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Allogeneic stem cell transplantation in X-linked lymphoproliferative disease: two cases in one family and review of the literature

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Summary:

X-linked lymphoproliferative disease (XLP) is a rare immunodeficiency caused by mutations in the signaling lymphocyte activating molecule-associated protein/ *SH2D1A* gene and characterized by a dysregulated immune response to Epstein–Barr virus and other pathogens. The clinical presentation is heterogeneous and includes fulminant infectious mononucleosis, lymphoma, hypogammaglobulinemia and aplastic anemia. XLP is associated with a high morbidity and overall outcome is poor. At present, allogeneic stem cell transplantation (alloSCT) is the only curative treatment. XLP patients may be recognized in various stages of disease and even when symptoms are not yet evident. We here present two related XLP patients in different stages of disease that were both treated successfully with alloSCT using a matched unrelated donor. In addition, we have reviewed all reported cases of alloSCTs in XLP patients. Based on these results and in order to improve the final outcome, we conclude that alloSCT should be recommended in both symptomatic and asymptomatic XLP patients.
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X-linked lymphoproliferative disease (XLP) is a rare primary immunodeficiency with an unfavorable long-term prognosis.[1,2] The first cases were described 30 years ago by Purtilo.[3] Six out of 18 male maternal cousins, who were born in one generation, died of fulminant infectious mononucleosis (FIM), while none of the sisters were affected. This disease was initially called Duncan's disease after the name of the original kindred. Although it was proposed to change the name to Purtilo's disease, David Purtilo himself suggested that the name of the disease should be closely related to the pathophysiology of the disorder. In the ensuing years, the disease became known as X-linked lymphoproliferative disease (XLP).

XLP usually presents in childhood or adolescence with a wide spectrum of clinical manifestations: malignant lymphoma, FIM, hypogammaglobulinemia, vasculitis and pulmonary lymphomatoid granulomatosis or aplastic anemia (AA).[1,3–6] Although phenotypically heterogeneous in their initial presentation, XLP patients usually die before the age of 20 without intervention by allogeneic stem cell transplantation (alloSCT).[1]

In the large majority of cases, Epstein–Barr virus (EBV) has been identified as the trigger for symptomatic disease. However, this is not a prerequisite since approximately 10% of the affected males may develop clinical XLP manifestations in the absence of EBV infection.[7] Several studies have suggested that a dysregulated cellular immune response to a primary EBV infection and probably other pathogens is responsible for the severe or fatal course observed in XLP patients.[8–11] However, until recently the cellular mechanism responsible for this dysregulated immunity remained unknown. The identification of the genetic defect has provided important insight into the molecular and immunological mechanisms of this disease.

The gene responsible for the majority of XLP cases has been identified in 1998 as the signaling lymphocyte activating molecule (SLAM)-associated protein (*SAP*)/ *SH2D1A* gene encoding a 128-amino-acid protein known as SAP.[12,13] Functional studies indicate that SAP acts as an adaptor molecule that regulates SLAM- and 2B4-mediated lymphocyte activation.[14] SLAM is a transmembrane receptor expressed in T and B lymphocytes, whereas 2B4 is expressed in T-lymphocytes and natural killer (NK) cells. Following lymphocyte activation, SAP mediates recruitment of the protein tyrosine kinase (PTK) Fyn to the tyrosine-phosphorylated cytoplasmic tail of SLAM as well as 2B4 that is essential for further cellular activation.[13,15,16] Different mutations of *SAP*/*SH2D1A* have been identified in XLP patients, all leading to a nonfunctional protein.[7,12,13,17,18] However, a clear genotype–phenotype correlation is lacking. The implication of the *SAP*/*SH2D1A* mutation on cellular immunity has been addressed in several recent studies. Analysis of NK cells from

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XLP patients has demonstrated defective 2B4-mediated cytotoxic lysis of EBV-transformed B-cell lines.[19] In addition, T-cell receptor and SLAM-mediated IFNγ and IL-2 secretion was shown to be severely impaired in $CD4^+$ T cells from XLP patients.[20] Recent, data show that *in vitro* generated EBV-specific cytotoxic T lymphocytes (CTLs) from XLP patients, although phenotypically normal, are functionally impaired and that this defect can be corrected by *SAP*/*SH2D1A* gene transfer.[21]

It should be noted that it is common to find a *SAP*/*SH2D1A* mutation in XLP patients with a positive family history, but rare in a sporadic XLP patient,[7] suggesting that mutations in other genes involved in the SLAM/SAP/Fyn signaling pathway may be found in XLP patients without a *SAP*/*SH2D1A* mutation.[14,22] In addition to the observations in typical EBV-triggered XLP patients, *SAP*/*SH2D1A* mutations have also been identified in patients with features of common variable immune deficiency,[23,24] as well as in patients with EBV-negative non-Hodgkin's lymphoma in early childhood.[17,25]

Most treatment modalities to control XLP manifestations, including intravenous immunoglobulins (IVIG), steroids, antiviral drugs and chemotherapy, have been unsuccessful and temporarily effective at best. Recently, evidence has been provided that elimination of B lymphocytes in the initial phase of EBV infection may reduce clinical manifestations.[26] At present, alloSCT is the only therapy available with a curative potential. The first bone marrow transplant was described in 1986 by Filipovich *et al*[27] and since then several additional cases have been published.[28–35] We here describe two cousins with the same *SAP*/*SH2D1A* mutation: one, a boy with typical EBV-induced XLP and the other, an EBV-negative boy with limited symptoms. Both boys were successfully transplanted with a matched unrelated donor. The results and the timing of alloSCT in these and other XLP patients reported in literature will be discussed.

Results

Diagnosis and treatment of two related XLP cases

Patient A presented in October 2001, at age 7 years, with FIM (hepatosplenomegaly, lymphadenopathy and pancytopenia). His medical history did not mention significant pathology other than moderate asthma for which he was treated with β2-mimetics and inhalation steroids. Adverse reactions to regular childhood immunizations were not observed. At the time of first presentation, there was no family history suggesting a primary immunodeficiency.

The acute infection with EBV was confirmed by the presence of viral capsid antigen (VCA) IgM and IgG and early antigen (EA) IgG antibodies. Antibodies against EBV nuclear-associated antigen (EBNA) were not detectable. EBV-DNA was repeatedly detected in peripheral blood by real-time quantitative PCR (RQ-PCR), as shown in Figure 1.[36] Cerebrospinal fluid analysis revealed a pleiocytosis of 183 lymphocytes/mm^3. The EBV infection was complicated by convulsions caused by an encephalitis with cerebral oedema for which he was ventilated in the intensive care unit. The patient recovered rapidly upon treatment with prednisolone and aciclovir. In December 2001, he presented with recurrent neutropenia and persistent diarrhoea. The latter was diagnosed as ulcerative colitis, that responded well to steroid treatment. An uncomplicated pneumonia (*Streptococcus pneumoniae*) was treated with antibiotics. In the same episode, dysgammaglobulinemia (IgG 4.2 g/L, IgM 2.9 g/L, IgA 3.6 g/L) was diagnosed and IVIG supplementation was initiated.

As XLP was suspected, molecular analysis of the *SAP*/*SH2D1A* gene was performed. A C→T substitution was identified at position 26 in exon 2, resulting in a premature stop codon at position 55 of the gene product.[12]

Owing to persistent high EBV load (10^3–10^4 copies(cp)/ml), he was treated with Rituximab 375 mg/m^2 and maintenance steroids to control symptomatic disease until alloSCT.

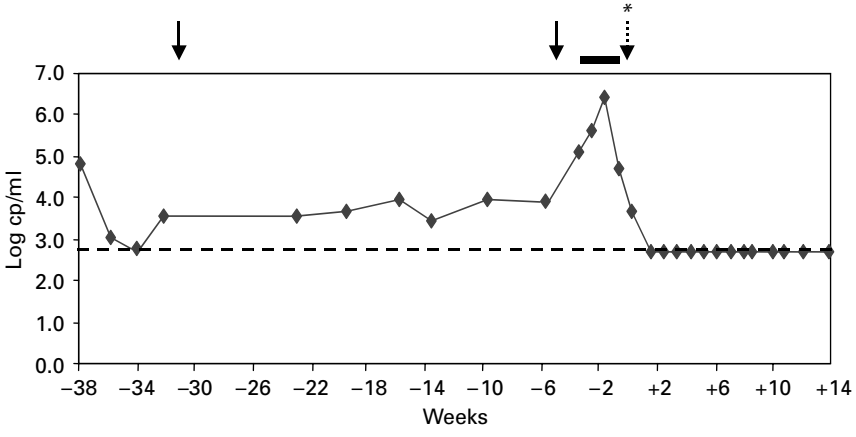

Figure 1 Kinetics of the EBV-DNA load (◆-◆) in patient A during the pre- and post-transplant period measured by RQ-PCR and depicted on the *Y*-axis as the log value in EBV-DNA cp/mL. The dotted line (—) at log value 2.7 represents the lower limit of reliable quantification by RQ-PCR. The two arrows (↓) indicate the administration of Rituximab, the arrow with asterisk (↓*) indicates the day of alloSCT. The black bar (■) represents the period of conditioning.

In this period, his 3-year-old brother, who was also diagnosed with the same *SAP*/*SH2D1A* mutation, died from rapidly progressive FIM in combination with a Gram-negative sepsis. In June 2002, our patient was stable and clinically well before undergoing alloSCT. Owing to a persistent EBV load (10^3–10^4 cp/ml), a second infusion of Rituximab (375 mg/m^2) was administered 4 weeks prior to alloSCT, and aciclovir treatment (30 mg/kg/day) was initiated. The preparative regimen consisted of Busulfan (16 mg/kg), Cyclophosphamide (200 mg/kg) and rabbit antithymocyte globulin (5 mg/kg, IMTIX SangStat, Amstelveen, Netherlands). Cyclosporin A (2 mg/kg starting at day −1) and a short course of methotrexate (10 mg/m^2 on days +1, +3 and +6) was administered as graft-versus-host disease (GvHD) prophylaxis. Nonmanipulated bone marrow from an EBV-seropositive fully matched (10/10 alleles) unrelated donor (containing 0.5×10^8 mononucleated cells/kg and 2.5×10^6 CD34+ cells/kg) was infused. Neutrophil engraftment ($>0.5 \times 10^9$/l) occurred at day 20, reticulocytes were over 5 promille at day 19, and thrombocytes were $>20 \times 10^9$/l at day 26. Cytogenetic analysis on peripheral blood leukocytes showed 100% donor chimerism at weeks 4 and 8 post transplant. On day 26, aciclovir treatment was discontinued because of repeatedly negative EBV PCRs. EBV-DNA was monitored every 1–2 weeks during the first six months post alloSCT and no reactivation was documented in this period (Figure 1). At day +40 the patient developed GvHD grade I, which was treated with prednisone and a rapid improvement was observed. Normal counts of peripheral blood lymphocytes and lymphocyte subpopulations, and normal proliferative responses of T-lymphocytes to mitogenic stimuli, were measured at 1 year post alloSCT. At that time, IgM, IgG and IgA levels were within the normal range, although IVIG was discontinued at 10 months post alloSCT. Persistance of full donor chimerism in peripheral blood leukocytes was confirmed at 1 and 2 years post alloSCT. Seroconversion was documented after vaccination and twice boosting with diphteria toxoid, tetanus toxoid and inactivated poliovirus (DTP) and *Haemophilus influenzae* type b conjugate. Normal EBV-specific immune reconstitution was demonstrated by the presence of EBV VCA and nuclear antigen (NA) IgG antibodies at 1 and 2 years post alloSCT. At present, 2 years post alloSCT, he is alive and well.

Patient B, a 2-year-old boy and cousin of patient A (the respective mothers are sisters), was suffering since the age of 1.5 years from recurrent upper airway infections. Screening of his immune function revealed a hypogammaglobulinemia (IgG 3.6 g/L, IgM <0.3 g/L, IgA <0.5 g/L) for which regular supplementation with IVIG was initiated. Serological screening for EBV-EA, -VCA and -NA antibodies, as well as repetitive analysis by EBV-DNA PCR on plasma was negative, indicating that he had most probably not yet encountered EBV. Based on the diagnosis in his cousin, XLP was suspected. Indeed, the same *SAP*/*SH2D1A* mutation was found. Following an episode of upper airway infection and fever, he developed AA, from which he partially recovered without any treatment. A concurrent Human Herpes Virus 6 infection was documented by PCR on plasma, which may have been the causative agent for the AA. Based on the clinical symptoms, the family history and the availability of a matched unrelated donor, the patient was scheduled for alloSCT. At the time of alloSCT, EBV serology was positive for EBNA and VCA antibodies that were passively acquired by IVIG. The EBV-DNA PCR remained negative. The preparative regimen and GvHD prophylaxis in patient B were the same as described for patient A. A nonmanipulated bone marrow graft from an EBV seropositive, fully matched (10/10 alleles) unrelated donor (1.6×10^8 mononucleated cells/kg and 9.3×10^6 CD34+ cells/kg) was administered. Transplant-related toxicity was limited to a mild veno-occlusive disease. Neutrophil engraftment ($>0.5 \times 10^9$/l) occurred at day 26, reticulocyte count was >5 promille at day 19 and thrombocytes were $>20 \times 10^9$/l on day 40. At day 35 after transplant he was discharged. Cytogenetic analysis on peripheral blood leukocytes showed 100% donor chimerism at weeks 4, 8 and 52 post-transplant. IVIG supplementation was discontinued at 6 months after alloSCT. Cellular and humoral immune recovery, including EBV serology, was similar to that described for patient A. EBV reactivation, monitored every 1–2 weeks by EBV-DNA PCR, did not occur. At present, 2 years post alloSCT, the patient is alive and well.

Further genetic analysis of the maternal pedigree confirmed that the mothers of the affected boys were carriers of the *SAP*/*SH2D1A* mutation. In addition, one of their two remaining sisters as well as their mother were also carriers of the mutation.

Reported cases of alloSCT in XLP

Since the first report of alloSCT for XLP in 1986 by Filipovich *et al*[27] and including our two patients, 14 cases have been published (Table 1). In 64% (9/14) of the cases EBV-related disease was documented either as typical (fulminant) IM, non-Hodgkin's lymphoma or hemophagocytic lymphohistiocytosis. In the remaining five cases, that presented as non-Hodgkin's lymphoma ($N=2$), hypogammaglobulinemia ($N=2$) and hypogammaglobulinemia plus marrow hypoplasia ($N=1$), no evidence was found for the presence of EBV either by serological tests or PCR analysis. Conditioning was based on total body irradiation (TBI) in five cases and on chemotherapy only in nine cases. In one case, a nonmyeloablative (NMA) regimen was used (patient 10 in Table 1). Different kinds of stem cell donors were used, including matched siblings ($N=7$), matched sibling cord blood (CB; $N=1$), matched unrelated donors ($N=4$) and unrelated CB ($N=2$). Based on the reported follow-up, overall survival was 71% (10/14). Survival was seen in four of seven cases with matched sibling donors, in three of four cases with unrelated donors and in all the three CB transplants. Patient 10 died at day 104 of progressive disease. The other three nonsurvivors died due to various forms of transplant-related toxicity between days 23 and 84 post-transplant, all receiving TBI-based conditioning regimens. The overall survival in patients with TBI-based *vs* chemotherapy-only conditioning regimens was 20% (1/5) and 89% (8/9), respectively. Although the nonsurvivors cluster in the TBI cohort, a causal relation is unlikely. The fact that the medical history of the TBI group was more

Table 1 Characteristics of reported cases of alloSCT in XLP patients

Study	*Pt*	*EBV status preSCT*	*XLP manifestation*	*Donor EBV status*	*Conditioning*	*GvH prophylaxis*	*AcV proph.*	*EBV status postSCT*	*Complications reported outcome*
Filipovich, 1986	1 19 years	VCA− NA− Culture+	IM, AA, Hg	MSD VCA/NA+	Cy/TBI/ATG	MTX/MP	No	VCA+ NA+	GVHD II, AdV Died at day +84
Williams, 1993	2 11 years	spont.LCL	NHL (EBV+), Hg	MSD VCA/NA+	Bu/Cy/AraC	CsA/MP	Yes	PCR+ Sero−	None Alive at +4 years
Vowels, 1993	3 2 years	Sero− PCR+	IM, Hg, LPD	CB EBV sero−	Cy/Mel/ATG	MTX/CsA	Yes	Sero−	GVHD I, convulsions Alive at +4 years
Pracher, 1994	4 6 years	VCA+ NA−	FIM	MSD VCA/NA+	Bu/Cy/Eto	MTX	Yes	VCA+ NA+	GVHD II Alive at +4.5 years
Gross, 1996	5 19 years	VCA+ NA−	IM, LPD	MSD VCA/NA+	Cy/TBI	MTX	Yes	n.d	GVHD II, MOF Died at day +61
	6 30 years	VCA+ NA−	LPD, Hg	MUD VCA/NA+	Cy/TBI	MTX/CsA	Yes	ND	GVHD II, MOF Died at day +23
	7 15 years	Sero− PCR−	NHL (EBV−)	MSD VCA/NA+	Cy/TBI	CsA	Yes	VCA+ NA+	GVHD II Alive at +3 years
Hoffmann, 1998	8 14 years	Sero−	NHL (EBV−)	MUD n.d	Cy/TBI/ATG	MTX/CsA	No	ND	GVHD I Alive at+21 months
Arkwright, 1998	9 7 years	VCA+ NA−	FIM	MSD NA+	HLH-94	ND	No	VCA+ NA+	GVHD II, AdV Alive
Amrolia, 2000	10 3 years	PCR+	HLH	MSD n.d	Flu/Mel/ALG	CsA/MP	Yes	PCR+	HLH/MC Died at day +104
Ziegner, 2001	11 8 months	Sero−	Hg	MUD CB EBV sero−	Bu/Cy/Eto/ATG	CsA/MP	No	Sero−	GVHD I Alive at+2 years
	12 4 years	Sero−	Hg	MUD CB EBV sero−	Bu/Cy/Eto/ATG	CsA/MP	No	Sero−	GVHD I Alive at+2 years
Lankester, 2004	13 3 years	PCR− VCA− NA−	Hg, AA	MUD VCA/NA+	Bu/Cy/ATG	MTX/CsA	No	VCA+ NA+ PCR−	VOD (mild) Alive at+2 years
	14 6 years	PCR+ VCA+ NA−	FIM	MUD VCA/NA+	Bu/Cy/ATG	MTX/CsA	Yes	VCA+ NA+ PCR−	GvHD I Alive at+2 years

EBV status: VCA = viral core antigen (antibodies); NA = nuclear antigen (antibodies); sero −, negative EBV serology documented without further specification; PCR = polymerase chain reaction; ND = not documented. Results on EBV serology were obtained in the absence of IVIG supplementation.
XLP manifestations: (F)IM = (fulminant) infectious mononucleosis; AA = aplastic anemia; Hg = hypogammaglobulinemia; NHL = non-Hodgkin's lymphoma; LPD = lymphoproliferative disease; HLH = hemophagocytic lymphohistiocytosis.
Donor: MSD = (HLA) matched sibling donor; (U)CB = (unrelated) cord blood; MUD = (HLA) matched unrelated donor.
Conditioning: Cy = cyclophosphamide; Bu = busulfan; Ara-C = cytosine-arabinoside; Mel = Melfalan; Flu = fludarabin; Eto = etoposide; TBI = total body irradiation; ATG = antithymocyte globulins; ALG = antilymphocyte globulins; HLH-94.[49]
GvH prophylaxis: MTX = methotrexate; MP = methylprednisolone; CsA = cyclosporin A.
AcV proph: aciclovir prophylaxis.
Complications: AdV = adenovirus infection; MOF = multiorgan failure; MC = mixed chimerism; VOD = veno-occlusive disease.

complicated by infections and organ toxicity because of previous treatment has probably had a major impact on the transplant outcome. This is also reflected by the higher mean age in the TBI group compared to the chemotherapy group (19.4 *vs* 4.7 years). Except for the CB transplants and one case where information was unavailable (patient 8), all donors were EBV seropositive. In 8/14 patients aciclovir prophylaxis was reported. Evidence for EBV reactivation post transplant was only documented in patient 10, in whom this coincided with mixed chimerism and recurrence of the initial hemophagocytic lymphohistiocytosis symptoms.

Discussion

Although alloSCT is considered to be the only curative treatment for XLP, only a limited number of cases have been reported in literature. XLP is heterogeneous in its clinical presentation and therefore early recognition in case of a nonsuspicious family history may be difficult. Most patients will have experienced a period of severe clinical disease as well as various forms of treatment that may be associated with toxicity, and thus have an impact on the transplant-related morbidity and mortality. This may explain the unfavorable outcome in the subgroup of older patients. A causal relation between TBI-based conditioning and poor outcome seems unlikely in the light of the causes of death, that is, infection, GvHD and multiorgan failure. The overall outcome in the majority of patients is surprisingly good and at least comparable with results obtained in other primary immunodeficiency syndromes.[37,38] Based on the reported experience, myeloablative chemotherapy seems to be the conditioning regimen of choice in not-critically-ill XLP patients. NMA regimens may be advantageous in order to reduce organ toxicity and late effects. However, substantial clinical experience in support of this approach in XLP is unavailable. At present, we would suggest that NMA regimens should be restricted to those patients with a severely compromised clinical condition. In these cases, especially in the window phase of mixed chimerism and impaired immune recovery, reactivation of the initial disease symptoms may be a considerable risk and patients should be monitored carefully, clinically as well as by EBV-specific RQ-PCR.

Interestingly, EBV-induced complications during conditioning and post transplant are negligible in this series of patients. Only in one of 14 cases has EBV reactivation been documented and appeared to be correlated with recurrence of the initial disease. Notably, this was the sole patient that had received NMA conditioning followed by an HLA-identical sibling transplant, a procedure that is known to frequently result in mixed chimerism and impaired recovery of T-cell immunity towards EBV and CMV.[39–41] In general, following myeloablative conditioning, symptomatic EBV reactivation is restricted to other than HLA-identical sibling transplants, patients receiving a T-cell-depleted graft and the usage of ATG.[42,43] In most cases of EBV reactivation, EBV-infected B cells appear to be of donor origin.[44,45] This observation is supported by the observation that, in case of EBV-positive donors, B-cell depletion of the stem cell graft reduces the risk for EBV-LPD with high efficacy.[46] In addition, our finding of a vanishing EBV-DNA load after myeloablative conditioning in patient A indicates that the possible persistence of recipient EBV post-transplant has probably no significant clinical consequences. In general, the beneficial effect of aciclovir prophylaxis to prevent EBV reactivation post-transplant has not been proven.[47] In this cohort, 8/14 XLP patients received aciclovir prophylaxis and in some cases this implicated that previously initiated treatment was continued. Although there may be a role for aciclovir to control EBV replication in the pretransplant period, its additive value post-transplant is probably limited. Altogether, the risk for symptomatic EBV reactivation following myeloablative conditioning in XLP patients is probably comparable with non-XLP patients and thus restricted to T-cell-depleted SCT and the use of ATG. The use of EBV seropositive donors may be beneficial to control reactivation of recipient EBV, especially in those cases where mixed chimerism and residual EBV disease are likely to occur. If EBV reactivation is considered to be a high risk due to the conditioning regime used or uncontrolled infection preceding transplant, the generation and use of donor-derived EBV-specific CTLs may be beneficial.[48]

Based on the favorable outcome in the reported patients, alloSCT should indeed be recommended in all cases with EBV-positive symptomatic XLP. Interestingly, alloSCT has been successfully performed in three EBV-negative XLP cases that presented with hypogammaglobulinemia (two) and hypogammaglobulinemia plus AA (one) and with a positive family history of fulminant IM. This limited experience suggests that alloSCT should also be seriously considered for XLP patients with less severe clinical manifestations. In this way, deterioration of the clinical condition by recurrent infections and particularly primary EBV infection may be circumvented. In these three cases a partially matched unrelated CB (two) and a matched unrelated marrow graft (one) were successfully used. Together, these results indicate that alloSCT should be recommended in both EBV-positive and -negative symptomatic XLP patients. The favorable outcome in the reported unrelated donor transplants supports the use of also other than HLA-identical sibling donors in this high-risk disease.

AlloSCT in asymptomatic XLP patients remains a more difficult decision that will be influenced by a positive family history of FIM or fatal disease. Based on the forementioned considerations concerning outcome in symptomatic XLP patients and the limited toxicity observed in the EBV-negative cases, we advocate that alloSCT should be strongly considered in asymptomatic patients soon after diagnosis. This will eliminate the risk for XLP manifestations that negatively impact on the transplant outcome and would most probably result in a favorable outcome in the large majority of patients. The clinical course in the sibling of patient A supports this policy. It is evident that it will be more easy to make this decision in case of an available HLA-matched donor. However, the limited experience so far suggests that other donors, even including unrelated cord blood, should be considered as well. Recently, evidence has been provided that depletion of B lympho-

cytes by administration of Rituximab in the acute phase of EBV infection in XLP patients may significantly reduce morbidity.[26] A similar approach may be considered in asymptomatic, EBV-negative, XLP patients to bridge the time to alloSCT and thus reduce the risk for rapidly deteriorating EBV infection.

Note added in proof

After submission of our manuscript Slatter *et al* (BMT 2005; **35**: 683–689) reported a case of NMA alloSCT in a XLP patient that resulted in mixed chimerism and post transplant EBV reactivation.

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