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Ⅳ　研究成果の刊行物・別刷

Bone Marrow Transplantation (2014) **49,** 1155–1161

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ORIGINAL ARTICLE

Cord blood transplantation is associated with rapid B-cell neogenesis compared with BM transplantation

K Nakatani[1], K Imai[2], M Shigeno[1], H Sato[3], M Tezuka[2], T Okawa[2], N Mitsuiki[1], T Isoda[2], D Tomizawa[2], M Takagi[2], M Nagasawa[2], M Kajiwara[4], M Yamamoto[5], A Arai[5], O Miura[5], C Kamae[6], N Nakagawa[6], K Honma[6], S Nonoyama[6], S Mizutani[1,2] and T Morio[1,2]

Hematopoietic cell transplantation (HCT) is used for treatment of hematopoietic diseases. Assessment of T- and B-cell reconstitution after HCT is crucial because poor immune recovery has a major effect on the clinical course. In this study, we retrospectively analyzed T-cell receptor excision circles (TRECs) as well as signal and coding joint kappa-deleting recombination excision circles (sjKRECs and cjKRECs, respectively) as markers of newly produced lymphocytes in 133 patients (56 primary immunodeficient and 77 malignant cases, median (range): 12 (0–62) years old). We analyzed the kinetics of TREC and KREC recovery and determined the factors that contributed to better immune recovery. KRECs became positive earlier than TRECs and increased thereafter. Younger recipient age had a favorable effect on recovery of sjKRECs and cjKRECs. Compared with BM and peripheral blood, our data suggested that cord blood (CB) provided rapid B-cell recovery. CB also provided better B-cell neogenesis in adult HCT recipients. Chronic GVHD was associated with low TRECs, but not increased sjKRECs/cjKRECs. Finally, positive sjKRECs 1 month after HCT were associated with fewer infectious episodes. Monitoring of TRECs and KRECs may serve as a useful tool for assessment of immune reconstitution post HCT.

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INTRODUCTION

Hematopoietic cell transplantation (HCT) serves as a curative treatment for diseases such as hematopoietic malignancy, congenital BM failure and primary immunodeficiency (PID). Selection of a suitable donor by HLA matching and/or an appropriate conditioning regimen has improved the outcome of HCT for leukemia patients[1] and PID patients.[2,3] Recently, successful outcomes of cord blood transplantation (CBT) and BM transplantation (BMT) have been observed even in HLA-mismatched conditions.[4–8]

Despite these improved outcomes, transplantation-related morbidities such as graft failure, GVHD and infection are still major problems that affect the prognosis and/or quality of life. Infection monitoring after HCT is important for the initiation of preemptive therapy at the appropriate time, while assessment of immune reconstitution is essential because it is considered to be associated with post-transplant infection, relapse of primary disease and OS.[9]

CD4+ T-cell counts, T-cell proliferative capacity, B-cell number and serum IgG have been used as parameters of immune recovery after HCT. Recently, more direct assessment of T- and B-cell neogenesis has become feasible by analyses of T-cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs), respectively.

DNA fragments between rearranging V, D and J gene segments are deleted as circular excision products during rearrangement of the T-cell receptor gene.[10] These products are called TRECs. Quantitative detection of TRECs enables direct measurement of thymic output. The recovery of TRECs is associated with survival and infection after HCT for treatment of malignancies.[11–13] In a previous study, TREC levels were lower in patients post CBT than in those receiving BMT or PBSC transplantation (PBSCT).[14]

KRECs are formed by Ig kappa-deleting rearrangement during B-cell development. Coding joint KRECs (cjKRECs) serve as an indicator of B-cell numbers,[15] and signal joint KRECs (sjKRECs) are an indicator of B-cell neogenesis. However, the kinetics of KREC recovery post HCT are largely unknown. A correlation between KRECs and survival or infection after HCT has not been reported previously. In addition, whether B-cell recovery as assessed by KRECs is different among graft sources is still unknown.

Here, we investigated the kinetics of TREC and KREC recovery post HCT and factors contributing to better recovery of TREC and KREC levels, mainly focusing on KRECs. We also assessed the association of KRECs with infection after HCT in patients with malignancies or PID.

MATERIALS AND METHODS

Patients

A total of 133 patients who underwent allogeneic HCT from March 1996 to August 2013 were enrolled in this study. The patients were followed up at the Department of Pediatrics or Department of Hematology at Tokyo Medical and Dental University or the Department of Pediatrics of the National Defense Medical College in Japan. The median age at transplantation was 12 years (range, 0–62 years). Table 1 shows the patient characteristics, information on HCT and events associated with transplantation. This study was approved by the ethics committees of

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Table 1. Patient characteristics and clinical course

	PID (56)	*Malignancy (77)*	*All (133)*
Patient characteristics			
Recipient age			
< 18 years	45	44	89
⩾ 18 years	11	33	44
Sex			
Male	42	44	86
Female	14	33	47
Conditioning			
RIC or minimal conditioning	33	10	43
MA	23	66	89
Donor age (BM)			
< 18 years	5	8	13
⩾ 18 years	25	38	63
Cell source			
BM	35	47	82
CB	19	17	36
PB	2	13	15
HLA allele			
⩽ 5/6	20	31	51
6/6	23	33	56
Relation			
Related	13	30	43
Unrelated	43	47	90
Steroid use	28 (52%)	32 (42%)	60 (46%)
ATG use	26 (48%)	5 (6%)	31 (24%)
Clinical course			
Acute GVHD			
Grade 0–2	44	53	97
Grade 3–4	8	10	18
Chronic GVHD	15 (28%)	35 (45%)	50 (38%)
Infection	29 (53%)	48 (62%)	77 (58%)
Bacterial infection	11 (20%)	20 (26%)	31 (23%)
Fungal infection	4 (7%)	6 (8%)	10 (8%)
Viral infection	18 (33%)	35 (45%)	53 (40%)
Relapse	—	30 (39%)	—
Survival	51 (91%)	49 (64%)	100 (75%)

Abbreviations: ATG = antithymocyte globulin; CB = cord blood; MA = myeloablative; PID = primary immunodeficiency; RIC = reduced-intensity conditioning.

Tokyo Medical and Dental University and National Defense Medical College. Informed consent was obtained in accordance with the Declaration of Helsinki.

Measurement of TREC and KREC levels

TREC, sjKREC and cjKREC levels were measured by real-time PCR as described previously[15–19] at 1, 3 and 6 months, and yearly after HCT. RNase P was used as an internal control. Primer and probe sequences are listed in Supplementary Table I. The minimum detectable limit was 10 copies/μg DNA. TRECs or KRECs < 10 copies/μg DNA were defined as negative, and TREC or KREC levels of > 10 copies/μg DNA were defined as positive.

Monitoring of infections

Genomic DNA of eight human herpes virus species, BK virus, JC virus and parvovirus B19 in peripheral blood was measured by multiplex PCR and real-time PCR as described previously.[20] Adenovirus, hepatitis A virus, hepatitis B virus, hepatitis E virus, Norwalk-like virus, Coxsackie virus, ECHO virus, enterovirus, human metapneumovirus and human bocavirus were measured by real-time PCR as described elsewhere.[21] The minimum detectable limit was at least 30 copies/μg DNA.

Definitions

Patients treated with a > 5 Gy single dose of TBI, > 8 Gy fractionated TBI or > 8 mg/kg body weight of BU in addition to other cytoreduction agents were categorized as receiving myeloablative (MA) regimens.[22] HLA typing was performed by genotyping for HLA-A, B and DRB1 loci. GVHD was graded according to standard criteria.[23] We defined the incidence of infection as having symptoms of infection with detectable pathogens and severity ⩾ grade 3 as defined in the Common Terminology Criteria for Adverse Event (CTCAE) version 4.0, National Institutes of Health and National Cancer Institute.

Statistical analysis

Recipient age, recipient sex, disease, conditioning regimen, donor age, cell source, HLA disparity, relationship, acute GVHD, chronic GVHD, and the use of steroids or antithymocyte globulin (ATG) were chosen as clinical parameters. We categorized the diseases of enrolled patients as PID or malignancy. A MA regime was evaluated in comparison with reduced-intensity conditioning regimens and minimal conditioning regimens. HLA-mismatched HCT was compared with 6/6 HLA-matched HCT. Acute GVHD was graded as 0–4 and divided into two groups (0–2 and 3–4). The proportion of surviving patients was estimated by the Kaplan–Meier method and compared using the log-rank test. Factors that were found to be significant ($P < 0.05$) in univariate analysis were included in the multivariate analysis. Multivariate analyses of factors contributing to better TREC/KREC recovery were performed by excluding or including acute and chronic GVHD, steroid use, and ATG use, because these factors are post-HCT events and associated with other factors. Donor age was also excluded because it is restricted for BMT.

RESULTS

Levels of sjKRECs and cjKRECs recover faster than those of TRECs

First, we evaluated the recovery of TREC, sjKREC and cjKREC levels post transplantation.

One month after HCT, TRECs, sjKRECs and cjKRECs were detectable in 17 (17.5%), 34 (35.1%),and 28 (28.9%) of 97 patients, respectively. The median copy number was low (< 10 copies/μg DNA) in all assays (Figures 1a and b).

Eighty-two patients were examined 3 months after HCT. TRECs were positive in 15 (18.3%) patients, whereas sjKRECs and cjKRECs were positive in 57 (69.5%) and 59 (72.0%) patients, respectively (Figures 1c and d).

TRECs became positive in 41.3% of patients at 6 months and in 66.7% of patients 1 year post HCT. SjKRECs and cjKRECs were positive in 77.8% of patients at 6 months and in > 90% of patients at 1 year post HCT. The median level of TRECs was < 10 copies/μg DNA at 6 months and reached up to 1270 copies/μg DNA at 1 year. Interestingly, sjKRECs continued to increase for at least 1 year, while cjKRECs peaked at 6 months, and then started to decline (Figures 1e–h).

The recovery of sjKREC and cjKREC levels correlated as shown in Figures 1b, d, f, and h. This finding indicates that B-cell maturation is intact once B-cell engraftment is achieved. On the other hand, a considerable number of patients exhibited B-cell neogenesis in the absence of T-cell neogenesis, especially at the early stage post HCT (Figures 1a, c, and e).

We examined the trend of TRECs and KRECs in individual patients, of whom 71% had positive sjKRECs at 1 month and showed increased sjKRECs at 3 months. Similarly, the levels of sjKRECs detectable at 1 month increased at 6 months in 80% of the patients (Figure 2b). On the other hand, positive TRECs at 1 month did not indicate further T-cell recovery at a later period. When we examined patients with positive TRECs at 3 months, 10 of the 11 patients had increased TREC levels at 6 months, suggesting that positive TRECs at 3 months may serve as a predictor of T-cell reconstitution after 6 months (Figure 2a).

Longitudinal analysis showed that the recovery course of TRECs from 1 month to 15 years post HCT is at least not inferior to CBT when compared with that of BMT and PBSCT. Compared to BMT,

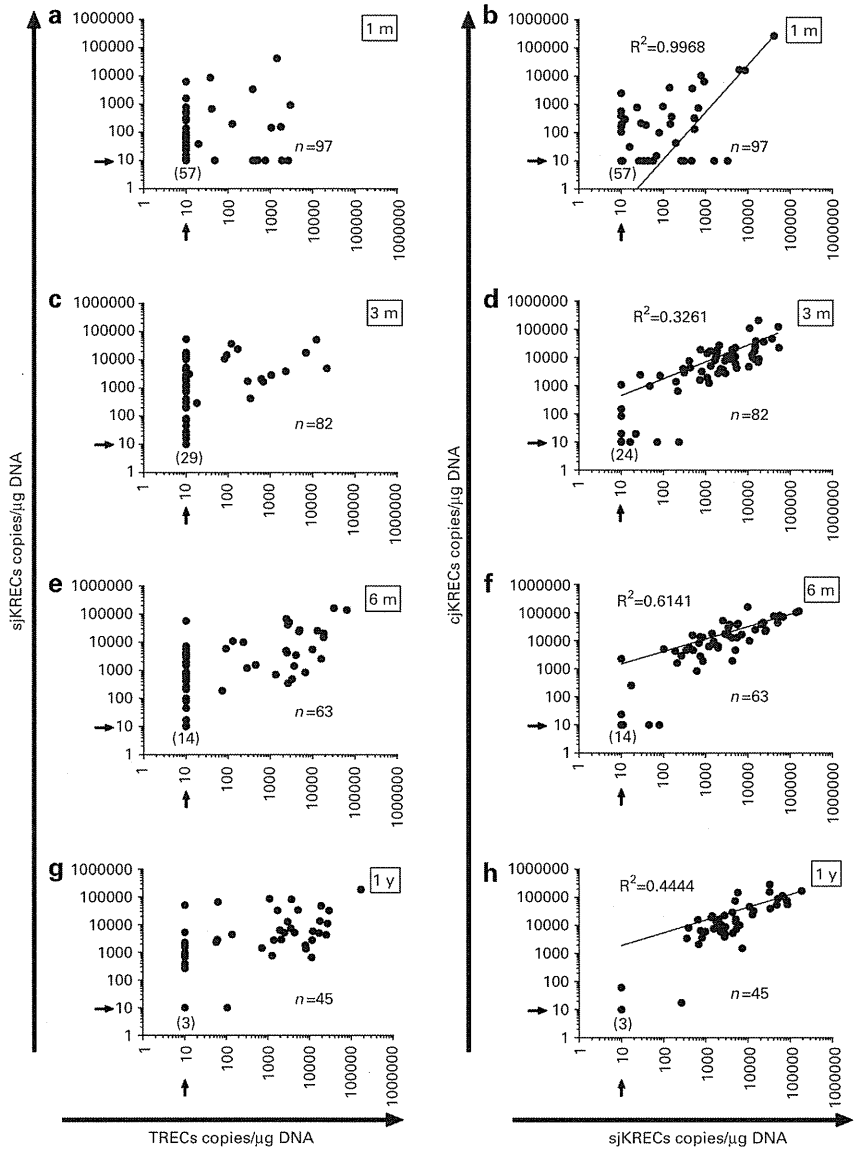

Figure 1. Recovery of TREC and KREC levels. Recovery of the levels of TRECs and sjKRECs, and sjKRECs and cjKRECs at 1 month (**a**, **b**), 3 months (**c**, **d**), 6 months (**e**, **f**) and 1 year (**g**, **h**) after HCT. Arrows show the detectable limit of the real-time PCR (10 copies/μg DNA). Values under the limit are considered 'negative'. Numbers in parentheses indicate the number of subjects who show a negative value (< 10 copies/μg DNA) for the indicated products.

KREC levels recovered more rapidly after CBT (Supplementary Figures 1 and 2). Final sjKREC/cjKREC levels reached the levels of the age-matched control when KRECs were fully recovered (data not shown).

Younger recipient age and CB favor increased levels of sjKRECs and cjKRECs

Next, we evaluated the factors that contributed to the levels of TRECs, sjKRECs and cjKRECs by regression analysis, including the factors listed in Materials and Methods (Supplementary Table II). A younger recipient and donor age was defined as < 18 years old.

In univariate analysis, a younger recipient age was a favorable factor for increased levels of TRECs, sjKRECs or cjKRECs post HCT. In fact, only the cjKREC levels of older recipients became close to those of younger recipients at 2 years after HCT (Figure 3). In BMT recipients, a younger donor age was a favorable factor for increased levels of TRECs, sjKRECs and cjKRECs (Supplementary Table II and Supplementary Figure 3). Compared with BM or PB, the use of CB was a favorable factor for increased levels of sjKRECs and cjKRECs after HCT (Figure 4 and Supplementary Table II).

A MA regime, PID, no or mild acute GVHD (grade 0–2), no chronic GVHD, no use of steroids, and no use of ATG were also favorable factors for increased levels of TRECs, sjKRECs or cjKRECs at various time points after HCT (Supplementary Table II).

On the basis of the results obtained from the univariate analysis, the following factors were used in multivariate analysis: recipient age, disease, conditioning regimen, cell source and relationship. Our results concerning TRECs largely reconfirmed previous reports,[13,24] indicating that a younger recipient age, no ATG use and a MA regime are associated with better TREC recovery. When focusing on B-cell recovery, we found that a younger recipient age was a favorable factor for increased levels of sjKRECs at 6 months to 2 years and cjKRECs at 6 months to 1 year after HCT. In addition, compared with BMT, CBT favored increased levels of sjKRECs at 1, 3 and 48 months. A MA regime was a favorable factor for increased levels of sjKRECs at 3 to 6 months (Table 2).

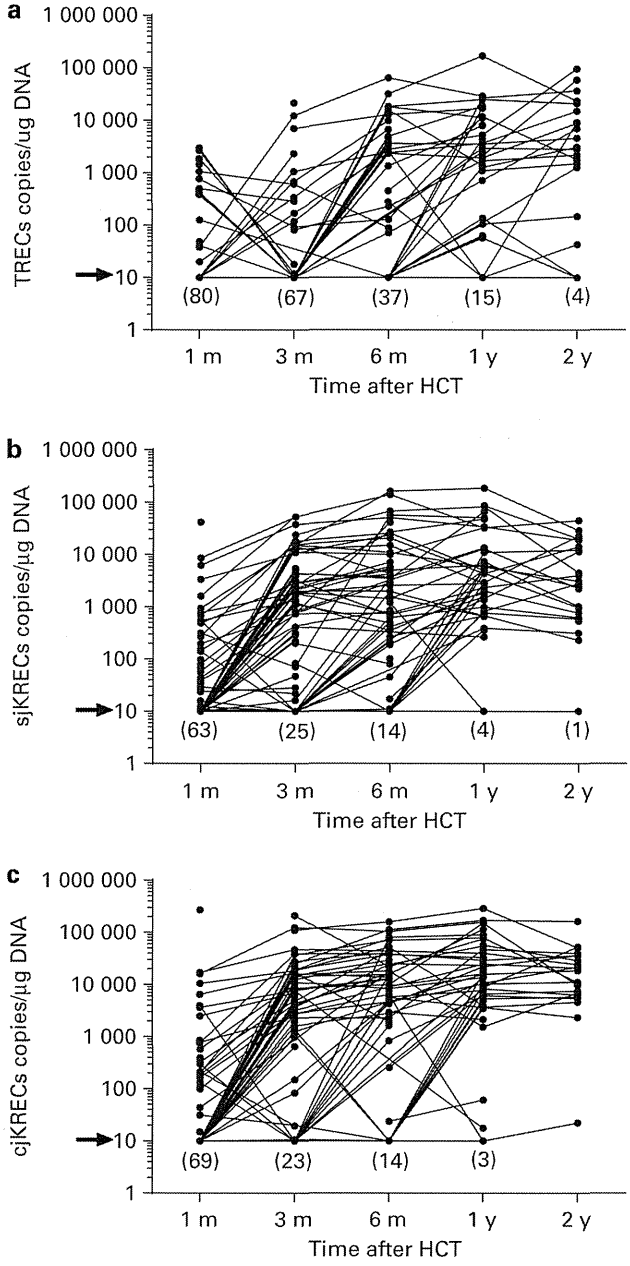

Figure 2. Levels of TRECs and KRECs after HCT. The levels of TRECs (**a**), sjKRECs (**b**) and cjKRECs (**c**) after HCT. The arrows show the detectable limit of the real-time PCR (10 copies/μg DNA). Values under this limit are considered 'negative'. Numbers in parentheses indicate the number of subjects who show a negative value (< 10 copies/μg DNA) for the indicated products.

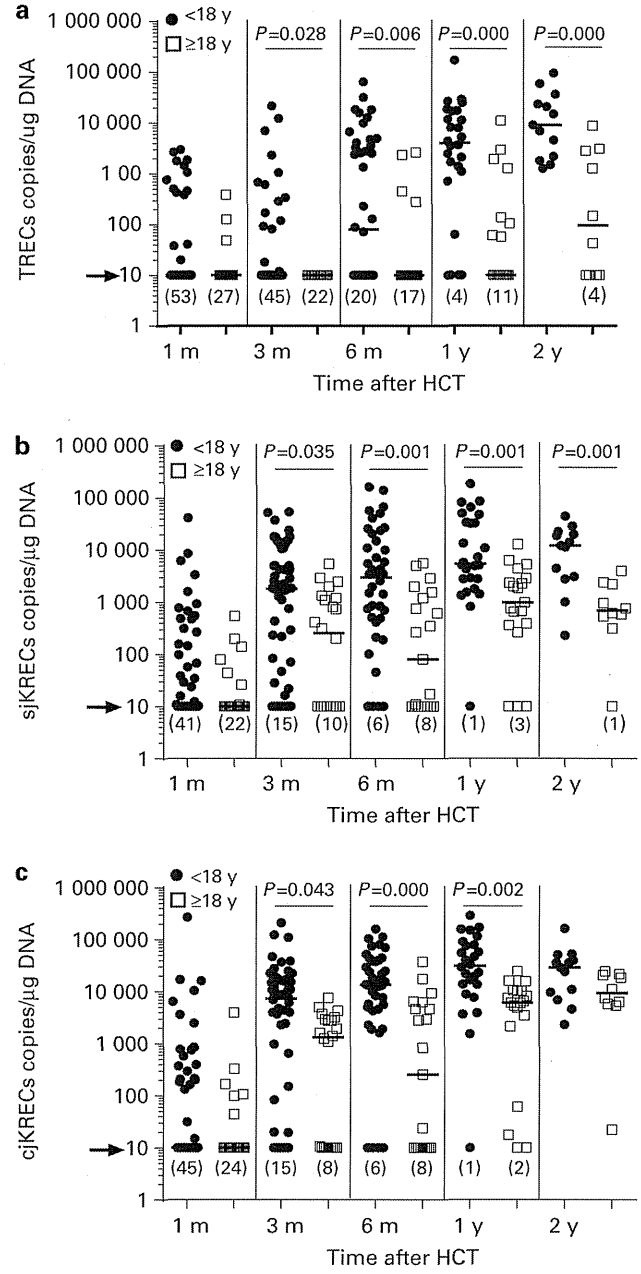

Figure 3. Recipient age and the levels of TRECs and KRECs. Recipient age and the levels of TRECs (**a**), sjKRECs (**b**) and cjKRECs (**c**). Closed circles indicate < 18 years old, and open squares indicate ⩾ 18 years old. Arrows show the detectable limit of the real-time PCR (10 copies/μg DNA). Values under this limit are considered 'negative'. Numbers in parentheses indicate the number of subjects who show a negative value (< 10 copies/μg DNA) for the indicated products.

By including acute GVHD, steroid use and ATG use in the analysis, grade 0–2 acute GVHD, no steroid use and no ATG use were identified as factors favoring better KREC recovery at various time points (Supplementary Table III). The analysis further including chronic GVHD suggested that the condition does not affect B-cell neoproduction (Supplementary Table IV).

We then performed multivariate analysis of a group of patients with malignancy. Compared with BM recipients, the results showed that sjKRECs and cjKRECs were more frequently detectable in CB recipients at 3 months (Supplementary Table V). Compared with BM recipients, in adult patients of ⩾ 18 years of age ($n = 44$), the use of CB was a favorable factor for increased levels of sjKRECs at 1 month (Supplementary Table VI). These data show that CB use contributes to early recovery of neogenesis. In contrast, we observed no significant difference of T-cell recovery in adult patients when CB use was compared with BM at any time point after HCT (Supplementary Figure 4).

Positivity for sjKRECs 1 month after HCT is associated with decreased infectious episodes

We next investigated whether the levels of TRECs, sjKRECs or cjKRECs were associated with the occurrence of infections. We found that positive sjKRECs or TRECs 1 month after HCT correlated

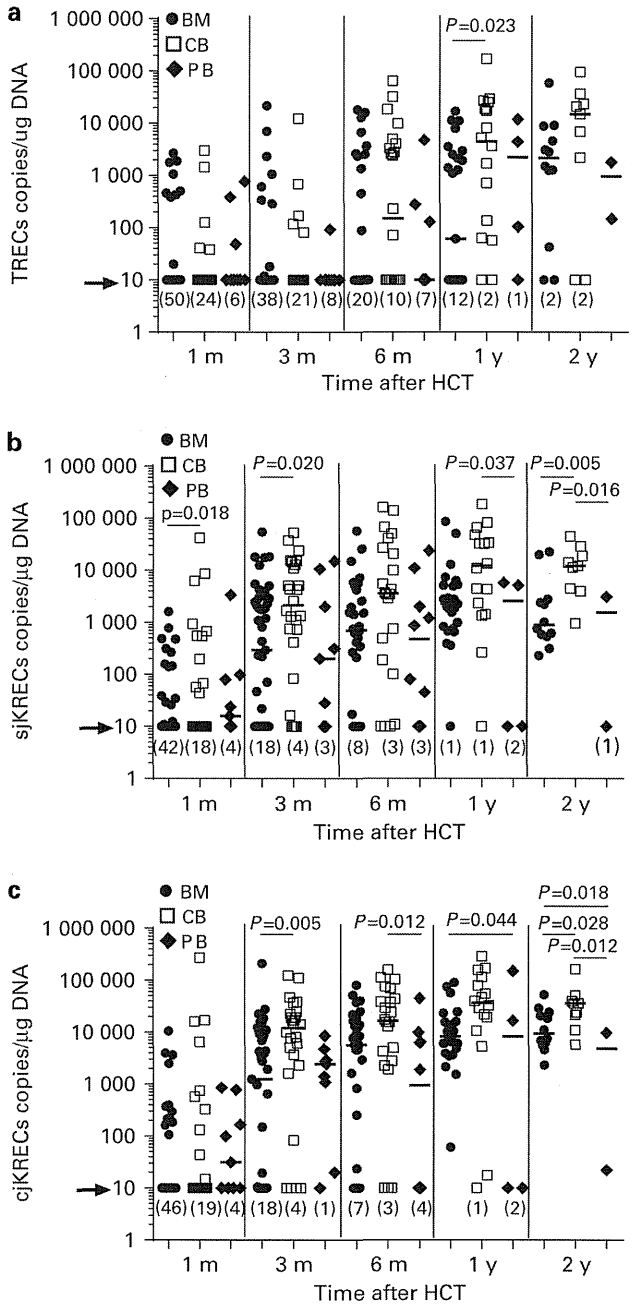

Figure 4. Cell source and the levels of TRECs and KRECs. Cell source and the levels of TRECs (**a**), sjKRECs (**b**) and cjKRECs (**c**). Closed circles indicate BM, open squares indicate cord blood and closed diamonds indicate peripheral blood. Arrows show the detectable limit of the real-time PCR (10 copies/μg DNA). Values under the limit are considered 'negative'. Numbers in parentheses indicate the number of subjects who show a negative value (< 10 copies/μg DNA) for the indicated products.

with decreased infectious episodes (Figure 5). Sixteen out of 34 patients who were positive for sjKRECs suffered from infections, whereas 43 of 63 patients who were negative for sjKRECs acquired infections (Figure 5b, $P = 0.033$).

We also examined the association between each index and the incidence of infectious episodes caused by bacteria, fungi or viruses. Although there was a tendency toward less bacterial infections in sjKREC- or cjKREC-positive groups, we found no statistical significance. Cumulative incidence of each infection did

Table 2. Multivariate analysis of factors that contributed to the levels of TRECs, sjKRECs and cjKRECs

Factors	*1 month*		*3 months*		*6 months*		*1 year*		*2 years*	
	β (95% CI)	*P-value*	*β (95% CI)*	*P-value*	*β (95% CI)*	*P-value*	*β (95% CI)*	*P-value*	*β (95% CI)*	*P-value*
TRECs										
Younger recipient age	0.084 (−0.213 to 0.449)	0.481	0.140 (−0.161 to 0.633)	0.241	0.231 (−0.133 to 1.400)	0.104	**0.379 (0.263 to 1.829)**	**0.010**[a]	**0.629 (0.662 to 2.511)**	**0.002**[a]
PID	0.217 (−0.077 to 0.665)	0.119	0.120 (−0.221 to 0.583)	0.373	0.126 (−0.505 to 1.158)	0.435	**0.348 (0.155 to 1.769)**	**0.021**[a]	0.355 (−0.254 to 2.118)	0.115
MA	**0.354 (0.137 to 0.853)**	**0.007**[a]	0.166 (−0.134 to 0.648)	0.194	0.049 (−0.635 to 0.893)	0.737	0.154 (−0.338 to 1.180)	0.269	−0.117 (−1.217 to 0.632)	0.512
BM (compared with CB)	0.088 (−0.194 to 0.428)	0.456	−0.071 (−0.488 to 0.275)	0.580	−0.183 (−1.241 to 0.296)	0.223	−0.256 (−1.514 to 0.111)	0.088	−0.018 (−0.925 to 0.837)	0.916
PB (compared with CB)	0.223 (−0.114 to 1.113)	0.109	−0.254 (−1.338 to 0.130)	0.105	−0.286 (−2.415 to 0.391)	0.154	−0.106 (−2.046 to 1.030)	0.508	0.033 (−1.742 to 2.032)	0.873
Relation	−0.075 (−0.491 to 0.272)	0.571	**0.344 (0.115 to 1.000)**	**0.014**[a]	0.220 (−0.345 to 1.568)	0.206	0.195 (−0.312 to 1.487)	0.194	0.116 (−0.735 to 1.367)	0.532
sjKRECs										
Younger recipient age	0.070 (−0.282 to 0.552)	0.553	0.072 (−0.427 to 0.841)	0.517	**0.339 (0.241 to 1.618)**	**0.009**[a]	**0.328 (0.060 to 1.292)**	**0.032**[a]	**0.414 (0.071 to 1.333)**	**0.031**[a]
PID	0.160 (−0.187 to 0.715)	0.248	0.178 (−0.186 to 1.098)	0.161	−0.053 (−0.883 to 0.610)	0.716	0.236 (−0.147 to 1.123)	0.128	0.282 (−0.310 to 1.308)	0.210
MA	0.167 (−0.151 to 0.719)	0.198	**0.263 (0.069 to 1.318)**	**0.030**[a]	**0.299 (0.098 to 1.471)**	**0.026**[a]	0.107 (−0.378 to 0.817)	0.462	0.069 (−0.514 to 0.747)	0.701
BM (compared with CB)	**−0.258 (−0.796 to −0.041)**	**0.030**[b]	**−0.372 (−1.566 to −0.347)**	**0.003**[b]	−0.230 (−1.284 to 0.095)	0.090	−0.181 (−1.010 to 0.268)	0.247	**−0.370 (−1.224 to −0.022)**	**0.043**[b]
PB (compared with CB)	−0.098 (−1.012 to 0.479)	0.479	**−0.407 (−2.824 to −0.480)**	**0.006**[b]	−0.349 (−2.495 to 0.025)	0.055	**−0.447 (−2.809 to 0.389)**	**0.011**[b]	−0.420 (−2.540 to 0.034)	0.056
Relation	0.149 (−0.200 to 0.727)	0.262	**0.394 (0.381 to 1.793)**	**0.003**[a]	0.194 (−0.320 to 1.398)	0.214	0.218 (−0.219 to 1.197)	0.170	−0.019 (−0.751 to 0.682)	0.920
cjKRECs										
Younger recipient age	0.017 (−0.450 to 0.519)	0.887	0.085 (−0.429 to 0.967)	0.445	**0.352 (0.309 to 1.822)**	**0.007**[a]	**0.383 (0.144 to 1.563)**	**0.020**[a]	0.171 (−0.375 to 0.869)	0.412
PID	**0.287 (0.024 to 1.112)**	**0.041**[a]	0.148 (−0.285 to 1.128)	0.239	−0.052 (−0.969 to 0.671)	0.717	0.145 (−0.407 to 1.055)	0.376	0.305 (−0.339 to 1.257)	0.240
MA	0.216 (−0.083 to 0.966)	0.098	**0.281 (0.136 to 1.510)**	**0.020**[a]	0.257 (−0.007 to 1.500)	0.052	0.015 (−0.654 to 0.721)	0.921	0.071 (−0.520 to 0.724)	0.733
BM (compared with CB)	−0.164 (−0.775 to 0.135)	0.166	**−0.427 (−1.890 to −0.548)**	**0.001**[b]	−0.189 (−1.297 to 0.219)	0.160	−0.033 (−0.810 to 0.662)	0.840	−0.261 (−0.967 to 0.218)	0.199
PB (compared with CB)	−0.026 (−0.985 to 0.812)	0.849	**−0.329 (−2.770 to −0.190)**	**0.025**[b]	**−0.422 (−3.035 to −0.267)**	**0.020**[b]	−0.346 (−2.730 to 0.056)	0.059	**−0.580 (−2.747 to −0.208)**	**0.025**[b]
Relation	0.158 (−0.225 to 0.893)	0.238	**0.336 (0.253 to 1.808)**	**0.010**[a]	0.160 (−0.454 to 1.433)	0.303	0.154 (−0.441 to 1.188)	0.359	0.001 (−0.706 to 0.708)	0.998

Abbreviations: CB = cord blood; cjKREC = coding joint kappa-deleting recombination excision circle; MA = myeloablative; PB = peripheral blood; PID = primary immunodeficiency; sjKREC = signal joint kappa-deleting recombination excision circle; TREC = T-cell receptor excision circle. Bold letters indicate significant factors. [a]Significant favorable factors that contributed to the levels of TRECs, sjKRECs and cjKRECs. [b]Significant unfavorable factors that contributed to the levels of TRECs, sjKRECs and cjKRECs.

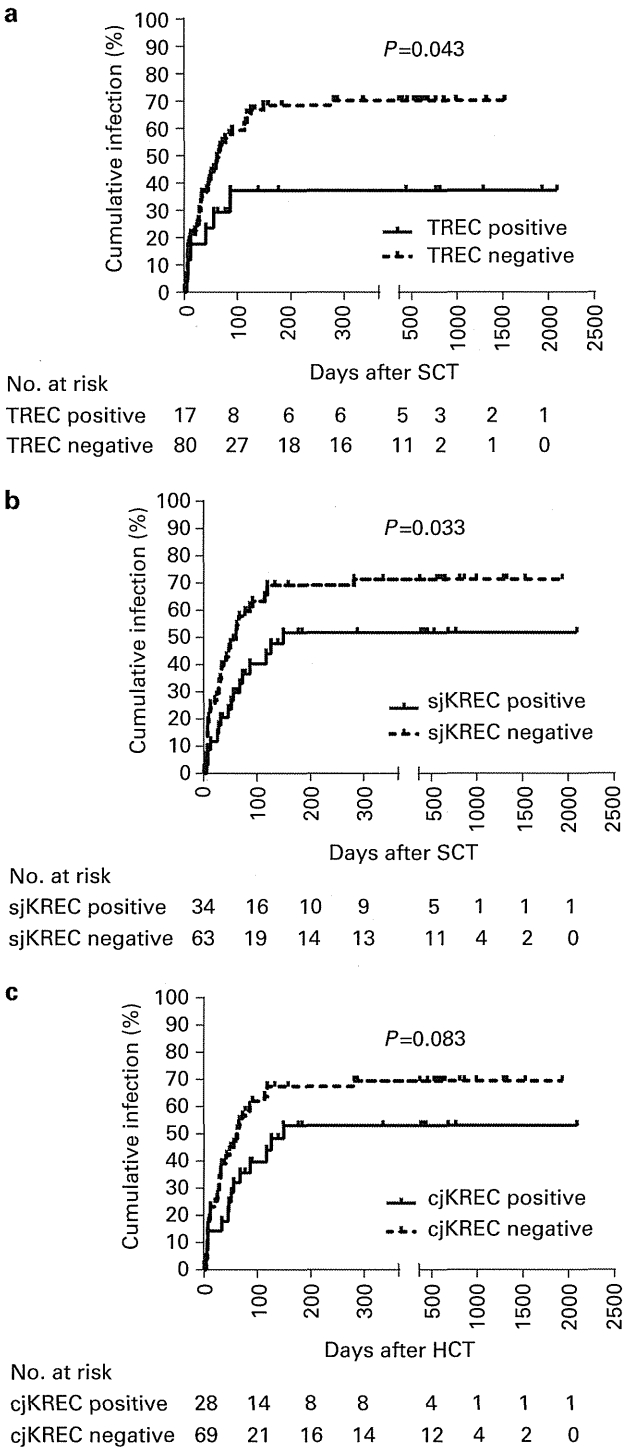

Figure 5. Cumulative incidence of infection after HCT. Cumulative incidence of infection and the levels of TRECs (**a**), sjKRECs (**b**) and cjKRECs (**c**) 1 month after HCT. Solid lines indicate the positive levels of TRECs (**a**), sjKRECs (**b**) and cjKRECs (**c**), and the dotted lines indicate their negative levels.

not correlate with negative TRECs, sjKRECs or cjKRECs at 3 months after HCT (data not shown).

DISCUSSION

In this study, we examined TRECs and sjKRECs/cjKRECs in post-transplantation patients with malignancies or PID. Our data showed the following. (1) The levels of sjKRECs and cjKRECs increase earlier than those of TRECs. (2) A younger recipient age is favorable for better recovery of sjKRECs and cjKRECs post HCT. (3) The use of CB achieves rapid recovery of sjKRECs and cjKRECs compared with that of BM or PB as a graft source. (4) Detectable sjKRECs 1 month after HCT is related to a decreased frequency of infectious episodes.

Patients with positive sjKRECs at 1 month had increased levels of sjKRECs at 3 and 6 months, suggesting that positivity can predict sound B-cell immune reconstitution. In addition, the levels of sjKRECs and cjKRECs increased earlier than those of TRECs (Figure 1, Supplementary Figures 1 and 2).

There have been no reports of the factors that contribute to better KREC reconstitution. Compared with BM and PB, we found that the levels of sjKRECs and cjKRECs recovered rapidly in patients who received CB. Faster B-cell reconstitution after CBT has been reported previously.[14,25] CB itself does not have high sjKREC/cjKREC levels. Our results suggest that rapid B-cell recovery by CBT is because of B-cell neogenesis and not B-cell expansion in the periphery.

A previous study has demonstrated that sjKREC levels are the highest in < 1-year-olds and then declines with age in healthy children.[17] Thus, it is likely that younger donors have an advantage in terms of B-cell reconstitution. Our results indicated that a younger recipient age also contributed to increased levels of sjKRECs and cjKRECs.

In addition, our data showed that acute 0–2 GVHD, no steroid use and no ATG use were associated with positive sjKRECs and cjKRECs (Supplementary Tables III and IV). These data indicate that steroid or ATG use affects not only T-cell recovery but also B-cell immune reconstitution.

As expected, patients with chronic GVHD showed significantly lower levels of TRECs at 6 months and 1 year. On the other hand, and in contrast to our expectation, we observed lower sjKRECs and cjKRECs from 3 months to 2 years in patients with chronic GVHD (Supplementary Figure 5). This observation does not support the data of Allen *et al.*,[26] which revealed increased numbers of B cells and expression of BAFF (B-cell-activating factor belonging to the TNF family) in patients with chronic GVHD. This discrepancy may be because the patients with chronic GVHD were on more active immunosuppressants compared with those without chronic GVHD. Additionally, there may be relatively high levels of KRECs in patients with the severe extensive type of chronic GVHD. However, we would need more patients and additional analyses of B-cell numbers and activation to reach a conclusion.

Our study suggests that patients with positivity for TRECs or sjKRECs at 1 month are less likely to develop post-transplant infections. The contribution of earlier B-cell recovery to overall immunity, especially anti-microbial immunity, needs further investigation. Patients with early B-cell neogenesis may attain early myeloid recovery. B-cells may also serve as antigen-presenting cells in addition to antibody-producing cells.

A correlation between KREC levels and prognosis has not been addressed previously. Although there was a tendency toward better survival for the KREC-positive group at 1 month, we observed no statistical significance. Further study with a larger cohort is required to determine whether the difference can be significant.

It is still unclear whether TREC levels are lower in patients post CBT than in those receiving BMT.[25] Our data focusing on adult patients showed that T-cell recovery was at least not inferior and appeared to be similar in CB and BM recipients (data not shown). On the other hand, compared with BM and PB, CB was superior for B-cell recovery. This observation suggests quantitative superiority of B-cell recovery following CBT. Further study should investigate the repertoire diversity and somatic hypermutation of B-cell receptors to evaluate qualitative differences and determine whether rapid qualitative maturation has an effect on improved

outcomes. In combination with *in vitro* immunological data and clinical data such as long-term infection, autoimmunity and immunological findings, KRECs and TRECs may serve as useful tools for immunological monitoring after HCT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ASTUTE CLINICIAN REPORT

Hematopoietic Stem Cell Transplantation for X-Linked Thrombocytopenia With Mutations in the *WAS* gene

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Abstract X-linked thrombocytopenia (XLT) is a mild form of the Wiskott-Aldrich syndrome (WAS) caused by mutations in the *WAS* gene. A recent retrospective study of the clinical outcome and molecular basis of a large cohort of XLT patients demonstrated that although overall survival is excellent, event free survival is severely affected with conservative treatment. To answer the question whether hematopoietic stem cell transplantation (HSCT) offers a viable alternative therapeutic option in XLT, we retrospectively investigated the outcome of HSCT in a cohort of 24 XLT patients who received HSCT

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between 1990 and 2011 at 14 transplant centers in the United States, Italy, Germany, Canada, and Japan. The engraftment rate was 100 % and the overall survival rate was 83.3 %. Of the four non-survivors, 2 underwent splenectomy prior to HSCT and died of sepsis, and two of aspergillus infections associated with severe GVHD. In all but one patient, pretransplant complications were resolved by HSCT. Our data indicate that HSCT following myeloablative conditioning is curative and associated with acceptable risks as a treatment option for XLT.

Keywords X-linked thrombocytopenia · hematopoietic stem cell transplantation · Wiskott-Aldrich syndrome

Introduction

X-linked thrombocytopenia (XLT), caused by mutations in the Wiskott-Aldrich syndrome (*WAS*) gene [1], is defined as a mild WAS phenotype with symptoms limited to thrombocytopenia, absent or mild eczema and mild to moderate infections (WAS-score 1–2 [2]). While hematopoietic stem cell transplantation (HSCT) is curative and the treatment of choice for patients with classic WAS (WAS-score 3–4) [3–5], the therapeutic recommendations for XLT are less clear and include avoiding contact sports, wearing a helmet, and possibly undergoing splenectomy or HSCT. A recent retrospective study of the clinical outcome and molecular basis of a large cohort of XLT patients demonstrated that although overall survival is excellent, event free survival is severely affected [6]. With conservative treatment, nearly 75 % of XLT patients reported complications throughout the course of the disease, including life threatening intra-cranial hemorrhage, systemic infections after splenectomy, autoimmune diseases and malignancies [6]. This observation raises the question whether HSCT offers a viable alternative therapeutic option in XLT. Here, we retrospectively investigated the outcome of HSCT in a cohort of 24 patients with the XLT phenotype and mutations in the *WAS* gene.

Methods

Data Accrual

We enrolled patients with clinically defined XLT who had reliable information and were transplanted between 1990 and 2011 at 14 transplant centers in the United States, Italy, Germany, Canada, and Japan including those reported previously [6, 7]. Patient data were acquired by retrospective chart review and then anonymized by the submitting physicians. The study was either approved or a waiver of consent was issued by the local ethics committee of each institution.

Patients

To be enrolled, patients had to meet all of the following criteria [6].

(I) classified by their treating physician as XLT,
(II) *WAS* gene mutations consistent with an XLT phenotype (either previously reported for XLT [6], or missense mutations in exon 1–3, or variant splice site mutations),
(III) WAS score of 1, 2 or 5; we included patients with a score of 5 if they were scored as 1 or 2 before the development of autoimmunity or malignancy.

In this study we wanted to enroll all patients with a mutation in the *WAS* gene and a mild WAS phenotype (Score 1–2) at the time of HSCT, so patients<2 years of age were included.

Definitions

Life threatening infections were defined as requiring hospitalization such as meningitis, or pneumonia needing oxygen supply or mechanical ventilation. Serious bleeding was defined as a fatal or life-threatening bleeding episode requiring hospitalization or red blood cell transfusion. Other serious complications were a diagnosis of autoimmunity, malignancy or death. Mutations are described according to the nomenclature recommended by den Dunnen and Antonarakis [8].

Statistical Analysis

Kaplan Meier survival estimates were compared using the log rank test (GraphPad Prism Version 5.0a; GraphPad Software, San Diego, California, USA).

Results and Discussion

To be enrolled, patients had to meet the criteria described in the method section, including an initial WAS/XLT score of 1 or 2 [2,6].

Patient characteristics are shown in Table 1. Three patients (patient 19, 20 and 24) had progressed to a score of 5 before undergoing HSCT because they developed autoimmunity or malignancy. WASp was expressed in all 19 patients studied by Western blot or FACS, while the remaining 5 patients had mutations expected to express at least some WAS protein [9]. Median age at the time of HSCT was 7.5 years (range, 1.3–37 years). All 24 patients underwent myeloablative conditioning before receiving fully or partially matched hematopoietic stem cells (Table 2). Post-transplantation follow-up ranged from 21 to 204 months (median, 50 months). 20/24 patients (83.3 %) enrolled are alive without serious post

Table 1 Patient characteristics and disease status

No.	cDNA mutation (predicted protein change)	WASP	Pre-transplant complications[a]				Age and year of HSCT	Donor and TNC ($x10^8$/kg)	HLA match
			Severe hemorrhage	Infections	Eczema	Others			
1	464-2A>G (splice defect[c])	positive	–	–	–	–	1.3 2005	BM-URD 4.1	11/12
2	559+5G>A (splice defect[d])	positive	Melena	–	+	absence of isohemagglutinins	1.5 1998	UCB-URD 0.7	5/6
3	1508-1511delGAGT (X502Trp)	positive	–	–	–	–	1.8 2004	BM-URD 2.5	11/12
4	91 G>A (Gly31Lys)	positive	ICH	mild pox lesions after chickenpox vaccine	+	anti-platelet antibody	1.8 2010	UCB-URD 0.47	5/6
5	919A>G (Met307Val)	not done	–	–	–	–	2 2007	BM-URD 6.6	12/12
6	256C>T (Arg86Lys)	positive	–	–	–	–	2.8 2011	BM-URD 7.9	10/10
7	257G>A (Arg86His)	positive	Melena	MRSA enterocolitis, CMV hepatitis	+	–	3 2001	BM-URD 4.2	6/6
8	314 T>C (Leu105Phe)	positive	–	–	–	–	3.3 2002	BM-URD 4.5	8/8
9	361-1G>A (splice defect[e])	positive	Melena	–	+	–	3.5 1997	UCB-MSD 1.0	7/7
10	116 T>C (Leu39Pro)	positive	Melena	–	+	–	4 1999	BM-URD 6.5	9/9
11	256C>T (Arg86Cys)	positive	–	–	+	reactive airway disease	4 1998	BM-URD 4.4	5/6
12	1075-1079delC (Pro360HisfsX444)	not done	–	–	–	–	7 1999	UCB-MSD 0.7	8/8
13	256C>T (Arg86Cys)	positive	–	CMV hepatitis	–	–	8 2005	BM-MSD 4.2	6/6
14	559+5G>A (splice defect[d])	positive	ICH	viral encephalitis	+	–	10 2006	BM-MSD 4.1	6/6
15	18G>A (Met6Ile)	positive	ICH	Pneumococcal meningitis	–	MDS, splenectomy	11 2002	BM-URD 2.3	5/6
16	559+5G>A (splice defect[d])	positive	–	–	–	–	11 2011	BM-URD 2.4	10/10
17	559+5G>A (splice defect[d])	not done	ICH	–	–	–	11.7 1998	BM-MSD 1.4	8/8
18	399G>T (Glu133Asp)	not done	–	–	–	–	11.8 1998	BM-URD 1.8	7/8
19[b]	1453G>A (Asp485Asn)	positive	–	–	+	IgA nephropathy, atypical LPD, urticaria	14 2008	BM-URD 0.34 (MNC)	6/6
20	71C>T (Ser24Phe)	positive	Melena	–	+	IgA nephropathy, HSP, arthritis	19 1999	BM-MSD 3.2	6/6
21	257G>A (Arg86His)	positive	–	bacterial lymphadenitis, pneumonia	+	–	19 1995	BM-URD 4.7	6/6

Table 1 (continued)

No.	cDNA mutation (predicted protein change)	WASP	Pre-transplant complications[a]				Age and year of HSCT	Donor and TNC ($x10^8$/kg)	HLA match
			Severe hemorrhage	Infections	Eczema	Others			
22	777+1G>A (splice defect[f])	not done	–	–	+	vasculitis	20 2012	BM-URD 1.4	10/10
23	1075-1079delC (Pro360HisfsX444)	positive	Melena	Pneumococcal pneumonia	–	–	21 2005	BM-MMFD 2.7	5/6
24	143C>T (Thr48Ile)	positive	–	multiple episodes of sepsis and meningitis	–	BCP-ALL, splenectomy	37 2004	PB-MSD 16	6/6

ICH intracranial hemorrhage; *MDS* myelodysplastic syndrome; *CMV* cytomegalovirus; *MRSA* methicillin-resistant Staphylococcus aureus; *HSP* Henoch-Schönlein purpura; *BCP-ALL* B-cell precursor acute lymphoblastic leukemia; *LPD* lymphoproliferative disease

[a] Complications other than mild upper respiratory infections, eczema, and petechiae

[b] Reported by Otsubo et al. [10]

[c] This splice site defect is expected to result in truncated WASP

[d] This splice site defect in intron 6 that results in the production of some normal WASP in addition to a insertion of 36 nt from intron 6, resulting in frameshift and stop at amino acid 190 (ref 9)

[e] This splice site defect results in the insertion of intron 3, resulting frameshift and stop at amino acid 201 (ref. 9)

[f] This splice site defect results in the deletion of exon 8, resulting frameshift and stop at amino acid 246 (ref 9)

transplantation events (Fig. 1). There was no statistically significant difference in survival between patients<10 years of age (85.1 %) and those 10 or older (78.8 %). Engraftment rate was 100 % and 23/24 patients achieved more than 95.8 % donor chimerism (supplementary table 1). Although the number of patients suffering from grade II–IV acute GVHD (10/24, 42 %) and chronic GVHD (10/24, 42 %) was high, all surviving patients are presently free from chronic GVHD, are off immunosuppressive medications, are fully immune reconstituted and immunoglobulin independent. All 4 patients who developed extensive chronic GVHD following HSCT (patients 2, 11, 15, 21) had received grafts from unrelated donors who were typed only at 6 HLA-loci. The 7 patients (patients 1, 3, 5, 6, 10, 16, 22) who received grafts that matched better than 9/10 HLA-loci did not develop extensive chronic GVHD. It is therefore possible that the 4 patients with extensive chronic GVHD had a donor that was mismatched by today's standards. Patient 24, who had a splenectomy during infancy, died at age 37 years of overwhelming pneumococcal sepsis 9 months after successful HSCT for acute B cell lymphoblastic leukemia, while off prophylactic antibiotics. Patient 15, who was splenectomized at age 6 years, died of overwhelming pseudomonas sepsis 12 months post-transplantation. Because his GVHD treatment had to be reduced due to tacrolimus-induced encephalopathy, his GVHD exacerbated, causing profound immunosuppression, which, in combination with splenectomy, likely contributed to sepsis, despite being on prophylactic antibiotics. Patient 2 and 11, who had severe chronic GVHD, died of Aspergillus infections 8 and 24 months post-transplantation, respectively. Thus, three of the 4 patients who died post-transplantation had extensive chronic GVHD, and the two who had been splenectomized prior to HSCT succumbed to systemic infections. Splenectomy prior to HSCT and chronic GVHD after HSCT have been recognized as risk factors for premature death following transplantation especially from overwhelming sepsis [4]. Moreover, the recent retrospective study of 173 XLT patients demonstrated the association of splenectomy with severe infections, independent of HSCT: 14 of 41 splenectomized patients developed systemic infections, two being fatal [6]. Risks and benefits of splenectomy should therefore be weighed carefully in XLT patients, especially if HSCT is being considered as a future treatment. If splenectomized prior to HSCT, long term, possibly life-long, antibiotic prophylaxis has to be considered.

None of the transplanted patients developed lymphoproliferative [10] or autoimmune diseases following HSCT, and pretransplant complications, such as hemorrhage secondary to thrombocytopenia and recurrent mild to moderate infections resolved in all but one patient. Patient 23 had one episode of pneumococcal pneumonia after HSCT, but responded promptly to intravenous antibiotics. The median platelet count at last post-transplant visit was 246×10^3 /μl (range, 39–387×10^3 /μl) and all

Table 2 Patient transplant details

No.	Conditioning[a]	GVHD prophylaxis, treatment	Transplant related toxicity	Symptoms[b] (post-transplant)	Acute GVHD	Chronic GVHD	Outcome (cause of death)
1	BU (20)/CY (200)/ATG (10)	CyA	none	none	none	none	Alive, 40 months
2	BU (20)/CY (200)/ATG (90)	CyA+mPSL	none	E.coli sepsis	2	extensive (no response to therapy)	Dead, 8 months (Disseminated Aspergillosis)
3	BU (20)/CY (200)/ATG (10)	CyA	none	none	none	none	Alive, 45 months
4	BU (16)/CY (200)/ATG (90)	CyA+MMF	MRSA Cellulitis	none	2	limited (resolved)	Alive 50 months
5	BU (16)/CY (200)	CyA+MTX	none	none	1	none	Alive, 60 months
6	Mel (140)/FLU (150)/ Campath (45)	CyA+mPSL+MTX, Rituximab	Acute Nephritis	none	1	AIHA (resolved)	Aive 21 months
7	BU (16)/CY (200)	CyA+MTX	none	none	none	none	Alive, 105 months
8	BU (20)/CY (200)/ATG (10)	CyA	none	none	1	none	Alive, 60 months
9	BU (20)/CY (200)/ATG (90)	CyA+PSL, PUVA	none	none	2	limited (resolved)	Alive, 204 months
10	BU (16)/CY (150)/ATG (30[c])	CyA+PSL, MMF	none	none	2	none	Alive, 132 months
11	BU (16)/CY (200)/ATG (60)	CyA+MTX+ATG	Polyserositis, Adenovirus hemorrhagic cystitis, CMV viremia, Pulmonary aspergillosis	none	4	extensive (no response to therapy)	Dead, 24 months (Aspergillus pneumonia)
12	BU (16)/CY (200)	CyA	none	none	2	none	Alive, 124 months
13	BU (16)/CY (200)	CyA+MTX	none	none	none	none	Alive, 62 months
14	BU (16)/CY (200)	CyA+MTX	none	none	none	none	Alive, 36 months
15	BU (16)/CY (200)/ATG (40)	Tacrolimus+MTX	Tacrolimus-induced encephalopathy, Bronchiolitis obliterans, TMA	none	3	extensive (no response to therapy)	Dead, 12 months (Pseudomonas aeruginosa sepsis)
16	BU (16)/FLU (160)/ Alemtuzumab (0.6)	CyA+MMF	none	none	none	none	Alive, 34 months
17	BU (16)/CY (200)/ATG (60)	CyA+PSL	Brief GI bleeding	none	none	none	Alive, 144 months
18	BU (16)/CY (200)/ATG (60)	CyA+PSL	Adenovirus infection	none	2	limited (resolved)	Alive, 89 months
19	BU (12.8)/CY (200)/TBI (3Gy)	Tacrolimus+MTX	viral cystitis	none	2	none	Alive, 38 months
20	BU (16)/CY (200)	CyA+MTX	an inflammation of the glans penis and the prepuce	none	none	extensive (resolved)	Alive, 124 months
21	BU (16)/CY (200)	Tacrolimus[d]	PRCA, ARF, hemorrhagic cystitis	none	1	extensive[e] (resolved)	Alive, 144 months
22	BU (16)/FLU (160)/ Alemtuzumab (0.6)	CyA+MMF	hemorrhagic cystitis, BK virus nephropathy, CMV viremia	none	none	none	Alive, 25 months
23	BU (16)/CY (200)/ATG (40)	Tacrolimus+MTX	hemorrhagic cystitis, TMA	Pneumococcal pneumonia	2	extensive (resolved)	Alive, 50 months