatible with autoimmune disease.[8,9] Autoimmune syndromes are occasionally seen in patients with myelodysplastic syndromes, including chronic myelomonocytic leukemia.[10] These previous findings may suggest a close relationship of autoimmune disease and JMML. Because KRAS G13D has been identified in JMML,[11-13] it is tempting to speculate that KRAS G13D mutation is involved in JMML as well as RALD. In JMML, erythroid cells reportedly carry mutant RAS, whereas B- and T-cell involvement was variable.[13] In both of our cases, myeloid cells and T cells carried mutant RAS, whereas B cells were affected variably. These findings would support a hypothesis that the clinical and hematologic features are related to the differentiation stages of HSCs where RAS mutation is acquired. JMML-like myelomonocytic proliferation may predict an involvement of RAS mutation in myeloid stem/precursor cell level, whereas ALPS-like phenotype may predict that of stem/precursor cells of lymphoid lineage, especially of T cells. Under the light of subtle differences between the 2 cases presented, their hematologic and clinical features may reflect the characteristics of the stem cell level where *KRAS* mutation is acquired. Involvement of the precursors with higher propensity toward lymphoid lineage may lead to autoimmune phenotypes, whereas involvement of those with propensity toward the myeloid lineage may lead to GM-CSF hypersensitivity while still sharing some overlapping autoimmune characteristics.

One may argue from the other viewpoints with regard to the clinicopathologic features of these disorders. First, transformation in fetal HSCs might be obligatory for the development of JMML[14] and, in HSCs later in life, may not have the same consequences. Second, certain KRAS mutations may be more potent than others. Codon 13 mutations are generally less deleterious biochemically than codon 12 substitutions, and patients with JMML with codon 13 mutations have been reported to show spontaneous hematologic improvement.[12,15] Thus, further studies are needed to reveal in-depth clinicopathologic characteristics in this type of lymphomyeloproliferative disorder.

KRAS mutation may initiate the oncogenic pathway as one of the first genetic hits but is insufficient to cause frank malignancy by itself.[16,17] Considering recent findings that additional mutations of the genes involved in DNA repair, cell cycle arrest, and apoptosis are required for full malignant transformation, one can argue that RALD patients will also develop malignancies during the course of the disease. Occasional association of myeloid blast crisis in JMML and that of lymphoid malignancies in ALPS will support this notion. Thus, the 2 patients are now being followed up carefully. It was recently revealed that half of the patients diagnosed with Evans syndrome, an autoimmune disease presenting with hemolytic anemia and thrombocytopenia, met the criteria for ALPS diagnosis.[18,19] In this study, FAS-mediated apoptosis analysis was used for the screening. Considering the cases we presented, it will be intriguing to reevaluate Evans syndrome by IL-2 depletion-dependent apoptosis assay focusing on the overlapping autoimmunity with RALD.

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Authorship

Contribution: Masatoshi Takagi and S.M. designed entire experiments and wrote the manuscript; K.S., N.M., and Mari Takagi treated patients and designed clinical laboratory test; J.P. performed experiments described in Figure 1B-F; K.M., H.M., and S.D. performed colony and mutational analysis; and M.N., T.M., K.K., S.K., Y.K., and A.T. supervised clinical and immunologic experiments or coordinated clinical information.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Early and Rapid Detection of X-Linked Lymphoproliferative Syndrome with *SH2D1A* Mutations by Flow Cytometry

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Background: X-linked lymphoproliferative syndrome (XLP) is a rare immunodeficiency with extreme vulnerability to Epstein-Barr virus (EBV) infection. It presents with fatal infectious mononucleosis, lymphoproliferative disorder, or dysgammaglobulinemia. The majority of affected males have mutations in the *SH2D1A/SLAM-associated protein* (SAP) gene. We previously generated an antihuman SAP monoclonal antibody (KST-3) for a flow cytometric assay and described the activation of T cells to be necessary for the flow cytometric assessment of the SAP expression using an FITC-conjugated secondary antibody.

Methods: Between 2005 and 2008, we recruited 23 male patients with suspected XLP, including mainly EBV-associated hemophagocytic lymphohistiocytosis (HLH), and attempted to evaluate SAP expression in fresh lymphoid cells using Alexa Fluor 488-conjugated secondary antibody instead of an FITC-conjugated one.

Results: The method demonstrated that SAP was intensely expressed in $CD8^+$ T cells and NK cells in normal fresh blood samples, thus suggesting the possible rapid identification of individuals with SAP deficiency. *SH2D1A* mutations were identified in six patients with SAP deficiency, but not in patients with normal SAP expression.

Conclusion: The outcomes from this trial were verified by a flow cytometric assay using KST-3 and Alexa Fluor 488 secondary antibody. Based on the demonstration SAP deficiency in patients with suspected XLP, including mainly EBV-associated HLH, this approach could serve as a method for the early and rapid detection of patients with XLP-1.

Key terms: flow cytometry; hemophagocytic lymphohistiocytosis; SLAM-associated protein; *SH2D1A*; X-linked lymphoproliferative syndrome; genetic analysis

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X-linked lymphoproliferative syndrome (XLP) is a rare inherited immunodeficiency estimated to affect approximately one in one million males, though it may be under-diagnosed (1). Most XLP patients 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. Rapid definitive diagnosis and appropriate treatment are extremely significant for life-saving and improved prognosis for XLP patients (3). The responsible gene is termed the *SH2D1A/SLAM-associated protein* (SAP) gene (4–6). In contrast, some presumed XLP patients do not harbor *SH2D1A* mutations, although they are clinically and even historically similar to XLP patients with *SH2D1A* mutations. Recently, Rigaud et al. (7) identified the second causative gene for XLP, the *BIRC4* gene, which encodes the X-linked inhibitor of apoptosis protein (XIAP). Therefore, XLP is now divided into two distinct diseases, XLP-1 and XLP-2.

Regarding a rapid diagnosis of XLP-1, we previously generated a rat monoclonal antibody (mAb) specific for human SAP protein, termed KST-3, to develop a flow cytometric analysis of SAP deficiency seen in XLP patients with *SH2D1A* mutations (8). In the present study, we attempt to evaluate possible SAP expression in fresh lymphoid cells with a flow cytometric assay employing Alexa Fluor 488-labled secondary antibody, which is much brighter than conventional FITC antibodies (9). Between 2005 and 2008, we used a flow cytometric determination of SAP deficiency in $CD8^+$ T and NK cells to test 23 male patients with suspected XLP, including mainly EBV-associated hemophagocytic lymphohistiocytosis (HLH). *SH2D1A* mutations were identified in six patients with SAP deficiency, but not in the other patients with normal SAP expression. These results demonstrate that a flow cytometric assay using KST-3 and Alexa Fluor 488 secondary antibody can achieve the early and rapid detection of patients with XLP-1.

MATERIALS AND METHODS

Study Subjects

The subjects in this study were largely male patients with EBV-associated HLH. In addition, a few male patients with lymphoma or hypo-γglobulinemia of unknown genetic origin were studied. A total of 23 Japanese male patients between 4 months and 40 years of age with suspected XLP-1 were tested between 2005 and 2008. Normal donors included healthy adult volunteers 24–42 years of age, and children 1–14 years of age without immunologic and hematologic diseases. After written informed consent was obtained, 5–10 mL of venous blood was collected into heparin-containing syringes and subjected to investigation within 24 h. The study was approved by the Ethics Committee of the University of Toyama.

Flow Cytometric Analysis of SAP Expression

We performed a flow cytometric analysis of SAP expressed in lymphoid cells using a rat antihuman SAP mAb, termed KST-3, as previously described (8). We employed the Alexa Fluor 488-conjugated secondary antibody to examine the possible flow cytometric assessment of SAP expression in fresh lymphoid cells. 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. These cells were incubated with 2 μg/ml of KST-3 (rat IgG1) or irreverent rat IgG1 for 20 min on ice and further stained with a 1:1,000 dilution of Alexa Fluor 488-conjugated goat anti-rat antibody (Molecular Probes, Eugene, OR) for 20 min on ice. To evaluate SAP expression in $CD8^+$ T cells, $CD4^+$ T cells, NK cells, and B cells, PBMC were stained with phycoerythrin-conjugated anti-CD8, anti-CD4, anti-CD56 or anti-CD19 mAbs (DAKO Japan, Kyoto, Japan), respectively, before cellular fixation and permeabilization. In some experiments, we used phycoerythrin-Texas Red (ECD)-conjugated anti-CD45RO (Immunotech, Marseille, France). We analyzed the stained cells with a flow cytometer (EPICS XL-MCL; Beckman Coulter KK, Tokyo, Japan).

SH2D1A Mutation Detection

The *SH2D1A* mutations were detected by the direct sequencing. Genomic DNA was purified from PBMC with a QIAamp Blood Kit (Qiagen, Hilden, Germany), and each of the four exon-intron boundaries of the *SH2D1A* gene was amplified by PCR using the following primers: exon 1, forward, 5′-GCC CTA CGT AGT GGG TCC ACA TAC CAA CAG-3′, and reverse 5′-GCA GGA GGC CCA GGG AAT GAA ATC CCC AGC-3′; exon 2, forward, 5′-GGA AAC TGT GGT TGG GCA GAT ACA ATA TGG-3′, and reverse, 5′-GGC TAA ACA GGA CTG GGA CCA AAA TTC TC-3′; exon 3, forward, 5′-GCTCCTCTTGCAGGGAAATTC AGC CAACC-3′, and reverse, 5′-GCT ACC TCT CAT TTG ACT TGC TGG CTA CAT C-3′; exon 4, forward, 5′-GAC AGG GAC CTA GGC TCAGGC ATA AAC TGA C-3′, and reverse, 5′-ATG TAC AAA AGTCCATTT CAG CTT TGAC-3′ as previously described (6). We used the BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) with an automated ABI PRISM 310 DNA sequencer (Applied Biosystems) to carry out the sequence reaction.

RESULTS

SAP Expression in Normal Donors

We examined whether a flow cytometric analysis employing an Alexa Fluor 488-conjugated secondary antibody instead of an FITC-conjugated one could assess possible SAP expression in fresh lymphoid cells. We used this method to examine normal donors for SAP expression of $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and B cells in fresh blood samples. A representative profile in a healthy adult donor is shown in Figure 1. It has been shown that the SAP protein is basically expressed in all major T cell subsets and NK cells (6,8,10,11). Consistent with these observations, we demonstrated that $CD8^+$ T cells and NK cells expressed SAP intensely,

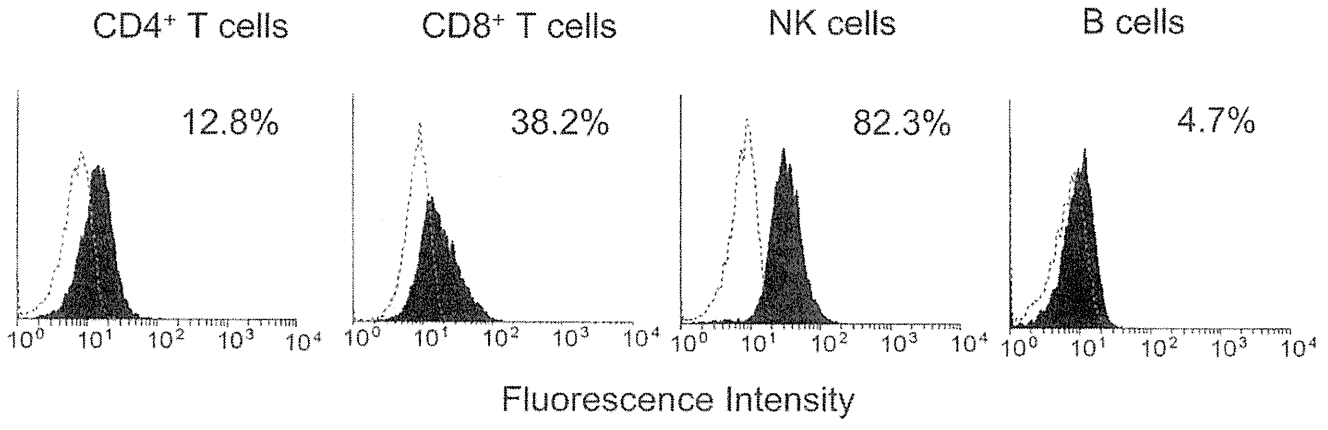

FIG. 1. The SAP expression in T, B, and NK cells in a normal adult donor. A flow cytometric analysis showed that $CD8^+$ T cells and NK cells expressed SAP relatively intensely, $CD4^+$ T cells weakly, and B cells negligibly. The dotted lines and shaded areas indicate staining by the control antibody and anti-SAP mAb (KST-3), respectively.

$CD4^+$ T cells relatively weakly, and B cells negligibly. We observed that SAP expression in $CD8^+$ T cells and $CD4^+$ T cells varied from donor to donor. We assumed that this variation might be due to individual differences in proportions of $CD45RO^+$ (memory/activated) subsets among $CD8^+$ T cells and $CD4^+$ T cells. A three-color analysis demonstrated that $CD45RO^+$ populations of T cell subsets showed enhanced SAP expression, especially of $CD8^+$ T cells (Fig. 2).

SAP Expression and *SH2D1A* Mutations in Patients with Suspected XLP

Based on the above observations in normal donors, we chose a flow cytometric analysis of SAP expression in $CD8^+$ T cells and NK cells to screen for SAP deficiency seen in XLP patients with *SH2D1A* mutations. Representative flow cytometric profiles are shown in Figure 3. All patients were simultaneously examined for a genetic analysis of the *SH2D1A* gene. The results of SAP expression and *SH2D1A* mutation analyses obtained from 23 patients with suspected XLP are summarized in Table 1. Six patients (P1–P6) demonstrated a marked reduction of SAP expression in $CD8^+$ T cells and NK cells. The percentages of SAP protein in $CD8^+$ T cells and NK cells in these patients were only 0.5–3.7% and 1.2–3.1%, respectively. *SH2D1A* mutations were confirmed in the patients with SAP deficiency. The mutations included g.23917insA, g.19528G > A (IVS2 + G>A) in sibling cases, g.357insG, deletion of exons 3–4, and g.352G > T (Ala3Ser). In contrast,

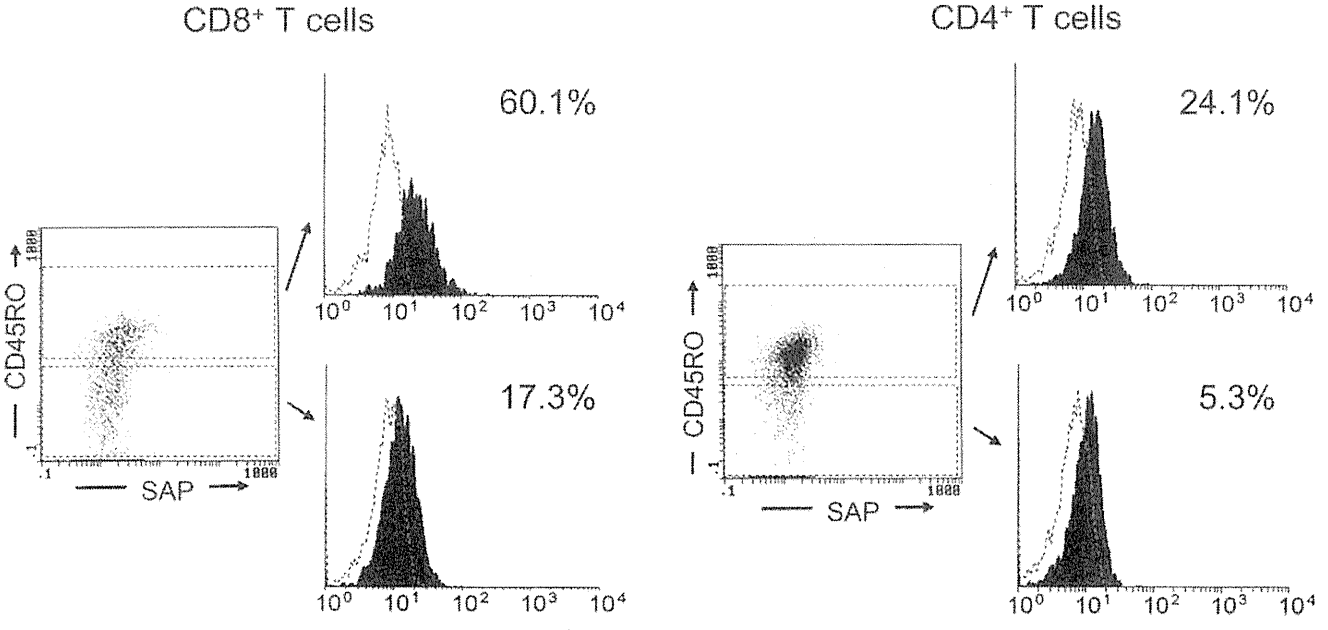

FIG. 2. An increased SAP expression in $CD45RO^+$ T cell subsets. $CD45RO^+$ (memory/activated) populations of T cells subsets, especially of $CD8^+$ T cells, exhibited an enhanced SAP expression.

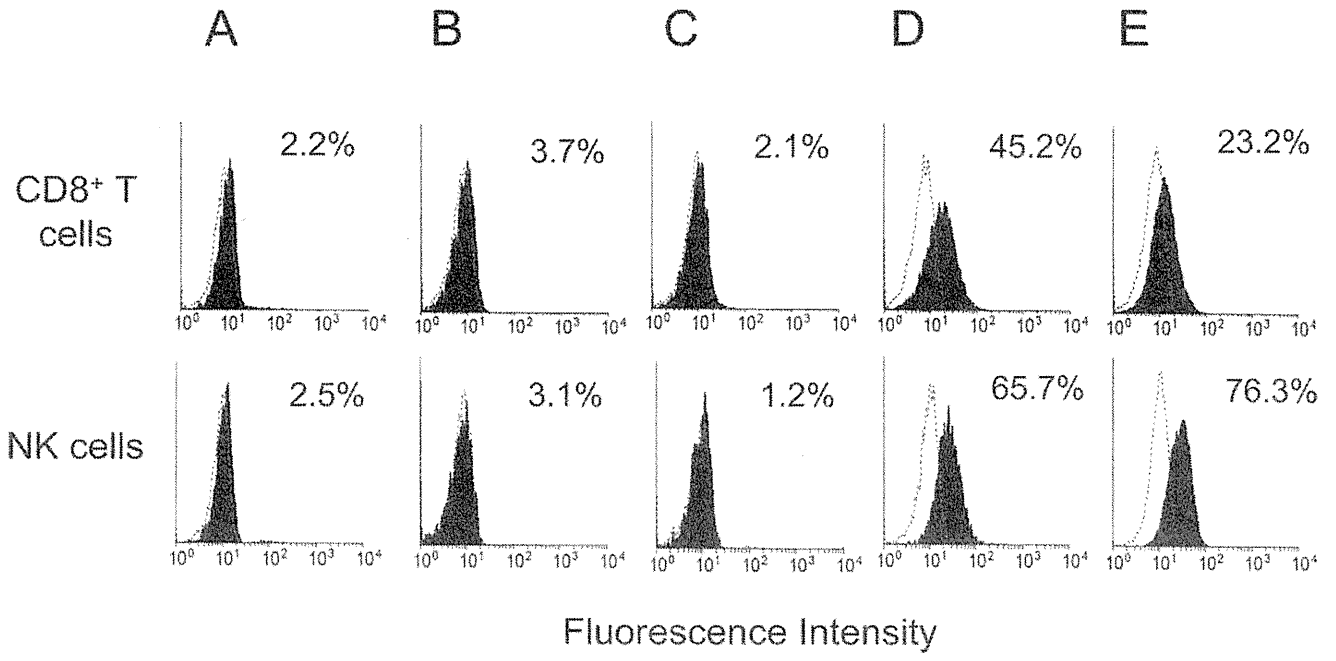

FIG. 3. The SAP expression in suspected patients with XLP. A marked SAP deficiency in both CD8+ T cells and NK cells was notable in some patients (A–C), but not in others (D, E). **A**, **B**, **C**, **D**, and **E** indicate P1, P2, P4, P10, and P11, respectively.

SH2D1A mutations were not seen in the other 17 patients (P7–P23), all of whom showed almost normal SAP expression in $CD8^+$ T cells (7.2–88.8%) and NK cells (24.3–98.1%). It is important to take into account the possibility that the flow cytometric assessment of SAP expression in $CD8^+$ T cells may be age-dependent,

Table 1
Clinical Characteristics and Immunological Data of the Patients Examined in this Study

					%SAP$^-$ cells in		*SH2D1A* mutation	
Patient	Age	Clinical presentation	EBV	Prognosis	$CD8^+$T cells	NK cells	Nucleotide	Amino acid
P1	10 y	hypo-γ, HLH	–	After BMT	2.2	2.5	g.23917insA	Frameshift
P2	2 y	HLH	+	Dead	3.7	3.1	g.19528G > A	Frameshift
P3	2 y	ADEM	HHV-6	After BMT	0.5	1.2	g.19528G > A	Frameshift
P4	6 y	hypo-γ	–	After BMT	2.1	1.2	g.357insG	Frameshift
P5	14 y	hypo-γ, HLH, lymphoma	+	After BMT	2.2	2.7	Deletion of exons 3-4	
P6	40 y	HLH	+	Dead	2.7	NE	g.352G > T	Ala3Ser
P7	1 y	HLH	+	Alive	7.2	54.1	None	
P8	19 y	hypo-γ, gastritis	+	Alive	35.8	65.2	None	
P9	1 y	HLH	+	Alive	88.8	85.2	None	
P10	2 y	HLH	+	Alive	45.2	65.7	None	
P11	8 y	HLH	+	After CBT	23.2	76.3	None	
P12	10 mo	HLH	+	Alive	48.6	68.5	None	
P13	3 y	Lymphoma, HLH	–	Dead	70.4	98.1	None	
P14	6 y	HLH	+	Alive	35.4	55.3	None	
P15	4 mo	HLH	+	Alive	20.4	32.0	None	
P16	1 y	HLH	–	Alive	41.7	57.7	None	
P17	1 y	HLH	+	Alive	27.7	36.5	None	
P18	1 y	HLH	+	Alive	13.5	32.6	None	
P19	5 y	HLH	+	Alive	64.1	48.1	None	
P20	7 y	HLH	+	Alive	51.0	49.9	None	
P21	1 y	HLH	+	Alive	16.0	28.7	None	
P22	1 y	HLH	–	Alive	47.4	54.0	None	
P23	1 y	HLH	+	Alive	30.2	24.3	None	
Normal (n = 12)				Mean (range)	48.5 (21.6–90.8)	53.8 (23.1–94.5)		

P2 and P3 are monozygotic twins. y, years; mo, months; hypo-γ, hypogammaglobulinemia; HLH, hemophagocytic lymphohistiocytosis; ADEM, acute disseminated encephalomyelitis; HHV-6, human herpesvirus-6; BMT, bone marrow transplantation; CBT, cord blood transplantation; and NE, not examined.

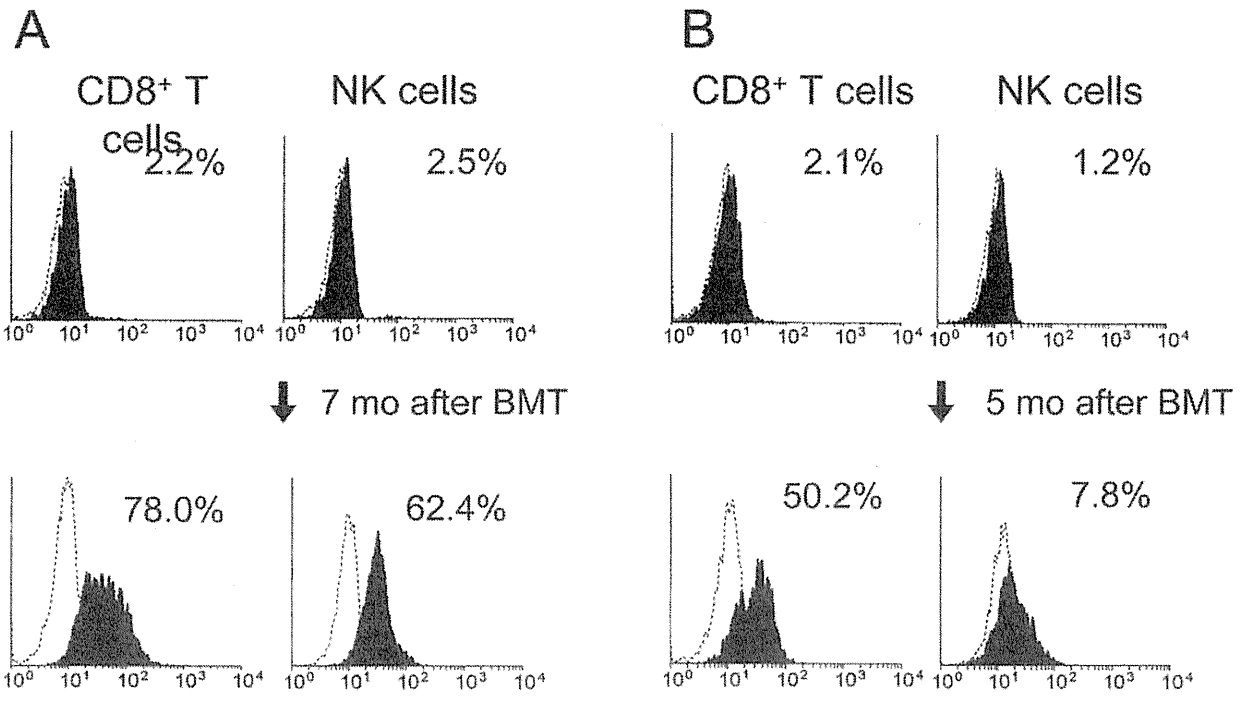

FIG. 4. The SAP expression in XLP patients after HSCT. A flow cytometric analysis demonstrated an increased SAP expression in CD8+ T cells and NK cells in 2 XLP patients after they have undergone HSCT. A and B indicate P1 and P4, respectively.

as exemplified in a one-year-old patient (P7) with no *SH2D1A* mutation. In this patient, the SAP expression in $CD8^+$ T cells was much weaker than in normal donors, thus suggesting a SAP deficiency, but the SAP expression in NK cells was comparable with the expression observed in normal donors.

Monitoring of the SAP Expression in XLP Patients After HSCT

In this series, four patients (P1, P3, P4, and P5) with XLP underwent HSCT. A flow cytometric assay was conducted to evaluate SAP expression in $CD8^+$ T cells and NK cells after HSCT. Representative cases are shown in Figure 4. All of the patients demonstrated increases of SAP expression in $CD8^+$ T cells and NK cells after undergoing HSCT. These outcomes therefore appear to validate the success of HSCT.

DISCUSSION

XLP is a rare but life-threatening disease. Most patients with XLP die by 40 years of age, and more than 70% of them die before 10 years of age (2). Early recognition in nonfamilial cases may be difficult because XLP phenotypes are heterogeneous in their clinical presentation. The ability to rapidly screen and accurately diagnose XLP patients facilitates the initiation of life-saving treatment and preparation for HSCT. Currently, XLP is divided into two distinct diseases, XLP-1 and XLP-2. The former is caused by mutations in the *SH2D1A* gene, whereas the latter is caused by mutations in the *BIRC4* gene. The majority of XLP patients have XLP-1 (7). In a previous study, we generated a rat mAb (KST-3) against human SAP protein. It was applied to the flow cytometric evaluation of SAP deficiency seen in XLP-1 patients (8). We found that activation of T cells in vitro for approximately 4 days was necessary for flow cytometric assessment of SAP expression using FITC-conjugated secondary antibody. The present study demonstrated that a flow cytometric analysis of lymphoid SAP expression was feasible in fresh blood samples by employing the Alexa Fluor 488-conjugated secondary antibody instead of the FITC-conjugated one. The Alexa Fluor 488-conjugated secondary antibody provides more intense fluorescence than the conventional one, and it can clearly discriminate positive cells from negative ones (9). Therefore, this method might lead to early and rapid detection of XLP patients with the *SH2D1A* gene.

Our flow cytometric analysis of SAP expression in $CD8^+$ T and NK cells identified SAP deficiency in 6 out of 23 patients with suspected XLP. As expected, all six patients with SAP deficiency (P1–P6) were shown to have mutations in the *SH2D1A* gene. As shown in previous studies of flow cytometry (8,11), all the missense, nonsense, and frameshift mutations in the *SH2D1A* gene resulted in deficient expression of SAP protein. Although XLP-1 patients with some missense mutations may show normal SAP expression, SAP deficiency can be demonstrated in most XLP-1 patients by flow cytometry. No *SH2D1A* mutations were identified in the remaining 17 patients with normal SAP expression. The suspected

XLP patients with normal SAP expression might have XLP-2, however, no *BIRC4* mutations were identified in these patients.

Among six patients diagnosed as having XLP-1, three patients (P2, P5, and P6) showed EBV-associated HLH, but HLH in P1 was not associated with EBV infection. Two patients (P1 and P4) showed hypo-γglobulinemia followed by acute EBV infection. P5 had EBV-negative malignant lymphoma in his brain. Interestingly, one patient (P3, a sibling of P2) had human herpesvirus-6 (HHV-6)-induced acute disseminated encephalomyelitis (ADEM). XLP is generally considered susceptible to EBV infection, but it might be vulnerable to infections from other herpesviruses as well. ADEM, is a rare manifestation in XLP, that might be a variant form of cerebellar vasculitis. Regarding clinical outcomes, two patients (P2 and P6) died of EBV-associated HLH, but four patients (P1, P3, P4, and P5) recovered after undergoing HSCT.

In conclusion, this study verified the clinical utility of a flow cytometric evaluation of lymphoid SAP expression for the detection of patients with XLP-1. Compared with the conventional Western blot technique, a flow cytometric assay can be more quickly performed with less blood, and multi-color analysis can reveal the protein expression in each cell lineage. It might be useful for detecting revertants and somatic mutations. In fact, Tabata et al. (11) demonstrated a mosaic expression of SAP in $CD8^+$ T cells, thus suggesting that the XLP-1 patient might have a revertant of $CD8^+$ T cells. Flow cytometric analysis of SAP protein is also useful to monitor a cellular reconstitution after HSCT in XLP-1 patients. Recently, a rapid flow cytometric screening method for XLP-2 has also reported (12). A male with any of the clinical phenotypes of XLP, with or without EBV infection, should initially be examined with a flow cytometric assay using both anti-SAP and anti-XIAP mAbs. Needless to say, a mutation analysis is the gold standard for confirming a definite diagnosis.

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X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease

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X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease

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X-linked lymphoproliferative disease (XLP1) is a rare immunodeficiency characterized by severe immune dysregulation and caused by mutations in the *SH2D1A*/SAP gene. Clinical manifestations are varied and include hemophagocytic lymphohistiocytosis (HLH), lymphoma and dysgammaglobulinemia, often triggered by Epstein-Barr virus infection. Historical data published before improved treatment regimens shows very poor outcome. We describe a large cohort of 91 genetically defined XLP1 patients collected from centers worldwide and report characteristics and outcome data for 43 patients receiving hematopoietic stem cell transplant (HSCT) and 48 untransplanted patients. The advent of better treatment strategies for HLH and malignancy has greatly reduced mortality for these patients, but HLH still remains the most severe feature of XLP1. Survival after allogeneic HSCT is 81.4% with good immune reconstitution in the large majority of patients and little evidence of posttransplant lymphoproliferative disease. However, survival falls to 50% in patients with HLH as a feature of disease. Untransplanted patients have an overall survival of 62.5% with the majority on immunoglobulin replacement therapy, but the outcome for those untransplanted after HLH is extremely poor (18.8%). HSCT should be undertaken in all patients with HLH, because outcome without transplant is extremely poor. The outcome of HSCT for other manifestations of XLP1 is very good, and if HSCT is not undertaken immediately, patients must be monitored closely for evidence of disease progression. (*Blood*. 2011;117(1):53-62)

Introduction

X-linked lymphoproliferative disease (XLP) is a rare primary immunodeficiency first described in 1975 by Purtilo[1] and characterized by severe immune dysregulation often after viral infection (typically with Epstein-Barr virus [EBV]). Since XLP was first

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described, our understanding of the molecular and cellular pathogenesis of the disease has greatly improved. However, clinically, it is still difficult to determine optimal management and prognosis for patients due to the variability of clinical presentation, lack of genotype-phenotype correlation, and rarity of the disease. Purtilo established an XLP registry in 1980, and by 1995 more than 270 boys had been identified in 80 kindreds.[2] To date this registry has provided the only data on clinical phenotype and prognosis for this patient group. Overall mortality in this group was 75%, with 70% of boys succumbing before 10 years of age. However, current outcomes for XLP may be very different due to the availability of unambiguous molecular diagnosis, improved viral monitoring, and the improvement in treatment regimens for disease manifestations.

XLP affects 1 to 3 million boys,[3,4] and most commonly presents in childhood or early adolescence. Presentation may be acute in the case of fulminant infectious mononucleosis (FIM)/hemophagocytic lymphohistiocytosis (HLH) or lymphoma or less aggressive with dysgammaglobulinemia or recurrent infections. Patients often manifest more than one phenotype and may progress from one phenotype to another, for example presenting with hypogammaglobulinemia and progressing to lymphoma, and different clinical features are often present in families highlighting the lack of genotype-phenotype correlation. Other rare but well-described presenting features include aplastic anemia, vasculitis, and chronic gastritis.[2,5-8] It is now known that the clinical syndrome of XLP arises from 2 different genetic defects in *SH2D1A* (XLP1, by far the most common and the focus of this report) and the *BIRC/XIAP* gene (XLP2). The gene responsible for XLP1 is the *SH2D1A* gene found on the X chromosome at position Xq25,[9-11] which encodes the cytoplasmic protein SAP (signaling lymphocyte activation molecule or SLAM-associated protein). SAP is a key regulator of normal immune function in T cells,[12-14] natural killer (NK) cells,[15-18] NKT cells,[19,20] and possibly B cells,[21] and defects in this protein lead to the varied immune defects described in XLP1 patients.[20,22] Humoral defects seen in this disease are thought to arise from impaired $CD4^+$ T-cell interaction with B cells and not an intrinsic B-cell deficit.[23]

Although it has always been presumed that EBV infection plays a crucial role in the development of clinical features in XLP1 patients, it is now clear that a proportion of boys are EBV negative at presentation and remain so. Indeed, 10% of patients have immunological abnormalities before any evidence of EBV exposure.[4,24] XLP1 is therefore a disorder of immune dysregulation rather than a disorder specifically associated with EBV infection.

Before 1994, acute management of FIM and HLH included antiviral medications, high-dose intravenous immunoglobulin (Ig), immunosuppressants, and other immune modulators such as interferon-α. These treatments proved disappointing[25] and the XLP registry data showed a survival of only 4% for boys presenting with these manifestations. Improved chemotherapy regimes for lymphoma and immunosuppressive protocols to treat HLH (including rituximab) may reduce the mortality rate for XLP1 patients and allow stabilization before hematopoietic stem cell transplant (HSCT).[26] Our report provides valuable outcome data collected since the introduction of current HLH treatment protocols, focusing on XLP1 patients with mutations in the *SH2D1A* gene.

Allogeneic HSCT remains the only curative option for XLP1 at present although large scale outcome studies are not available. Recently, Lankester et al reviewed 14 cases in the literature who had undergone HSCT and found an overall survival of 71% (10/14) with little evidence of EBV reactivation and posttransplant lymphoproliferative disease.[27] We describe here outcome data for a much larger cohort of patients transplanted since 1997.

There is no consensus on whether clinically stable XLP1 patients should undergo HSCT as the natural history of the disease is so variable, even within the same family. Treatment and management of the disease is severely hampered by the lack of data of a large cohort of patients and previously published outcome data are based on historical data, which may represent patients with conditions other than XLP1 as inclusion was based on clinical and not genetic diagnosis. Also, little recent data exist for patients who remain untransplanted. Hence, we describe a large cohort of genetically defined XLP1 patients collected from centers worldwide. The data presented will allow for better counseling of affected families regarding prognosis and management options, particularly in relation to timing of transplant.

Methods

Data collection

Questionnaires regarding patient demographics, transplant characteristics, and outcome were sent to centers worldwide identified through the European Society for Immunodeficiencies/European Bone Marrow Transplantation Registry, published case reports or centers known to perform pediatric HSCT. Retrospective analysis was performed using data collected for 91 patients from 32 centers worldwide. The number of cases from each center varied between 1 and 27 but was on average 1-2 cases. Patients included in this study were born between 1941 and 2005; 63 were born in or after 1990 (24 untransplanted patients and 39 transplanted patients). Only patients with a confirmed mutation in the *SH2D1A* gene were included in this series. Patients with mutations in other XLP-associated genes such as *XIAP/BIRC-4* were excluded, as were patients with abnormal SAP expression but no confirmed mutation in *SH2D1A*. EBV status was determined by polymerase chain reaction to avoid variable serology results in XLP1 patients and especially in those with dysgammaglobulinemia. Questionnaires offered reporting of FIM and HLH separately; thus, some centers with experience in this area reported patient data accordingly, and it is presented as such.

Data in various forms from 11 patients have been previously published[5,27-32] but standardized information was recollected in this study and added to the series.

Management of HLH and lymphoma

Patients who presented with HLH were managed predominantly in accordance with HLH 94 or HLH 2004 protocols. Additional or alternative treatment included antiviral therapy (aciclovir, ganciclovir, or foscarnet, n = 6), high-dose intravenous immunoglobulin (n = 9), immunosuppression (steroids, cyclosporine, and etoposide, n = 12), or anti-CD20 antibody (rituximab, n = 10). Intrathecal therapy was used where central nervous system involvement was suspected. Ten patients who proceeded to transplant received rituximab therapy before transplant, either as treatment for HLH or during conditioning.

Regimes for the treatment of lymphoma varied in line with appropriate national guidelines (eg, COPAD [cyclophosphamide, vincristine, prednisone, and doxorubicin]) study, Berlin-Frankfurt-Munster Group, Associazione Italiana Ematologia Oncologia Pediatrica, or United Kingdom Children's Cancer Study Group guidelines) and only occasionally involved surgical management.

Statistical analysis

Kaplan-Meier curves were used to analyze survival figures. The log rank test (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests were used to compare survival between different groups. Statistical analysis including hazard ratio calculation was performed using GraphPad Prism Version 5.00 for Windows.

Table 1. Presenting symptoms and features of XLP1 patients with associated mortality

	Incidence	Mortality
Presenting symptom		
HLH	31.9%	65.5%
FIM	7.7%	14.3%
Lymphoma	14.3%	7.7%
Dysgammaglobulinemia	22%	5%
Family history of XLP1 alone	16.5%	20%
Other	7.7%	14.3%
Features occurring at any time		
HLH	35.2%	65.6%
FIM	9.9%	22.2%
Lymphoma	24.2%	9%
Dysgammaglobulinemia	50.5%	13%
Other	15.4%	28.6%

Results

Data from 91 patients (64 pedigrees) in 32 centers worldwide were included in this report. The overall survival of XLP1 patients was 71.4% (65/91), and patients displayed a heterogeneous clinical phenotype. Due to the heterogeneity of the group, data were analyzed according to presentation with HLH, EBV status, and whether patients had received HSCT, allowing characterization of outcome after transplant.

Spectrum of XLP1 mutations

In keeping with previous publications, no genotype/phenotype correlation was evident, and the most frequently reported mutation involved the arginine residue at position 55 (exon 2) found in 11 patients from 9 different families. Detailed genetic information was available for 62 patients (50 pedigrees; supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Exon 2 had the most mutations with missense mutations accounting for the majority but nonsense, frameshift, and splice site mutations were also reported. Large gene deletions (up to 11 Mb) including those involving the whole gene were identified in 5 families. Three of these larger deletions were associated with gastrointestinal symptoms of colitis and gastritis. Such symptoms were not found in patients with other mutations apart from a patient with diarrhea as a feature (missense mutation exon 1, 62 T > C). In a further 29 patients, detailed genetic data were not supplied but a SAP/*SH2D1A* gene defect was confirmed by the documenting center.

Clinical manifestations of XLP1

Table 1 shows the presenting features of disease as well as features of disease manifesting throughout the course of the condition. HLH remained the most common presenting feature (39.6%), although dysgammaglobulinemia was the manifestation seen most commonly in patients during the course of the illness.

Although clinical features have remained similar to previously published data,[2] the survival associated with XLP1 is 71.4%, which is significantly improved over historical survival of 25%. The survival associated with different phenotypes has also changed significantly with mortality associated with HLH decreased from 96% to 65%, lymphoproliferative disease from 35% to 8%, and dysgammaglobulinemia from 55% to 5%.

Twenty-two patients suffered from malignant lymphoproliferative disease, with eighteen patients (81.8%) diagnosed with B-cell non-Hodgkin lymphoma mainly of the abdomen and cervical region. In 5 patients the disease was recurrent, with 1 patient experiencing a cerebral tumor. Only 1 patient was reported with cerebral T-cell lymphoma. Data on tumor histology is lacking in 3 patients.

Immunological abnormalities at diagnosis

Details of immune function were available for 57 patients, although in some cases, data were only available after the onset of disease manifestations that may have influenced immunoglobulin and lymphocyte subset levels. Immunoglobulin levels were recorded in 49 patients, and 32 of these showed varying degrees of abnormal immunoglobulin levels. Twelve children presented with neutropenia. Lymphocyte subset data were available for 47 patients; 19 showed a reduced percentage of B cells, 26 showed low NK cell numbers, and 12 had a reversed CD4:CD8 ratio.

Presentation with HLH

The mortality for patients presenting with HLH was 65.6%, with a median age at presentation of 3 years 2 months (range 8 months to 9 years). Of the 32 patients with HLH, 16 underwent transplant, of whom 8 survived (50%; Figure 1). Of those who did not receive a transplant, only 3 survived (18.8%), confirming previous reports that the prognosis for patients with HLH associated with a genetic defect is extremely poor and that HSCT is necessary.

EBV status

EBV status was documented in 79 patients showing that 51 (64.6%) were EBV positive at presentation or diagnosis (Table 2 and supplemental Figure 1). The median age of presentation in this group was 4 years (range 8 months to 40 years), and the overall mortality was 35.2% (18/51). There was no significant difference in mortality between patients with (35.2%) and without (28.6%) documented EBV infection.

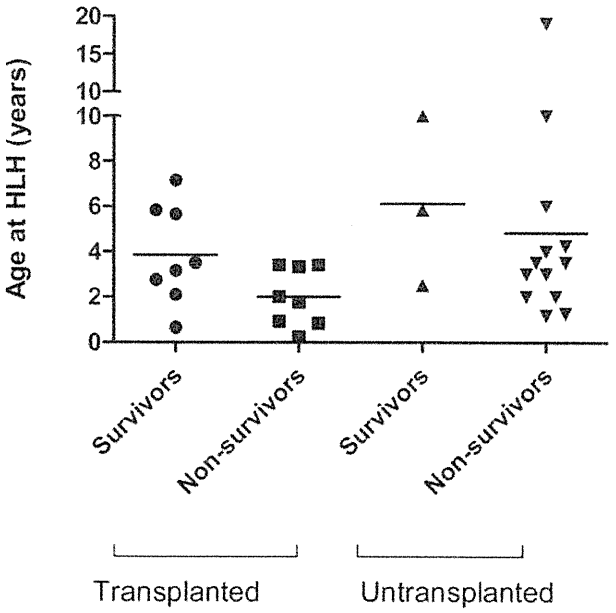

Figure 1. Outcome of patients with HLH during course of disease. Survival of patients who present with HLH—patients who remain untransplanted have a poor survival outcome with only 18.8% survival. By contrast the survival of those who undergo transplant is higher at 50%.

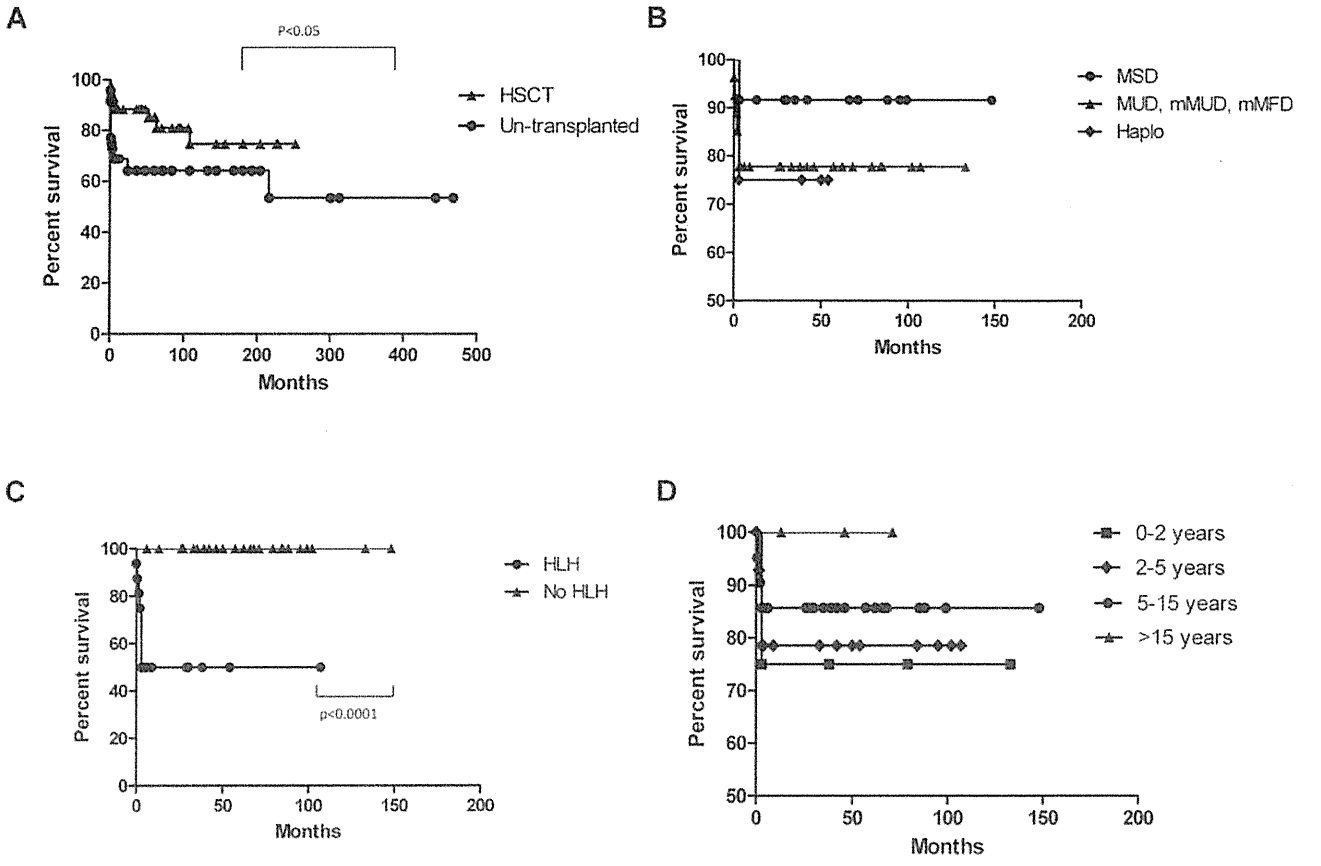

Figure 2. Survival in XLP1 related to different variables. (A) Overall survival of transplanted versus untransplanted patients. In the transplanted group this represents time from presentation and not transplant. (B) Survival according to donor source. (C) Survival after HSCT with relation to presence of HLH before transplant. (D) Survival according to age at transplant.

HLH/FIM was the most common feature in this group being seen in 35 patients (68.6%), with lymphoma present in 10 patients (19.6%), and dysgammaglobulinemia in 19 (37.2%). Nine EBV-positive patients had a family history of XLP1, and two others had a family history suggestive of an X-linked immunodeficiency. Of the 18 EBV-positive patients who died, the majority (14/18) died within 2 months of presentation due to disease progression. Three died in the early posttransplant period of infective complications and disease progression, and 1 died during treatment for lymphoma.

Twenty-eight patients were EBV negative at presentation or diagnosis. The median age of presentation for this group was 3 years (range birth to 31 years). Family history of XLP1 was the presenting feature for 12 patients, and a further 7 patients described a family history suggestive of an X-linked immunodeficiency or lymphoma. There was a higher rate of dysgammaglobulinemia (51.8%) in this group. Lymphoma was present in 7 patients. Fewer EBV negative patients presented with HLH/FIM, and this may suggest that at least for this manifestation a viral trigger is important. Information was sought on other viral infectious agents including cytomegalovirus and adenovirus, but data were not available for most patients. Other clinical features included aplastic anemia in 3 patients and vasculitis in 2 patients. The mortality for this EBV negative group was 28.6% (8/28); 3 patients died shortly after presentation before HSCT with central nervous system vasculitis (2) and HLH with enterococcal sepsis (1). One patient died 11 years after presentation following a complex course, and a further 4 patients died in the early posttransplant period (described in Table 5).

Table 2. Characteristics of EBV-positive and EBV-negative XLP1 patients

	EBV positive (64.6%, n = 51)	EBV negative (35.4%, n = 28)
Median age at presentation	4 y (8 mo-40 y)	3 y (0-31 y)
Family history of XLP1	17.6%	42.9%
HLH	51%	21.4%
FIM	17.6%	
Lymphoma	19.6%	25%
Dysgammaglobulinemia	37.2%	51.8%
Mortality	35.2%	28.6%
Median age at death	3 y 6 mo (14 mo-21 y)	5 y 11 mo (20 mo-31 y)

HSCT for XLP1

HSCT was undertaken in 22 centers (range of patients/center: 1-7) between 1997 and 2009 (Table 3). Forty-six transplants were performed on 43 patients, and the median age at transplant was 6.25 years (range 8 months to 19 years); 1 patient who had undergone a haploidentical transplant received a $CD34^+$ selected boost 1 year after initial transplant. One patient received an allogeneic HSCT to treat lymphoma before a diagnosis of XLP1 was established. Most patients received bone marrow or peripheral blood stem cells, and only 2 patients received umbilical cord HSCT. Donor grafts were from human leukocyte antigen-matched family donors in 14 cases, mismatched family donors or matched unrelated grafts in 28 cases, and haploidentical donors in 4 cases. Half of the transplant procedures (23/46) were performed using myeloablative conditioning regimes including combinations of

Table 3. Characteristics of XLP1 patients receiving allogeneic HSCT

	Percentage	Number	1-y survival	HR	95% CI	*P*
XLP1 features						
Previous HLH	37.2%	16/43	50%	23.93	5.31-108.0	< .0001
Previous NHL	27.9%	12/43	74.2%	0.23	0.05-1.06	.06
Previous dysgammaglobulinemia	46.5%	20/43	80%	1.2	0.29-4.96	.77
EBV+	51.2%	21/41	75%	1.37	0.36-5.3	.65
Age at HSCT	Mean 7 y (8 mo to 19 y 7 mo)					
0-2 y	9.3%	4/43	75%	5.75	0.11-302.1	.38
2-5 y	34.9%	15/43	78.6%	3.61	0.18-71.76	.40
5-15 y	48.8%	21/43	85.7%	3.16	0.11-90.83	.50
> 15 y	7%	3/43	100%			
Year of HSCT						
< 2000	7.0%	3/43	66.7%			
2000-2005	37.2%	16/43	87.5%			
2005-2009	55.8%	24/43	79.2%			
Donor Type						
MSD, MFD	30.4%	14/46	91.77%			
MUD, mMFD, mMUD	60.9%	28/46	77.8%	0.42	0.08-2.07	.27
Haplo	8.7%	4/46	75%	0.24	0.01-6.58	.4
Source						
Bone marrow	58.5%	24/41*	82.6%			
Peripheral blood	36.6%	15/41*	92.9%			
Umbilical cord	4.9%	2/41*	50%			
Conditioning						
MA	50%	23/46	82.9%			
NMA	50%	23/46	78.9%	1.25	0.30-5.2	.77
Serotherapy	30.4%	14/46				
GVHD	50%	19/38				
Grade 1	18.4%	7/38				
Grade 2-3	26.3%	10/38				
Grade 4	5.3%	2/38				
Chronic	5.3%	2/38				
Chimerism						
Full (> 98%)	92%	35/38	100%			
Mixed	8%	3/38	88.8%	2.98	0.06-151.0	.59
Replacement IVIg	20%	7/35†				
Alive	81.4%	35/43				
Follow up	6 wk to 148 mo					

*Data missing on 5 transplants, 1 died during conditioning.

†Three patients < 1 year after transplant.

CI indicates confidence interval; HR, hazard ratio; MSD, matched sibling donor; MFD, matched family donor; MUD, matched unrelated donor; mMFD, mismatched family donor; mMUD, mismatched unrelated donor; Haplo, haploidentical transplant; MA, myeloablative; and NMA, nonmyeloablative.

busulfan 12-20 mg/kg, cyclophosphamide 50-200 mg/kg, and total body irradiation 5-12 Gy. The other half of procedures used nonmyeloablative conditioning regimes consisting of fludarabine (30 mg/kg), melphalan (70-140 mg/kg), busulphan (4-12 mg/kg), or total body irradiation (3-5 Gy). Twenty-six patients received additional serotherapy with alemtuzumab, anti-thymocyte globulin, anti-CD3 antibody, and anti-CD20 antibody (rituximab). Graft-versus-host disease (GVHD) prophylaxis regimes differed between centers, but mostly involved combinations of cyclosporin with methotrexate, mycophenolate mofetil, steroids, and tacrolimus. T-cell depletion of the graft was used in 1 case.

Outcome for XLP1 patients who received allogeneic HSCT was good with 81.4% surviving the procedure (35/43) with a median follow up of 52 months. The majority of these patients (28/35 survivors) required no ongoing immunoglobulin replacement therapy. Tables 3 and 4 highlight details of transplanted patients, and Figure 2 describes survival according to several factors.

Sixteen patients were diagnosed with HLH before transplant and 12 patients had some form of lymphoproliferative disease (lymphoma). Only 51.2% of the cohort had documented evidence of EBV infection (by polymerase chain reaction) with survival rates in EBV+ patients similar to those without EBV infection (75% vs 80%). Most patients experienced some delay from first symptoms to diagnosis (average delay 2 years 7 months) but once a diagnosis of XLP1 was established time to transplant was generally less than 1 year. Median age at transplant was 6.25 years with a range of 8 months to 19 years.

Univariate analysis was performed to identify the major risk factors for survival after HSCT. The most important risk factor was prior HLH, which significantly decreased the survival outcome to 50%. A previous diagnosis of lymphoma had a near significant effect, but other variables were not shown to have a significant effect including importantly, previous evidence of EBV infection, the age at transplant, donor type, or the conditioning regime. It is also important to note that only patients who had HLH at some point before or during transplant died. Conversely, all patients without HLH (n = 27) survived the transplant procedure.

Half of the patients underwent a nonmyeloablative conditioning regime before HSCT and this did not impact on survival (nonmyeloablative vs myeloablative, 78.9% vs 82.9%) or long-term chimerism. More than 90% of patients achieved full donor chimerism, and

Table 4. Details of XLP1 patients surviving allogeneic HSCT

Year of HSCT	EBV	HLH	Age at HSCT	Donor	Conditioning/serotherapy/ graft manipulation	GVHD prophylaxis	GVHD	Chimerism	Follow up (mo)	Ig
1997	NK		7 y	MSD	Cy, TBI	MTX, CSA	1 S*	100%	148	
1998			1 y	MUD	Bu, Cy, ATG	MTX, CSA, P	1 S	100%	133	
2000	+		4 y	MUD	Bu, Cy, Campath	CSA	2 S	100%	102	
2000	+	Yes	3 y	mMUD	Bu, Cy	MTX, CSA	2 S, L	100%	107	
2001			4 y	MUD	Flu, Melph, ATG, TBI	MMF, CSA		100%	102	
2001	+		10 y	MSD	Bu, Cy, VP-16 (NHL)	MTX, CSA	2-3 GI	100%	99	
2001			4 y	MSD	Bu, Cy	CSA	2 S	100%	95	
2002			13 y	MSD	Thio, Flu, ATG	CSA		100%	88	
2002	+		7 y	MUD	Bu, Cy, ATG	MTX, CSA	1 S	100%	85	
2002			3 y	MUD	Bu, Cy, ATG	MTX, CSA		100%	84	
2003			8 mo	mMUD	Flu, Melph, ATG, TBI	TAC, MTX, P	S	100%	79	
2003			19 y	MSD	Thio, Flu, ATG	CSA		100%	71	
2003			11 y	mMUD	Flu, Melph, ATG	MMF, CSA		100%	68	
2004	+		5 y	MSD	Bu, Cy	MTX, CSA		20% PBMC	66	
2004	+		12 y	mMFD	Flu, Melph, Campath, 34+	MMF, CSA	4 S, L*	100%	62	Y
2004	+		8 y	mMUD	Flu, Melph, Campath	CSA	2-3 S, GI	100%	57	
2005	+	Yes	2 y	Haplo	Bu, Cy, ATG, 34+	CSA		100%	54	Y
2005			2 y	Haplo	Bu, Cy, ATG, 34+, top up 1 year	CSA		88% PBMC 97% M	50	Y
2005			12 y	mMUD	Flu, Melph, Campath, 34+	MMF, CSA	3 S	100%	46	
2005			18 y	MUD		NK		NK	46	NK
2006			5 y	MSD	Bu, Cy	CSA	1 G, 3 S	100%	42	
2006	NK		2 y	MUD	Bu, Flu, Campath	MTX		100%	42	
2006	+		7 y	Haplo	Flu, Melph, Thio, OKT3, ATG			100%, 75% CD3	39	
2006	+	Yes	1 y	MUD	Flu, Melph, Ritux	CSA	1 S	5%	38	
2006	+		11 y	MSD	Bu, Cy, ATG	MTX, CSA		100%	35	
2006			4 y	MUD	Bu, Cy, Campath	MMF, CSA	1 S	100%	33	
2007	+	Yes	6 y	MSD	Bu, Cy	MTX, CSA		99%	30	
2007	+	Yes	7 y	MSD	Bu, Cy	CSA	3 S, L, GI	100%	29	
2007	NK		7 y	MUD	Flu, Melph, TBI	TAC, MTX		98%	27	
2007			7 y	MSD/mMUD	Bu, Cy	CSA	2 S, GI	100%	26	
2008			17 y	MFD	Flu, Melp, Campath	MMF, CSA		100%	13	
2008		Yes	3 y	MUD	Bu, Flu	TAC, MTX		100%	9	Y
2009	+		7 y	MUD	Bu, Cy			100%	6	Y
2009	+	Yes	6 y	mMUD	Flu, Melph, Campath	CSA, MMF	1 S	100%	5	Y
2009	+	Yes	3 y	MUD	Thio, Cy, ATG	CSA, P		100%	4	Y

*Chronic GVHD.

PBMC indicates peripheral blood mononuclear cell; Flu, fludarabine; Melph, melphalan; 34+, CD34+ stem cell infusion; Bu, busulfan; Cy, cyclophosphamide; Thio, thiotepa; TBI, total body irradiation; CSA, cyclosporin A; MMF, mycophenalate mofetil; MTX, methotrexate; P, prednisolone; TAC, tacrolimus; S, skin; GI, gastrointestinal; L, lung; and Ig, replacement immunoglobulin.

those with a mixed or falling chimerism remained well with 1 patient still receiving replacement immunoglobulin.

Data were also collected on common posttransplant complications such as GVHD, infectious complications and toxicity attributable to chemotherapy. Half of the patients (50%) suffered from some form of GVHD; the majority of cases were grade 1-3 affecting the skin, liver, and gut. Two patients suffered grade 4 disease (of skin and liver), and 1 of these children died. Only 2 patients went on to develop chronic GVHD (see Table 3). One patient experienced both veno-occlusive disease and renal toxicity due to conditioning (busulfan, cyclophosphamide, and antithymocyte globulin), and this patient succumbed shortly after a haploidentical transplant.

In 3 patients with mixed chimerism in peripheral blood mononuclear cells, this remained stable in all but 1 patient, in whom it fell from 92% to 5%. However, this patient remains well 3 years posttransplant and does not require replacement immunoglobulin therapy. From this series, there is little evidence of viral reactivation posttransplant. Thirty-five patients are alive with 5 suffering some long-term effects including EBV viremia (managed with rituximab), bronchiectasis, autoimmune disease, chronic psoriasis, and neutropenia.

Eight patients did not survive after HSCT (see Table 5). Seven patients who died presented with HLH before HSCT (4/7 EBV+) compared with 8 of 35 survivors, but HLH was a feature of disease in all 8 nonsurvivors. The majority of nonsurvivors were ≤ 3 years old (5/8), and conditioning regime did not appear to play a role as 5/8 patients received a full myeloablative regime. The main cause of death in this group was sepsis, but disease progression accounted for 2 deaths. The 2 children dying with disease progression went into transplant with active disease; 1 died during conditioning and the other 3 days after HSCT. One further patient died 3 weeks after HSCT (7 months after presentation) from veno-occlusive disease (VOD), multiorgan failure, and renal toxicity attributable to chemotherapy. The remaining 5 patients died of sepsis (2 pseudomonal sepsis, 1 parainfluenza III infection, 1 with disseminated adenoviral infection, and 1 with EBV and fungal infection) within 3 months of HSCT.

Untransplanted patients

Data were available for 48 patients who did not receive HSCT (Table 6); 30 are alive, 4 of whom are actively awaiting transplant, and 3 who refused HSCT. One patient had received an autologous HSCT before diagnosis with XLP1, and this patient's data were

Table 5. Details of XLP1 patients not surviving allogeneic HSCT

EBV	HLH	Age at HSCT, y	Year of HSCT	Donor	Conditioning/serotherapy/ graft manipulation	GVHD prophylaxis	GVHD	Chimerism	Cause of death
−	Yes	2	2005	MMFD	Flu, TBI	N/A			Died during conditioning 6 wk from presentation
−	Yes	3	2003	MUD	Bu, Flu, Campath, Rituximab	CSA			Died 3 d after HSCT disease progression
	Yes	6	2005	MMFD	Bu, TBI	MMF. MTX, P			Died 14 d after HSCT MDR pseudomonal sepsis
−	Yes	3	2009	Haplo	Bu, Cy, ATG	TCD			Died 3 wk after HSCT VOD, MOF, renal toxicity
− (after HSCT)	Yes	5	2008	mMUD (cord plus PBSC 4 months later)	Bu, Flu, ATG then Flu, TBI	TAC, P		100%	Died 2 mo after second HSCT EBV, fungal, and ?PCP sepsis
	Yes	3	1998	MSD × 2	Flu, Melph	CSA, P		100%	Died 3 mo after HSCT *Pseudomonas* sepsis
−	Yes	12	2003	MUD	Bu, Cy, Flu, Campath		4 S	100%	Died 3 mo after HSCT disseminated adenovirus
	Yes	1	2007	MUD	Flu, Melph, ATG, 34$^-$	CSA	2-3 S, L	100%	Died 3 mo after HSCT paraflu III sepsis

PBSC indicates peripheral blood stem cell; Flu, fludarabine; Melph, melphalan; 34$^+$, CD34$^+$ stem cell infusion; Bu, busulphan; Cy, cyclophosphamide; Thio, thiotepa; TBI, total body irradiation; CSA, cyclosporine A; MMF, mycophenalate mofetil; MTX, methotrexate; P, prednisolone; TAC, tacrolimus; TCD, T-cell depletion; S, skin; L, lung; VOD, veno-occlusive disease; MOF, mullti-organ failure; MDR, multidrug resistant; and PCP, *Pneumocystis jiroveci*.

analyzed as though untransplanted. Less detailed information was available for this set of patients compared with those receiving HSCT. This may be because some patients died before EBV status and immune function could be established and any first symptoms may not have been recognized as a manifestation of XLP1. From data available, median age at presentation was 5 years, and delay in diagnosis ranged from a few weeks to 32 years.

Presentation was highly variable but as expected included HLH/FIM, dysgammaglobulinemia, and recurrent infection. More unusual presentations included 1 patient with central nervous system vasculitis, intracranial hemorrhage and myocardial fibrosis, and peripheral eosinophilia. The course of XLP1, both temporal and clinical, was extremely variable without any apparent correlation to family history or genetic mutation.

Table 6. Characteristics of XLP1 patients not receiving HSCT

		Number
Age at first symptom	8 y 8 mo (6 mo-40 y)	
Age at death	7.5 y (1-31 y)	
Time from presentation to death	17.3 mo (1 NK) 9 d-18 y	
Time from first symptom (in those patients alive)	12 y (1 NK) 1-39 y	
Presenting symptom		
HLH	31.3%	15/48
FIM	10.4%	5/48
Lymphoma	16.7%	8/48
Dysgammaglobulinemia	29.2%	14/48
Other	12.5%	6/48
Features		
HLH	33.3%	16/48
FIM	12.5%	6/48
Lymphoma	20.1%	10/48
Dysgammaglobulinemia	56.3%	27/48
Gut	8.3%	4/48
Other	14.6%	7/48
EBV status		
EBV$^+$	66.6%	32/48
EBV$^-$	14.6%	7/48
Unknown	18.8%	9/48
Mortality	37.5%	18/48 (4 EBV$^-$)
Associated with HLH	81.3%	13/16
Associated with FIM	33.3%	2/6
Associated with lymphoma	20%	2/10 1 had previous HLH and died during chemotherapy; 1 had recurrent lymphoma and many other problems
Immunoglobulin replacement		
Yes	70%	21/30
No	23.3%	7/30
Unknown	6.7%	2/30