分類不能型免疫不全症

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分類不能型免疫不全症は，抗体産生異常を主体とする原発性免疫不全症であるが，症例が多く，多彩な臨床症状を呈し，かついまだに原因が不明という点から暫定的に分類された疾患群である．10歳代以上に発症することが多く，自己免疫疾患や悪性腫瘍などの合併頻度も高い．その原因として，tumor necrosis factor(TNF)-TNF receptor(TNFR)ファミリー分子やCD19複合体分子の異常が同定されたが，これら以外にも10以上の原因が存在すると推測されている．責任遺伝子解明への戦略が重要である．

はじめに

Common variable immunodeficiency(CVID)は，従来分類不能型免疫不全症と翻訳されていたが，症例数が多く(common)，多彩な臨床症状をとる(variable)，分類不能な疾患であるために，暫定的につけられた名称がそのまま用いられている．

CVIDは，欧州免疫不全症学会(European Society for Immunodeficiencies：ESID)によれば，「2歳以上(多くは10歳代以降)で発症する低γグロブリン血症で，同種血球凝集素の欠損，あるいはワクチンへの低反応を示し，既知の免疫不全症ではない疾患」と定義されている．疾患概念は不明瞭であり，現時点において基本的には除外診断となっている(除外すべき免疫不全症については**表1**を参照)．30～50%が自己免疫疾患を合併すること，悪性腫瘍の発生も多いことなどの問題も内包する．多くの免疫不全症は小児科医が診療にあたっているが，CVIDの大半は成人であることに加えて，40歳代以降の発症もあり，患者の実態も明らかではない[1)～3)]．

近年，CVIDの病態が徐々に明らかになり，一部では責任遺伝子が判明しつつある．しかし，大半の責任遺伝子は特定されていない．また，各国からの報告から，CVIDと診断される患者のなかに，①X連鎖無γグロブリン血症(X-linked agammaglobulinemia：XLA)，②複合型免疫不全症(combined immunodeficiency)，③免疫グロブリンクラススイッチ異常症(高IgM症候群)，④X連鎖リンパ増殖性疾患(X-linked lymphoproliferative disorder)などの疾患が含まれていることが明らかになっている．

わが国では2009年に，厚生労働省難治性疾患克服事業において，「分類不能型免疫不全症に関する研究班」が立ち上がり，筆者らの全国調査や検査によりわが国におけるCVIDの実態や，免疫学的特徴が明らかになった．ここでは，既知の事項からはじめ，候補遺伝子を含めた今後の展開について概説する．

[キーワード]
CVID
記憶B細胞
KRECs/TRECs
whole exome sequencing

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表 1. CVIDの鑑別診断が必要な原発性免疫不全症

(1) B細胞欠損症**
BTK, IGHM, CD79A, CD79B, λ5, BLNK
(2) X連鎖リンパ増殖性疾患*
SAP, XIAP, ITK
(3) 複合型免疫不全症(SCIDを含む)**
ADA, CD25, STAT5b, ITK, DOCK8, LIG4, NHEJ1, RMRP, ATM
(4) 免疫グロブリンクラススイッチ異常症(高IgM症候群)***
CD40LG, CD40, AID, UNG, PMS2, RNF168, NEMO
(5) 胸腺腫を伴う免疫不全症(年長者が大半である)
(6) IgGサブクラス異常症
(7) その他
骨髄不全症候群(先天性角化異常症など)****
ICF, VODI, WHIM

*FACSにて第1次解析が可能であるもの(TACIを除く).
**KRECs, TRECs測定が有用なもの.
***CD40, CD40LG, NEMOなどはFACSにより検討することが可能である.
****特徴的な臨床症状あるいはマイトマイシンC(MMC)感受性, テロメア長の測定などが診断に有用である.

1. CVIDの臨床像と検査データ

CVIDでは, 特徴的な発症時期, 罹患しやすい病原体, 特徴的な身体所見は認めない. しかし, 多くは10歳代以降で発症するとされている. 2009年10月までに, 全国調査によって199例のCVIDについての情報が集まったが, 2歳未満で診断に至った症例は27例であった. これらの患者の大半は10歳代を超えていて, B細胞欠損やT細胞異常症の範疇に入らない疾患である. 一方, 50歳を超えて診断される患者も7例あり, 悪性腫瘍を含む合併疾患や薬物の影響の否定などが重要となってくる.

身体的特徴としては, 肝脾腫を呈する症例が比較的多い程度であり, 皮疹(アトピー様, 乾癬様, 多型滲出性紅斑様など), 神経症状, 発達遅滞などの合併も認める. 感染症としては, いわゆるsinopulmonary diseaseが多いが, Epstein-Barrウイルス(EBV)感染症, サイトメガロウイルス(CMV)感染症, パピローマウイルス感染症など, T細胞機能不全を疑わせる症例も散見される. 自己免疫疾患を合併するものは全体で19%, 40歳以上で36%, 悪性腫瘍の合併は全体で10%, 40歳以上で19%であった. 自己免疫疾患として最も多いのは, 自己免疫性溶血性貧血や自己免疫性血小板減少症であるが, 関節リウマチ, 炎症性腸疾患, 多発筋炎などさまざまな疾患を認める. 悪性腫瘍ではリンパ系悪性腫瘍が多いが, 甲状腺腫瘍, 子宮頸癌, 消化器系腫瘍も散見される.

2. CVIDの病因

CVIDでは, 現在までにCD19, CD21, CD81, CD20, B cell-activating factor (BAFF) receptor (BAFF-R), transmembrane activator and calcium modulator and cyclophillin ligand interactor (TACI), inducible T cell costimulator (ICOS)などが責任遺伝子として同定され, 新たな疾患として独立したが, いまだに分類上はCVIDの範疇に入っている[2)~9)]. ICOSを除き, B細胞上に発現する分子である. ICOSは活性化に伴いT細胞上に

表出され，B 細胞上の ICOS ligand(ICOS-L)と会合し，シグナルを伝えるが，ICOS 欠損では T 細胞に大きな欠陥が認められ，Th1，Th2，Th17，IL-10 producing requlatory T cell(Treg)の減少および IL-2，IL-22 を除くサイトカイン産生の低下を示した[3]．T 細胞全体としては，記憶 T 細胞の著減が特徴的である．同様の補助分子として，BAFF-R に会合する BAFF があげられる．また，BAFF には BAFF-R 以外に，TACI および B cell maturation antigen(BCMA)が会合する．さらに TACI，BCMA は，BAFF 類似分子である a proliferation-inducing ligand(APRIL)にも会合する．T-B 細胞相互作用に関与する分子群を**図 1** に示した．赤の点線で囲んだ分子(CD40，CD40L)の異常は，高 IgM 症候群としてクラススイッチ異常の表現型をとる．青の点線で囲んだ分子による免疫異常症(CVID)が明らかになっている．それぞれの分子の役割は当然異なるが，リガンドの分子の異常が同様の疾患を呈することは十分に考えられる．APRIL，BAFF，BCMA の遺伝子解析は精力的におこなわれているが，いまのところ分子異常は見つかっておらず，少なくとも CVID の major な集団ではないことは明らかである．

一方，CD19，CD81，CD21 は CD19 複合体を形成する分子群であり，B 細胞受容体を介したシグナルは減弱するが，B 細胞は存在する．しかし，分子欠損により，骨髄における正常な B 細胞の発生と成熟が侵されていると考えられている[5][6]．

3．CVID の病因探索

それでは，CVID の原因としてどのような分子異常が想定されるであろうか？われわれは，①臨床症状の特徴，②免疫担当細胞亜群分類，③B 細胞，T 細胞新生能，④候補遺伝子探索をもとに，

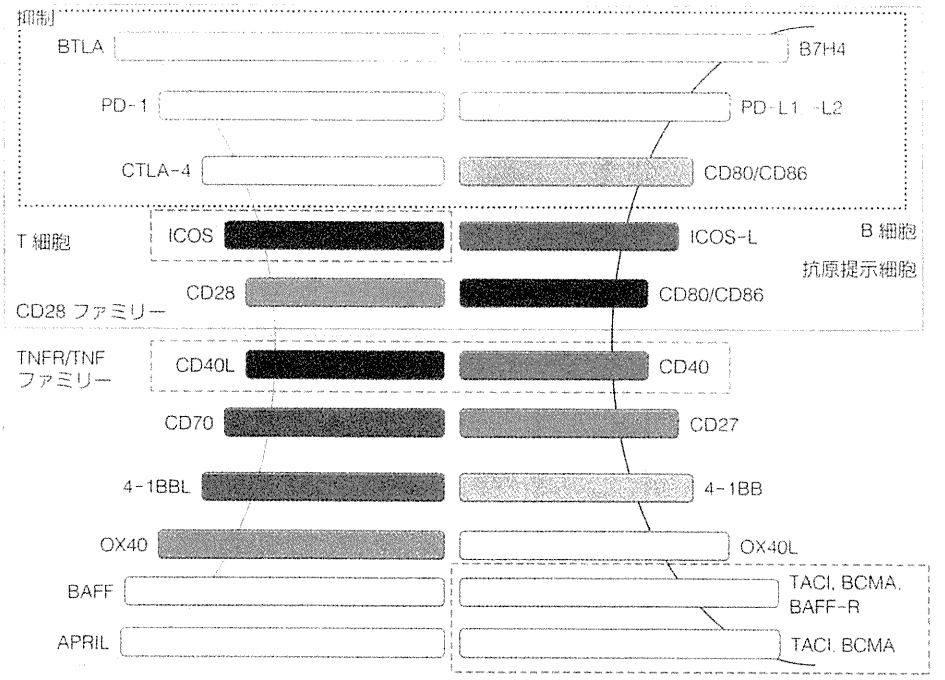

図 1．T 細胞と抗原提示細胞(および B 細胞)との相互作用
青の点線で囲った分子は，CVID の責任遺伝子として同定されている(BCMA を除く)．赤の点線で囲った分子は，高 IgM 症候群の責任遺伝子である．

CVIDにおける特徴を探り出し，またCVIDの亜群分類をおこなうことを試みた．

まず臨床上の特徴では，感染症や免疫異常を合併する若年発症例と，年長になってから採血などで偶然に発見される症例とでは，原因が異なることが想定される．また，精神・神経症状を伴う群，低身長を伴う群，自己免疫疾患が前面に出る群，特徴的な感染症を合併する群などは，おそらく機能が類似する分子の異常によって生じる疾患であろうかと推察される．

その結果，以前からの報告にあるように[1,2]，CVIDではCD27陽性クラススイッチ記憶B細胞が減少しており，それ以降の形質芽細胞の減少も認められた．一方，IgM陽性記憶B細胞はさまざまなデータを示した．ナイーブB細胞に至る前の移行B細胞などの検討結果も蓄積しつつある．T細胞では，ナチュラルキラーT(NKT)細胞が減少する群や，増加する群がある一方，T細胞受容体(T cell receptor：TCR)Vβレパトアは約50%で偏りを認めた．NK細胞も著減群と増加群を認める(**図2**)．

T細胞，B細胞新生能では，防衛医科大学小児科 今井，野々山らがマススクリニーニングなどへの応用研究を進めるT cell receptor excision circles (TRECs)，immunoglobulin kappa deleting recombination excision circles (KRECs)測定にて検討した[10]．その結果，TRECs，KRECsがともに低いものが12～15%．TRECs. KRECsが単独で減少する群が10%程度と，約2/3ではB細胞あるいはT細胞の新生能に欠陥があることが明らかになっている．

B細胞でのCD19，CD20発現，BAFF-R発現，刺激後のICOS発現をfluorescence-activated cell sorting(FACS)で検討するとともに(TACI

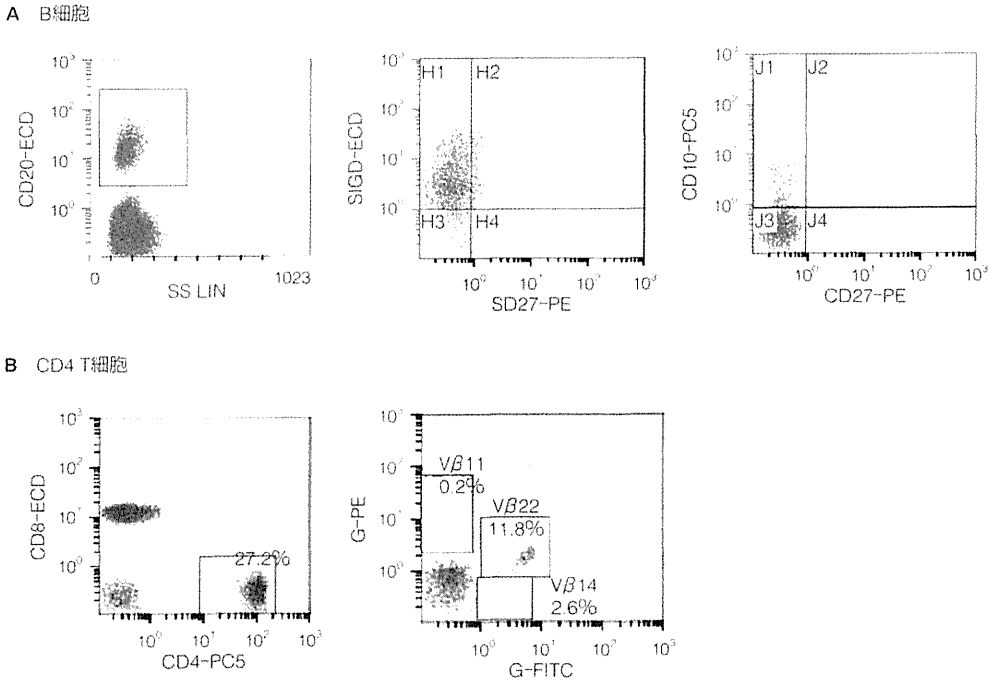

図 2. CVID末梢血リンパ球の典型的FACS解析所見

A：CD27陽性記憶B細胞の著減とCD10陽性移行期B細胞の増加が認められる．

B：CD4 T細胞比率はやや減少しており，そのなかでのVβレパトアに偏りが認められる．

異常症ではヘテロ異常が多くFACSデータはスクリーニングとならない)，候補遺伝子探索では，既知の遺伝子の塩基配列を決定し，男性ではBruton's tyrosine kinase(Btk)，Src homology 2 domain-containing protein 1A(SH2D1A)も検討し，30例程度のCVID患者で探索的にAPRIL，BAFF，BCMAを解析した．その結果，2例のICOS異常症が，1例のTACI異常症が明らかになった．それ以外のCVIDの原因は不明である．

それでは，どのような病因を念頭に置いて検索すべきであろう．問題となる分子としては，B細胞と相互作用し胚中心形成に重要なfollicular helper T cells，follicular dendritic cells，分化に重要な転写因子，B細胞の生存やアポトーシスに関与する分子，B細胞の2次リンパ組織への流入や，そこからの流出に関係する分子などが想定される．「問題となる分子」という定義には，分子自体の遺伝子変異に加えて，分子に対する阻害(自己抗体)の存在なども想定されることが話を複雑にする．

われわれは一時期，患者においてB細胞，CD4 T細胞，CD8 T細胞を高度に精製し，刺激後にmRNA expression profilingをおこなって，その差を示す分子を同定しようと試みたが，数例の解析で得られる有用な情報は少なかった．逆にICOS欠損症では，診断が確定してからこの手法を用いて解析し，発現が異なる分子の1つとしてICOSがピックアップされたが，その他の100以上の分子に埋もれる形になっていた．

今後の遺伝子同定の戦略としては，きわめて類似したCVIDの一群をピックアップし，その特徴的免疫担当細胞サブセットから，異常に関与する分子を想定する方法と，10例前後以上の患者において，あるいは家族例のあるCVIDにおいて免疫現象に関与する分子を網羅的に遺伝子解析する手法，さらにはwhole exome sequencing(マイクロRNAも含む)を用いる手法などが想定される[11]．家族例であれば，single nucleotide polymorphism(SNP)arrayなどで領域を狭めたうえで，deep sequencingを実施する方法も考えられる．いずれにせよ，これらの分子異常と表現型をつなぐためには，免疫機能の詳細なカタログ化が必須である．各国ではすでに大規模SNP arrayや，whole exome sequencingを導入し，いくつかのグループは責任遺伝子同定に近い立場にある．

おわりに

CVIDの病態がさらに明らかになることにより，その基準は変化するものと思われる．現時点でのCVIDの診断基準は誌面の都合上提示できないが，適宜分類不能型免疫不全症班研究のホームページ(準備中)などを通じてupdateしていく予定である．CVIDの解析から，自己免疫疾患や悪性腫瘍発生の分子機構が明らかになる可能性もあり，今後全国規模での症例の集積と詳細な解析による成果が期待される．

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In conclusion, the associations among asthma, biofilm-forming bacteria, and revision ESS are strong and robust after adjusting for other factors in patients with CRS from a tertiary medical center. Despite its limitations, this study may improve our understanding of refractory CRS pathogenesis, possibly leading to more effective treatment strategies, such as incorporating the treatments of asthma and biofilm infection into conventional CRS therapies. Prospective cohort studies in diverse populations are needed to assess the causality of these associations.

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Quantification of κ-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects

To the Editor:

X-linked agammaglobulinemia (XLA) is a primary immunodeficiency caused by severely decreased numbers of mature peripheral B lymphocytes as a result of a mutation in the *BTK* gene. Non-XLA is characterized by hypogammaglobulinemia with decreased B-cell counts (less than 2% of mature B cells) in the absence of the *BTK* gene mutation. Both XLA and non-XLA are caused by an early B-cell maturation defect.[1] In patients with XLA and non-XLA, recurrent infections appear between 3 and 18 months of age, whereas the mean age at diagnosis is 3 years.[2] This delayed diagnosis results in frequent hospitalization because of pneumonia, sepsis, meningitis, and other bacterial infections, which frequently require intravenous administration of antibiotics and can be fatal. Frequent pneumonia results in a high incidence of chronic lung diseases.[3] Thus, early diagnosis and early treatment, including periodical intravenous immunoglobulin replacement therapy, is essential to improve the prognosis and the quality of life of patients with XLA and non-XLA.

In the process of B-cell maturation, immunoglobulin κ-deleting recombination excision circles (KRECs) are produced during κ-deleting recombination allelic exclusion and isotypic exclusion of the λ chain.[4] Coding joint (cj) KRECs reside within the chromosome, whereas signal joint (sj) KRECs are excised from genomic DNA. cjKREC levels remain the same after B-cell division, whereas sjKREC levels decrease, because sjKRECs are not replicated during cell division.[5] Because the B-cell maturation defects in XLA and non-XLA occur before κ-deleting recombination, KRECs are not supposed to be produced. Therefore, measurements of KRECs have the potential to be applied to the identification of these types of B-cell deficiencies in patients, which consist of around 20% of all B-cell defects.[6] In addition, some types of combined immunodeficiencies show an arrest in B-cell maturation and can also be identified by this method. The success of newborn screening for T-cell deficiencies by measuring T-cell–receptor excision circles[7] prompted us to develop a newborn screening method for XLA and non-XLA by measuring KRECs derived from neonatal Guthrie cards.

The study protocol was approved by the National Defense Medical College institutional review board, and written informed consent was obtained from the parents of normal controls, the affected children, and adult patients, in accordance with the Declaration of Helsinki.

First, we determined the sensitivity of detection levels of cjKRECs and sjKRECs in Guthrie cards using real-time quantitative PCR.[5] Normal B cells from a healthy adult were isolated from peripheral blood (PB; mean purity, 88.5%). PB was also obtained from 1 patient with XLA (P20) whose B-cell number was 0.09 in 1 μL whole blood and who was negative for sjKRECs ($<1.0 \times 10^2$ copies/μg DNA). Various numbers of normal B cells were serially added to 1 mL whole PB obtained from this patient with XLA. The B-cell–added XLA whole blood was then applied to filter papers, and 3 punches (3 mm in diameter) of dried blood spots were used for DNA extraction. At least 3 DNA samples containing the same B-cell concentrations (0.09-400 B cells/μL) were used for the real-time quantitative PCR of cjKRECs and sjKRECs. The percentages of the positive samples ($>1.0 \times 10^2$ copies/μg DNA) of cjKRECs and sjKRECs increased constantly

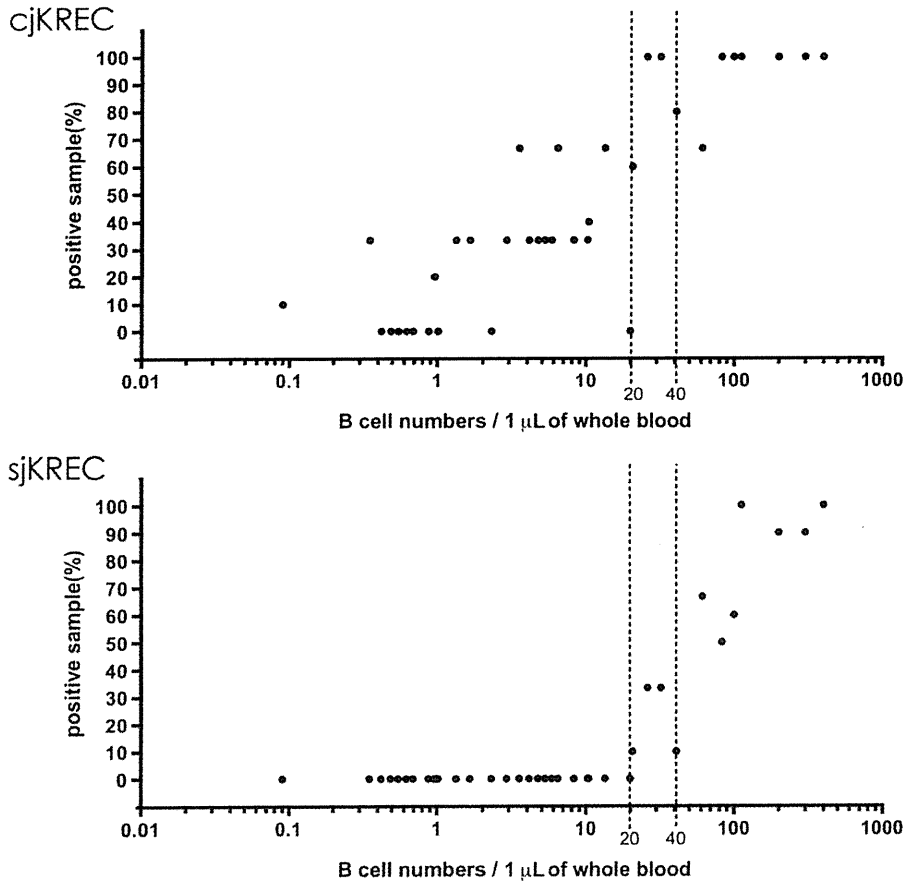

FIG 1. Sensitivity levels of cjKRECs and sjKRECs. Various numbers of purified normal B cells were serially added to whole PB from a patient with XLA (P20) to obtain B-cell–added XLA whole blood. cjKRECs and sjKRECs were measured in 3 to 10 samples of each concentration in triplicate. In all analyses, RNaseP (internal control) was positive ($2.3 \pm 0.2 \times 10^5$ copies/μg DNA). *X-axis,* B-cell numbers in 1 μL whole blood from a patient with XLA. *Y-axis,* Percentages of the KREC-positive results in the tests.

as the B-cell concentrations increased (Fig 1). None of the samples were positive for sjKRECs when the B-cell numbers were less than 20/μL, but cjKRECs were often positive. It has been reported that 90% of patients with XLA have less than 0.2% B cells in the PB at diagnosis.[1] Because peripheral lymphocyte numbers in neonates range from 1200 to 9800/μL,[8] the absolute B-cell numbers of 90% of patients with XLA are estimated to be 2.4 to 19.6/μL at the time of blood collection for Guthrie cards, although exact B-cell numbers of XLA in neonatal periods are not known at this moment. Because neonates are known to have fewer B cells than infants,[9] and we observed that B-cell numbers are constantly low in patients with XLA throughout infancy (Nakagawa, unpublished data, June 2010), which is consistent with the fact that BTK plays an essential role in B-cell maturation. It is likely that neonates with XLA also have severely decreased B cells. On the other hand, all samples obtained from 400 B cells/μL were positive for both cjKRECs and sjKRECs. We also observed that all healthy infants (1-11 months old; n = 15) were sjKREC-positive (Nakagawa, unpublished data, June 2010) and might have at least 600 B cells/μL whole blood.[9] From these data, it is assumed that at least 90% of patients with XLA are sjKREC-negative, and healthy neonates are positive for sjKRECs on neonatal Guthrie cards.

Next, we measured cjKRECs and sjKRECs in dried blood spots in filter papers or Guthrie cards from 30 patients with XLA and 5 patients with non-XLA and from 133 neonates born at the National Defense Medical College Hospital during this study period (August 2008 to October 2009) and 138 healthy subjects of various ages (1 month to 35 years old) to investigate the validity of this method. The levels of B cells of the patients ranged from 0.0% to 1.1% of total lymphocytes and 0.0 to 35.78/μL. IgG levels were 10 to 462 mg/dL (see this article's Tables E1 and E2 in the Online Repository at www.jacionline.org). Patients with leaky phenotypes[1,10] were included; 1 patient (P30) had more than 1% B cells and 34.22/μL total B cells, and 4 patients had more than 300 mg/dL serum IgG (P12, P30, P31, P33). All of the normal neonatal Guthrie cards were positive for both cjKRECs and sjKRECs ($7.2 \pm 0.7 \times 10^3$ and $4.8 \pm 0.6 \times 10^3$ copies/μg DNA, respectively). All healthy subjects of various ages were also positive for both cjKRECs and sjKRECs (Nakagawa, unpublished data, June 2010). In contrast, specimens from all 35 B-cell–deficient patients were sjKREC-negative ($<1.0 \times 10^2$ copies/μg DNA; Fig 2). All 5 patients with leaky phenotypes were also sjKREC-negative, which might be explained by the hypothesis that leaky B cells of patients with XLA are long-lived B cells that divided several times and have fewer sjKRECs than naive B cells.

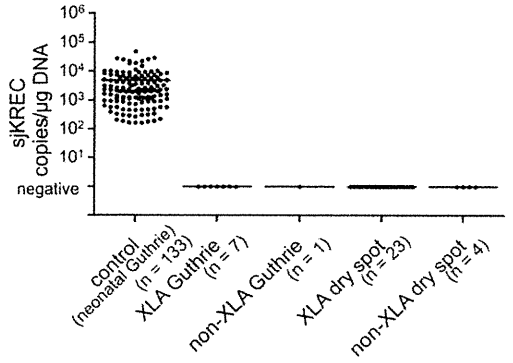

FIG 2. Copy numbers of sjKRECs measured in neonatal Guthrie cards or dried blood spots obtained from B-cell–deficient patients. On all samples from control, neonatal Guthrie cards (n = 133) were sjKREC-positive ($4.8 \pm 0.6 \times 10^3$ copies/μg DNA). B-cell–deficient patients were negative for sjKRECs in neonatal Guthrie cards (XLA, n = 7; non-XLA, n = 1) and dried blood spots (XLA, n = 23; non-XLA, n = 4).

One patient (P27) was positive for cjKRECs, but other patients were negative for it. *RPPH1* (internal control) was detectable at the same level as in normal controls in all samples.

These results indicate that sjKRECs are undetectable in XLA and non-XLA and suggest that measurement of sjKRECs in neonatal Guthrie cards has the potential for the use of newborn mass screening to identify neonates with early B-cell maturation defects. Greater numbers of neonatal Guthrie cards should be examined to confirm this potential, and the data obtained from dried blood spots on filter papers must be examined to prove that they truly reflect the data obtained from neonatal Guthrie cards. We should also examine whether screening can reduce the cost of treatment of the bacterial infections and chronic lung diseases in patients with XLA and non-XLA and increase the benefits for these patients. An anticipated pilot study using a large cohort of newborns must address these problems. We also found that T-cell–receptor excision circles and sjKRECs can be measured simultaneously on the same plate. Thus, a pilot study of neonatal screening for both T-cell and B-cell deficiencies could be performed simultaneously.

We thank the patients and their families who participated in this study. We also thank Ms Makiko Tanaka and Ms Kimiko Gasa for their skillful technical assistance and members of the Department of Obstetrics and Gynecology at the National Defense Medical College for collecting umbilical cord blood samples as well as Drs Wataru and Masuko Hirose. We are also indebted to Prof J. Patrick Barron, Chairman of the Department of International Medical Communications of Tokyo Medical University, for his *pro bono* linguistic review of this article.

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doi:10.1016/j.jaci.2011.01.052

TABLE E1. Characteristics of patients with XLA

Patient no.	Unique patient no.	Age (y)	Sex	Serum Ig (mg/dL)			$CD19^+$		*BTK* mutation			Source	
				IgG	IgA	IgM	% Lymph	/μL	Genomic DNA	cDNA	Amino acid	Guthrie	Dry spot
P1	670	0	M	87	<6	10	0.21	12.99	29269G>T	1178-1G>T	Splice acceptor defect	x	
P2	718	0	M	215	<10	<10	0.07	7.04	11593_11594 insA	144_145insA	Arg49 frameshift	x	
P3	722	0	M	80	<1	1	<1.00	NA	25644C>T	763C>T	Arg255X	x	
P4	727	8	M	295	59	57	0.11	3.52	29269G>T	1178-1G>T	Splice acceptor defect		x
P5	732	34	M	1140*	<6	8	0.02	0.24	11631T>A	182T>A	Ile61Asn		x
P6	811	24	M	458*	0	13	0.50	5.32	23570T>G	426T>G	Tyr142X		x
P7	813	18	M	628*	109	6	0.60	6.87	23570T>G	426T>G	Tyr142X		x
P8	814	19	M	260	0	NA	0.20	3.01	16180C>T	344C>T	Ser115Phe		x
P9	815	13	M	600*	<10	<5	0.08	1.72	11590G>T	142-1G>T	Splice acceptor defect		x
P10	816	11	M	12	0	5	0.00	0.00	150kb deletion of *BTK, TIMM8A, TAF7L, DRP2*				x
P11	817	10	M	10	2	24	0.80	35.78	36288C>T	1928C>T	Thr643Ile		x
P12	824	13	M	462	6	27	0.41	14.49	27518C>A	895-11C>A	Splice acceptor defect		x
P13	834	5	M	<237	<37	43	0.00	0.00	25715_26210del	776+57_839+73del	Exon 9 deletion		x
P14	838	21	M	<50	<5	7	0.00	0.00	31596G>C	1631+1G>C	Splice donor defect		x
P15	839	16	M	604*	<1	<2	0.04	0.66	31596G>C	1631+1G>C	Splice donor defect		x
P16	847	11	M	698*	26	11	0.08	1.86	25536delG	655delG	Val219 frameshift		x
P17	877	14	M	20	19	8	0.21	NA	32357T>C	1750+2T>C	Splice donor defect		x
P18	880	5	M	233	39	41	0.06	NA	10941-?_14592+?del	1-?_240+?del	Exon 1-3 deletion		x
P19	888	8	M	<212	<37	150	0.15	6.60	11023G>A	83G>A	Arg28His		x
P20	891	21	M	195	<6	37	0.02	0.09	32243C>G	1638C>G	Cys502Trp		x
P21	958	0	M	<50	<10	9	0.80	27.14	31544_31547 delGTTT	1580_1583del GTTT	Cys527 frameshift		x
P22	701	2	M	115	<2	4	0.09	1.99	16172C>A	336C>A	Tyr112X		x
P23	911	0	M	<10	<6	<4	0.00	0.00	29955A>C	1350-2A>C	Splice acceptor defect	x	
P24	937	0	M	60	<2	58	0.00	0.00	11022C>T	82C>T	Arg28Cys	x	
P25	938	0	M	<20	<4	<6	0.00	0.00	36269-?_36778+?del	1909-?_2418+?del	Exon 19 deletion	x	
P26	939	0	M	60	<2	22	0.00	0.00	11022C>T	82C>T	Arg28Cys	x	
P27	890	12	M	<237	<37	<20	0.03	NA	36261G>A	1909-8G>A	Splice acceptor defect		x
P28	944	6	M	12	<1	1	0.02	NA	36281C>T	1921C>T	Arg641Cys		x
P29	948	5	M	<237	<37	<20	0.01	0.70	36261G>A	1909-8G>A	Splice acceptor defect		x
P30	1053	5	M	386	5	113	1.10	34.22	32259A>C	1654A>C	Thr552Pro		x

Age, Age at analysis of KRECs; *$CD19^+$ % Lymph,* CD19-positive cell percentage in lymphocytes; *$CD19^+$ /μL,* CD19-positive cell number in 1 μL whole peripheral blood; *M,* male; *NA,* not available; *Serum Ig,* serum levels of immunoglobulins at diagnosis.

BTK mutation's reference sequences are NCBI NC_000023.9, NM_000061.2, and NP_000052.1.

*Trough level during intravenous immunoglobulin therapy.

TABLE E2. Characteristics of patients with non-XLA

Patient no.	Unique patient no.	Age (y)	Sex	Serum Ig (mg/dL)			$CD19^+$		*BTK* mutation	Source	
				IgG	IgA	IgM	% Lymph	/μL		Guthrie	Dry spot
P31	596	4	F	386	<6	6	0.42	21.27	Normal		x
P32	719	0	F	<50	<5	<5	0.00	0.00	Normal	x	
P33	835	8	M	311	323	20	0.09	1.88	Normal		x
P34	915	0	M	<212	<37	<20	0.00	0.00	Normal		x
P35	947	0	M	<21	<37	<39	0.00	0.00	Normal		x

Age, Age at analysis of KRECs; *$CD19^+$ % Lymph,* CD19-positive cell percentage in lymphocytes; *$CD19^+$ /μL,* CD19-positive cell number in 1 μL whole peripheral blood; *F,* female; *M,* male; *Serum Ig,* serum levels of immunoglobulins at diagnosis.

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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. © 2010 International Clinical Cytometry Society

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,

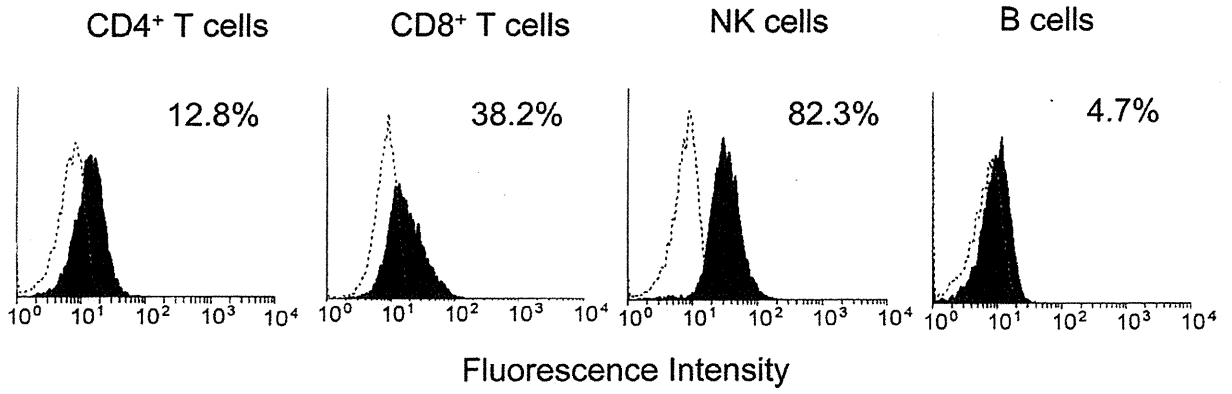

FIG. 1. The SAP expression in T, B, and NK cells in a normal adult donor. A flow cytometric analysis showed that $CD8^+$ T cells and NK cells expressed SAP relatively intensely, $CD4^+$ T cells weakly, and B cells negligibly. The dotted lines and shaded areas indicate staining by the control antibody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

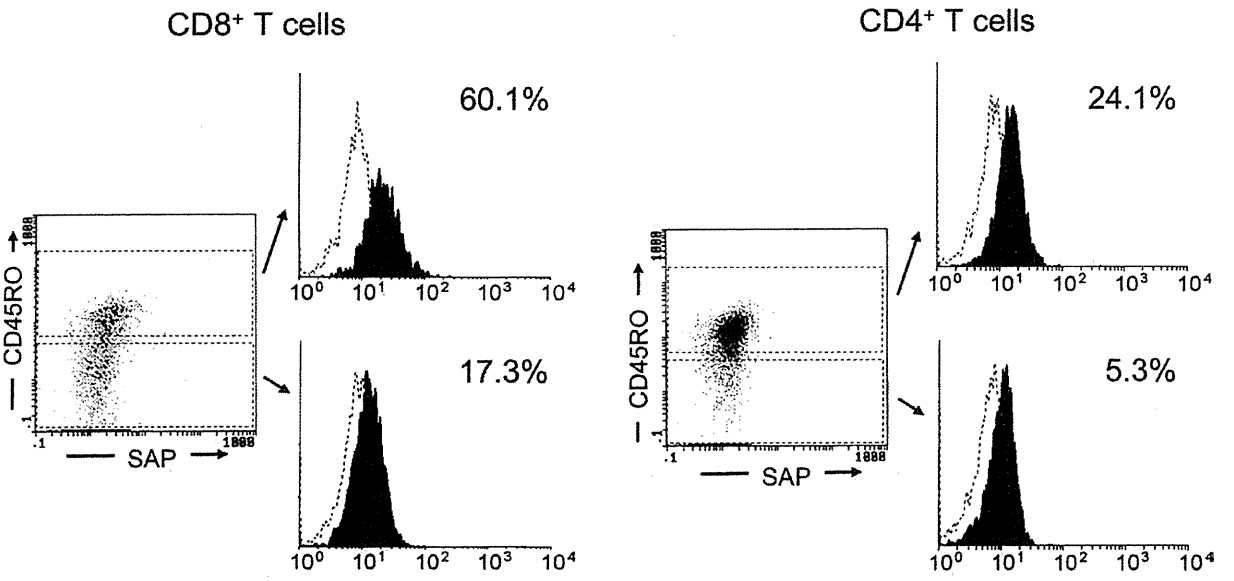

FIG. 2. An increased SAP expression in $CD45RO^+$ T cell subsets. $CD45RO^+$ (memory/activated) populations of T cells subsets, especially of $CD8^-$ T cells, exhibited an enhanced SAP expression.

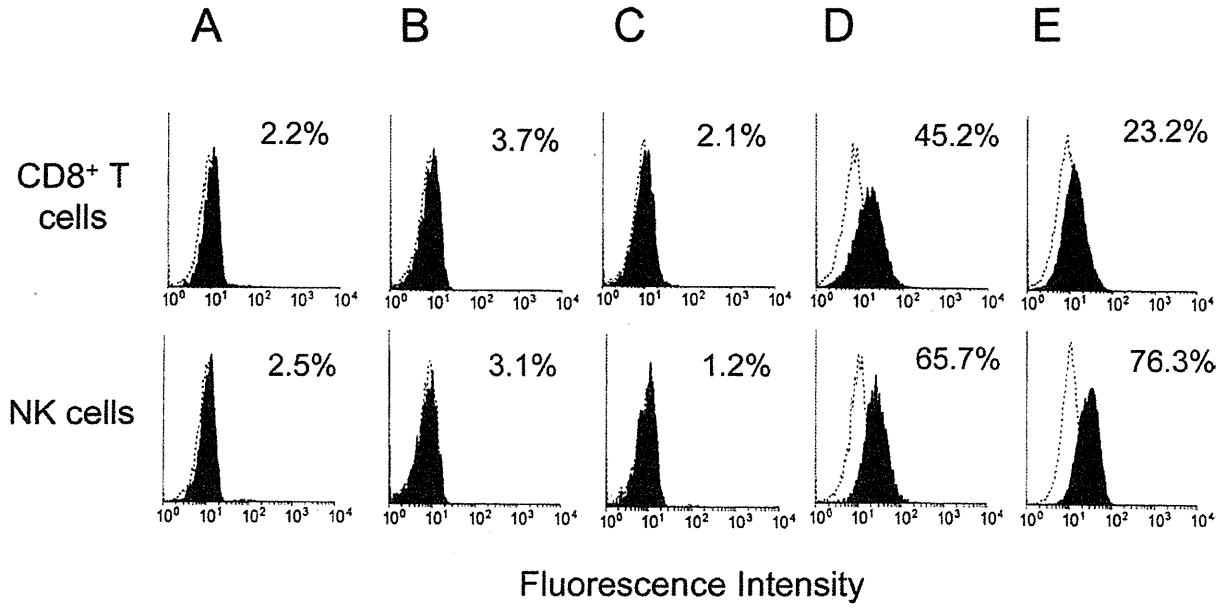

FIG. 3. The SAP expression in suspected patients with XLP. A marked SAP deficiency in both CD8+ T cells and NK cells was notable in some patients (A–C), but not in others (D, E). **A**, **B**, **C**, **D**, and **E** indicate P1, P2, P4, P10, and P11, respectively.

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	48.5	53.8		
				(range)	(21.6–90.8)	(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.

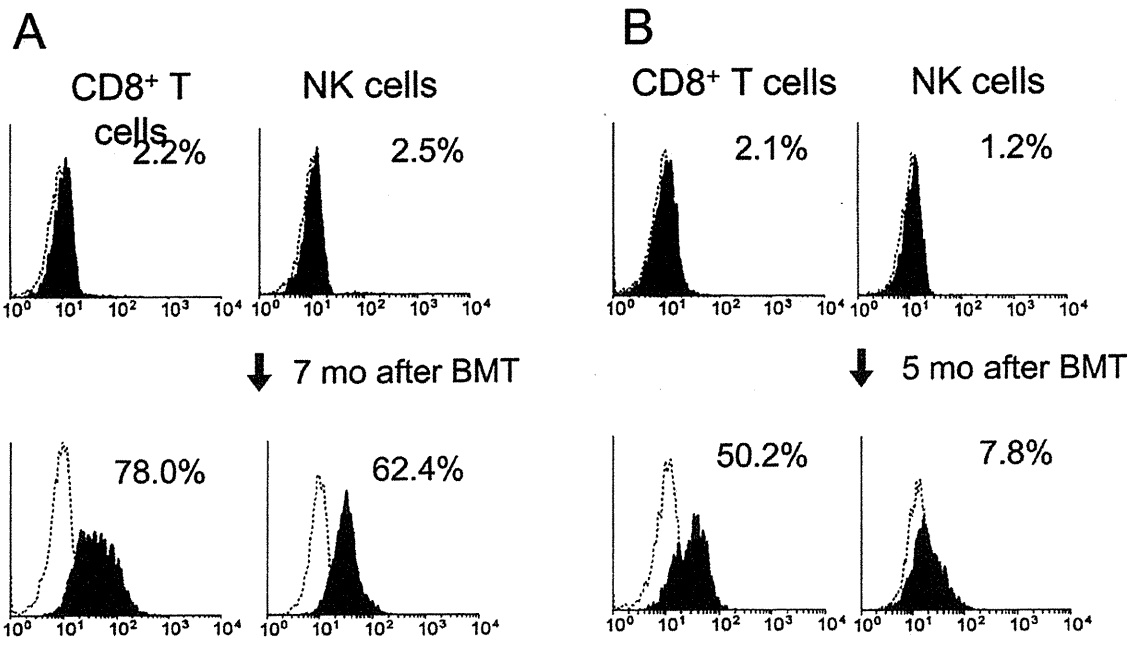

FIG. 4. The SAP expression in XLP patients after HSCT. A flow cytometric analysis demonstrated an increased SAP expression in CD8+ T cells and NK cells in 2 XLP patients after they have undergone HSCT. A and B indicate P1 and P4, respectively.

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 (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.

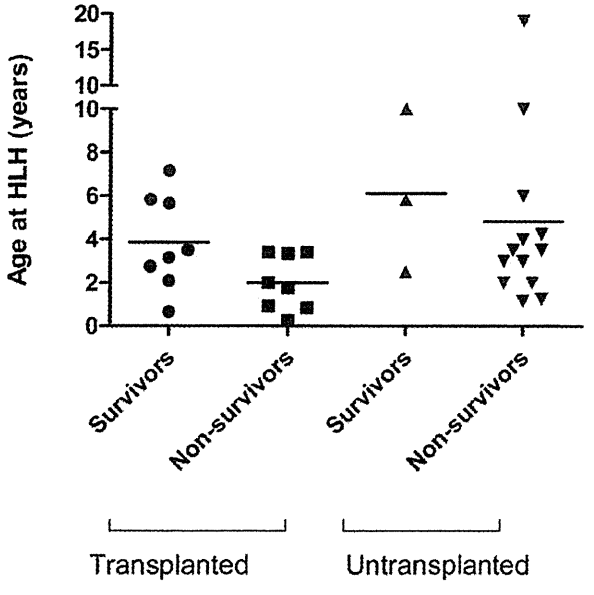

Figure 1. Outcome of patients with HLH during course of disease. Survival of patients who present with HLH—patients who remain untransplanted have a poor survival outcome with only 18.8% survival. By contrast the survival of those who undergo transplant is higher at 50%.