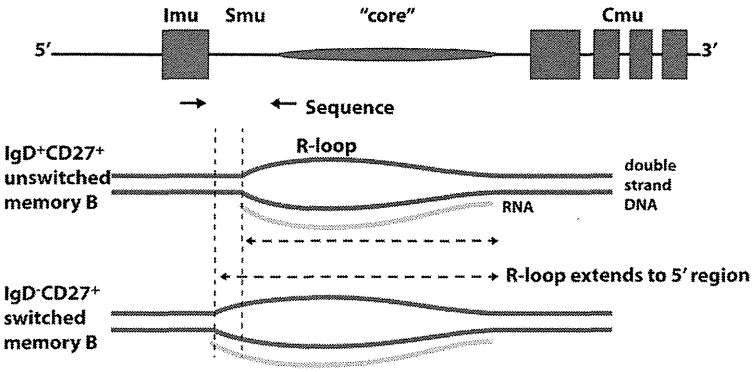


FIG E1. Smu-SHMs might correspond to the extension of the R-loop. We found more mutations in the 3′ part of the sequenced region, which is close to the Smu core region, than in the 5′ region in both unswitched and switched memory B cells. Furthermore, we found more mutations in the 5′ part of the sequenced region in switched memory B cells than in unswitched memory B cells. These findings might indicate the extension of the R-loop during CSR.

Bone Marrow Transplantation (2014) 49, 1155–1161

www.nature.com/bmt

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.

Bone Marrow Transplantation (2014) **49**, 1155–1161; doi:10.1038/bmt.2014.123; published online 30 June 2014

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

[1]Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences, Tokyo, Japan; [2]Department of Pediatrics, Tokyo Medical and Dental University Medical Hospital, Tokyo, Japan; [3]Department of Preventive Medicine and Public Health, National Defense Medical College, Saitama, Japan; [4]Department of Blood Transfusion, Tokyo Medical and Dental University Medical Hospital, Tokyo, Japan; [5]Department of Hematology, Tokyo Medical and Dental University Medical Hospital, Tokyo, Japan and [6]Department of Pediatrics, National Defense Medical College, Saitama, Japan. Correspondence: Dr T Morio, Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. E-mail: tmorio.ped@tmd.ac.jp
Received 17 July 2013; revised 29 April 2014; accepted 1 May 2014; published online 30 June 2014

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,

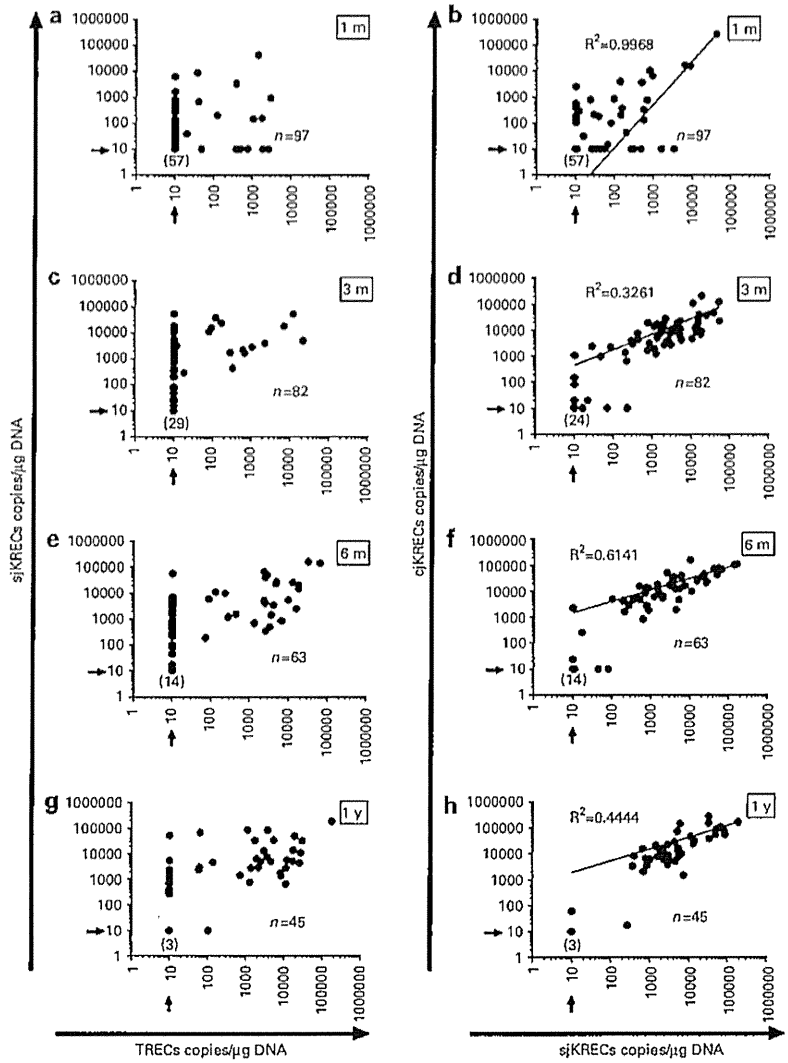


Figure 1. Recovery of TREC and KREC levels. Recovery of the levels of TRECs and sjKRECs, and sjKRECs and cjKRECs at 1 month (**a**, **b**), 3 months (**c**, **d**), 6 months (**e**, **f**) and 1 year (**g**, **h**) after HCT. Arrows show the detectable limit of the real-time PCR (10 copies/μg DNA). Values 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).

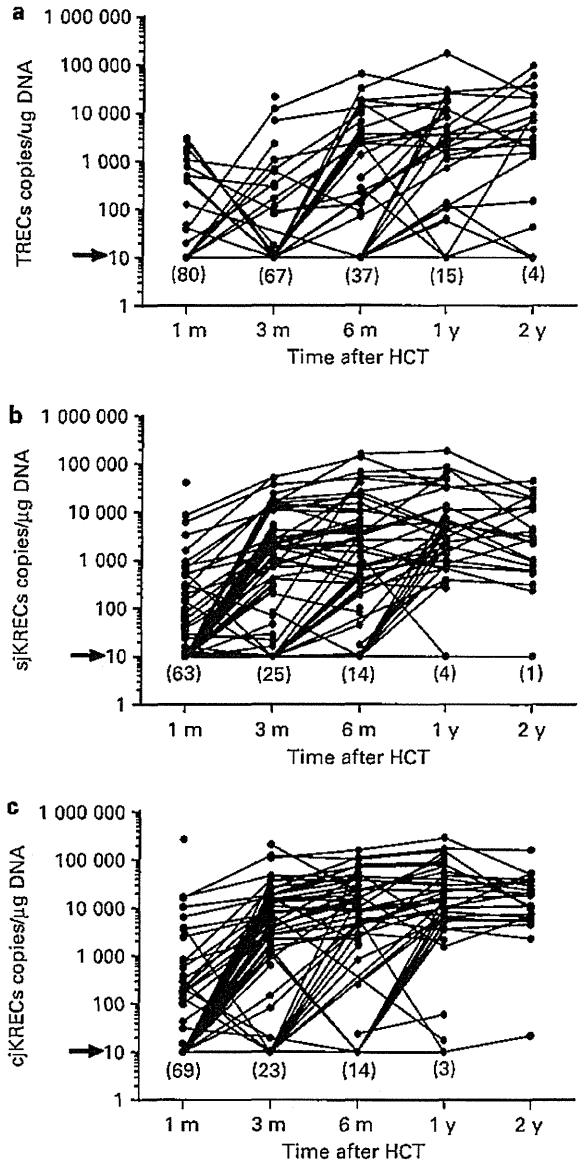


Figure 2. Levels of TRECs and KRECs after HCT. The levels of TRECs (a), sjKRECs (b) and cjKRECs (c) after HCT. The arrows show the detectable limit of the real-time PCR (10 copies/μg DNA). Values under this limit are considered 'negative'. Numbers in parentheses indicate the number of subjects who show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

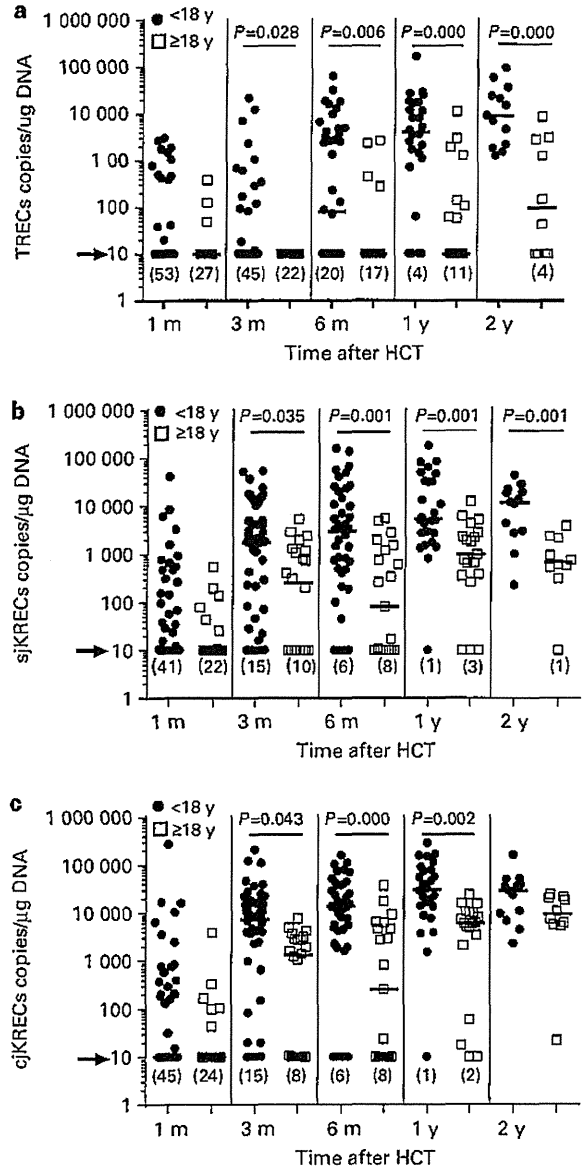


Figure 3. Recipient age and the levels of TRECs and KRECs. Recipient age and the levels of TRECs (a), sjKRECs (b) and cjKRECs (c). Closed circles indicate < 18 years old, and open squares indicate ≥ 18 years old. Arrows show the detectable limit of the real-time PCR (10 copies/μg DNA). Values under this 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

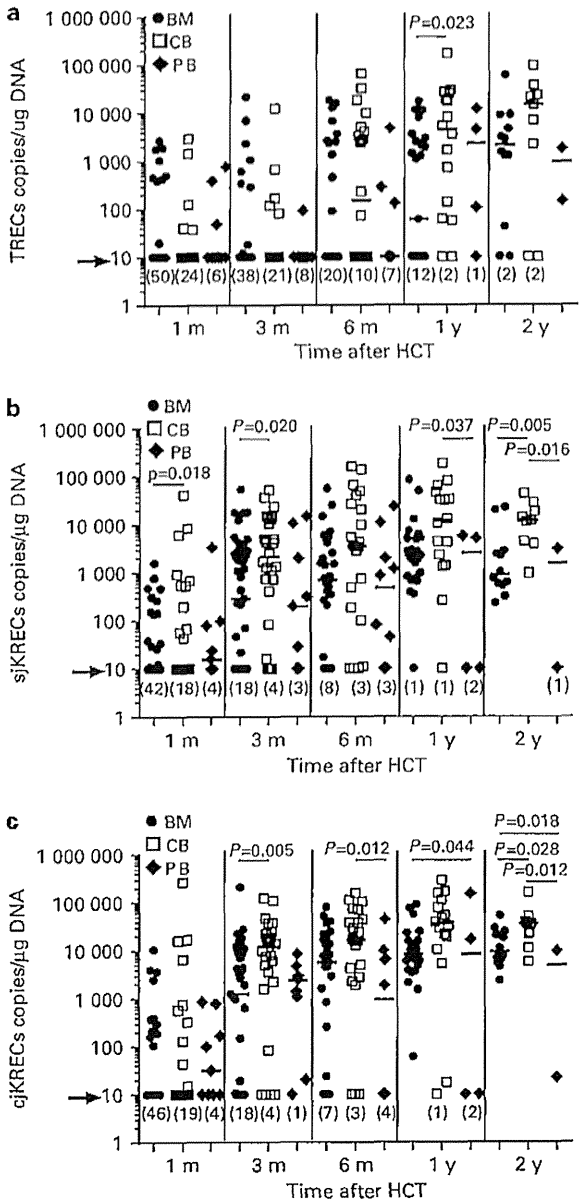


Figure 4. Cell source and the levels of TRECs and KRECs. Cell source and the levels of TRECs (**a**), sjKRECs (**b**) and cjKRECs (**c**). Closed circles indicate BM, open squares indicate cord blood and closed diamonds indicate peripheral blood. Arrows show the detectable limit of the real-time PCR (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	−0.285 (−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	0.235 (−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)	0.949	**−0.329 (−2.770 to −0.190)**	**0.025[b]**	**−0.422 (−3.035 to −0.267)**	**0.020[b]**	−0.345 (−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.

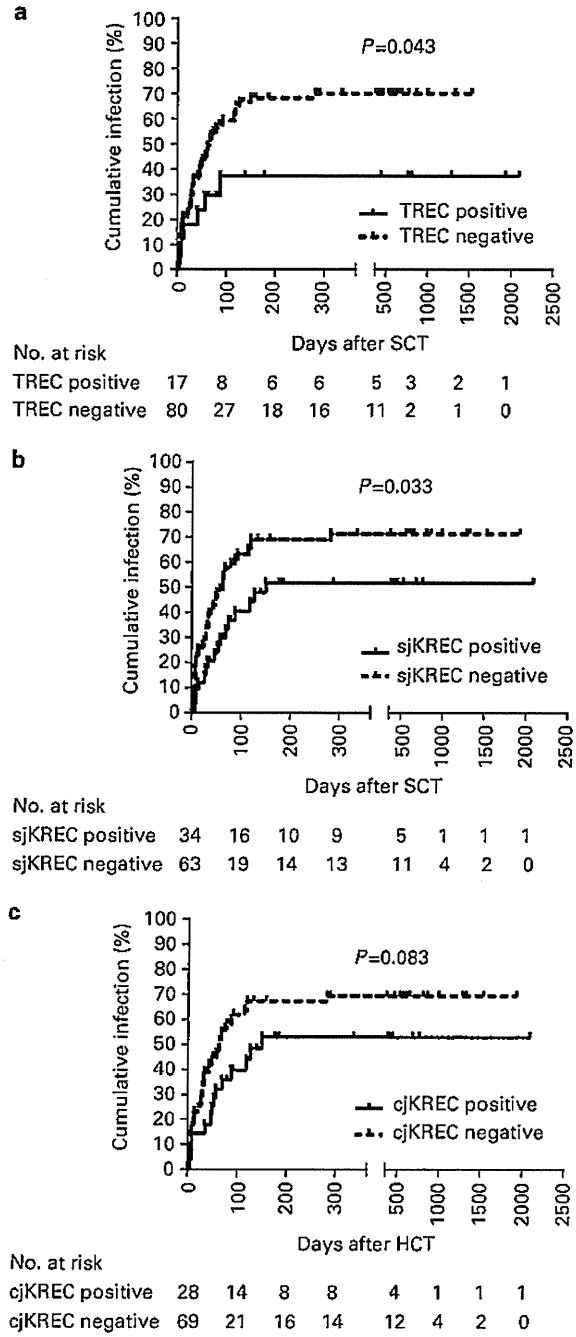


Figure 5. Cumulative incidence of infection after HCT. Cumulative incidence of infection and the levels of TRECs (**a**), sjKRECs (**b**) and cjKRECs (**c**) 1 month after HCT. Solid lines indicate the positive levels of TRECs (a), sjKRECs (b) and cjKRECs (c), and the dotted lines indicate their negative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

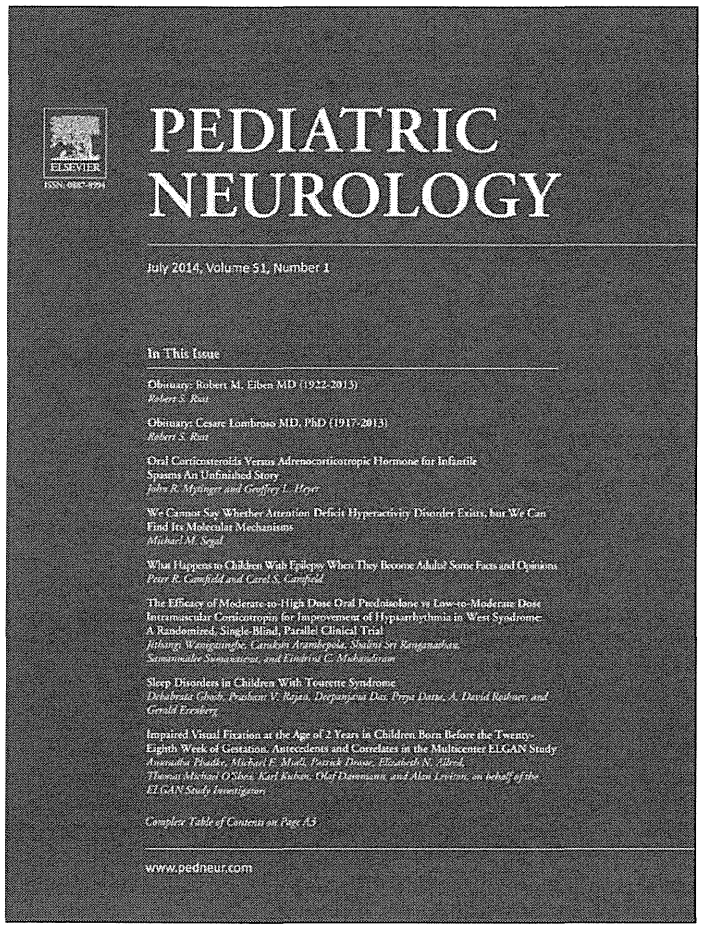
ACKNOWLEDGEMENTS

This work was in part supported by Health and Labour Sciences Research Grants for Intractable diseases (H23-003 and H24-008 to TM, and H24-013 to KI).

REFERENCES

1 Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M *et al.* Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med* 2010; **363**: 2091–2101.
2 Antoine C, Muller S, Cant A, Cavazzana-Calvo M, Veys P, Vossen J *et al.* Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. *Lancet* 2003; **361**: 553–560.
3 Dvorak CC, Cowan MJ. Hematopoietic stem cell transplantation for primary immunodeficiency disease. *Bone Marrow Transplant* 2008; **41**: 119–126.
4 Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R *et al.* Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. *N Engl J Med* 1997; **337**: 373–381.
5 Kurtzberg J, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC *et al.* Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. *N Engl J Med* 1996; **335**: 157–166.
6 Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE *et al.* Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 2004; **351**: 2265–2275.
7 Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A *et al.* Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 2004; **351**: 2276–2285.
8 Wagner JE, Rosenthal J, Sweetman R, Shu XO, Davies SM, Ramsay NK *et al.* Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. *Blood* 1996; **88**: 795–802.
9 Szabolcs P, Niedzwiecki D. Immune reconstitution in children after unrelated cord blood transplantation. *Biol Blood Marrow Transplant* 2008; **14**: 66–72.
10 Hazenberg MD, Verschuren MC, Hamann D, Miedema F, van Dongen JJ. T cell receptor excision circles as markers for recent thymic emigrants: basic aspects, technical approach, and guidelines for interpretation. *J Molec Med* 2001; **79**: 631–640.
11 Brown JA, Stevenson K, Kim HT, Cutler C, Ballen K, McDonough S *et al.* Clearance of CMV viremia and survival after double umbilical cord blood transplantation in adults depends on reconstitution of thymopoiesis. *Blood* 2010; **115**: 4111–4119.
12 Lewin SR, Heller G, Zhang L, Rodrigues E, Skulsky E, van den Brink MR *et al.* Direct evidence for new T-cell generation by patients after either T-cell-depleted or unmodified allogeneic hematopoietic stem cell transplantations. *Blood* 2002; **100**: 2235–2242.
13 Sairafi D, Mattsson J, Uhlin M, Uzunel M. Thymic function after allogeneic stem cell transplantation is dependent on graft source and predictive of long term survival. *Clin Immunol* 2012; **142**: 343–350.
14 Komanduri KV, St John LS, de Lima M, McMannis J, Rosinski S, McNiece I *et al.* Delayed immune reconstitution after cord blood transplantation is characterized by impaired thymopoiesis and late memory T-cell skewing. *Blood* 2007; **110**: 4543–4551.
15 van Zelm MC, Szczepanski T, van der Burg M, van Dongen JJ. Replication history of B lymphocytes reveals homeostatic proliferation and extensive antigen-induced B cell expansion. *J Exp Med* 2007; **204**: 645–655.
16 Hazenberg MD, Otto SA, Cohen Stuart JW, Verschuren MC, Borleffs JC, Boucher CA *et al.* Increased cell division but not thymic dysfunction rapidly affects the T-cell receptor excision circle content of the naive T cell population in HIV-1 infection. *Nat Med* 2000; **6**: 1036–1042.
17 Kamae C, Nakagawa N, Sato H, Honma K, Mitsuiki N, Ohara O *et al.* Common variable immunodeficiency classification by quantifying T-cell receptor and immunoglobulin kappa-deleting recombination excision circles. *J Allergy Clin Immunol* 2012; **131**: 1437–1440 e5.
18 Morinishi Y, Imai K, Nakagawa N, Sato H, Horiuchi K, Ohtsuka Y *et al.* Identification of severe combined immunodeficiency by T-cell receptor excision circles quantification using neonatal guthrie cards. *J Pediatr* 2009; **155**: 829–833.
19 Nakagawa N, Imai K, Kanegane H, Sato H, Yamada M, Kondoh K *et al.* Quantification of kappa-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects. *J Allergy Clin Immunol* 2011; **128**: 223–225 e2.
20 Sugita S, Shimizu N, Watanabe K, Mizukami M, Morio T, Sugamoto Y *et al.* Use of multiplex PCR and real-time PCR to detect human herpes virus genome in ocular fluids of patients with uveitis. *Br J Ophthalmol* 2008; **92**: 928–932.
21 Ono A, Mochizuki M, Yamaguchi K, Miyata N, Watanabe T. Increased number of circulating HTLV-1 infected cells in peripheral blood mononuclear cells of HTLV-1 uveitis patients: a quantitative polymerase chain reaction study. *Br J Ophthalmol* 1995; **79**: 270–276.
22 Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V *et al.* Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant* 2009; **15**: 1628–1633.
23 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
24 Clave E, Busson M, Douay C, Peffault de Latour R, Berrou J, Rabian C *et al.* Acute graft-versus-host disease transiently impairs thymic output in young patients after allogeneic hematopoietic stem cell transplantation. *Blood* 2009; **113**: 6477–6484.
25 Bartelink IH, Belitser SV, Knibbe CA, Danhof M, de Pagter PJ, Egberts AC *et al.* Immune reconstitution kinetics as an early predictor for mortality using various hematopoietic stem cell sources in children. *Biol Blood Marrow Transplant* 2012; **19**: 305–313.
26 Allen JL, Fore MS, Wooten J, Roehrs PA, Bhuiya NS, Hoffert T *et al.* B cells from patients with chronic GVHD are activated and primed for survival via BAFF-mediated pathways. *Blood* 2012; **120**: 2529–2536.

Supplementary Information accompanies this paper on Bone Marrow Transplantation website (http://www.nature.com/bmt)



Pediatric Neurology 51 (2014) 170–175

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Clinical Observations

Chromosome 9q33q34 Microdeletion With Early Infantile Epileptic Encephalopathy, Severe Dystonia, Abnormal Eye Movements, and Nephroureteral Malformations

Hiroshi Matsumoto MD, PhD [a,*], Kiyotaka Zaha MD [a], Yasuko Nakamura MD [a], Shin Hayashi MD, PhD [b], Johji Inazawa MD, PhD [b], Shigeaki Nonoyama MD, PhD [a]

[a] *Department of Pediatrics, National Defense Medical College, Tokorozawa, Japan*
[b] *Department of Molecular Cytogenetics, Medical Research Institute and School of Biomedical Science, Tokyo Medical and Dental University, Tokyo, Japan*

ABSTRACT

BACKGROUND: Microdeletion of chromosome 9q33q34 is an emerging disease disorder associated with early infantile epileptic encephalopathy, intellectual disability, and a variety of movement disorders. **PATIENT:** We describe a male infant with early infantile epileptic encephalopathy with suppression-burst (Ohtahara syndrome) who carried a *de novo* 2.0-Mb microdeletion in chromosome 9q33q34, including *STXBP1*. The previously reported examples of 9q33q34 microdeletion including *STXBP1* are reviewed. **RESULTS:** The patient developed infantile spasms at 4 months of age, and these were refractory to multiple antiepileptic drugs. He also developed severe dystonia during infancy, rotatory nystagmus, and nephroureteral malformations. Immunoglobulin and clobazam administered at 11 months were effective for the spasms, but profound psychomotor retardation remained. A comparative genomic hybridization array analysis and the fluorescence in situ hybridization analysis revealed a de novo 2.0-Mb microdeletion in chromosome 9q33q34, which encompasses *STXBP1*, *ENG*, *SPTAN1*, and 52 other genes. A total of 14 patients (13 from the literature) with a 9q33q24 microdeletion including *STXBP1* were reviewed, five of them displayed early infantile epileptic encephalopathy with suppression-burst, and six of them had early-onset epilepsy but not early infantile epileptic encephalopathy. Dystonia has been previously described in 9q33q34 deletions involving *TOR1A* but not *STXBP1*. Neither abnormal eye movements nor nephroureteral malformations has been previously described. **CONCLUSIONS:** This patient adds unique clinical presentations of neurological and nephroureteral abnormalities to the features of 9q33q34 microdeletion.

Keywords: 9q33q34 microdeletion, *STXBP1*, early infantile epileptic encephalopathy, dystonia, nystagmus, nephroureteral malformations

Pediatr Neurol 2014; 51: 170-175

Introduction

Early infantile epileptic encephalopathy (EIEE) with suppression-burst, also known as Ohtahara syndrome, is characterized by early onset of tonic seizures, suppression-burst patterns on electroencephalography (EEG), and poor outcome with severe psychomotor retardation. Early infantile epileptic encephalopathy is a genetically heterogeneous disorder, and at least 12 genes have been identified to have causative mutations.[1] Haploinsufficiency of syntaxin-binding protein 1 (*STXBP1*) causes epileptic encephalopathy, early infantile 4 (MIM [Mendelian Inheritance in Man] #612164).[2] Saitsu et al.[3] also reported that dominant negative mutations in spectrin, alpha, non-erythrocytic 1 (*SPTAN1*) cause West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delays, which is classified as epileptic encephalopathy, early infantile 5 (MIM

Article History:
Received January 12, 2014; Accepted in final form March 15, 2014

* Communications should be addressed to: Dr. Matsumoto; Department of Pediatrics; National Defense Medical College; 3-2 Namiki; Tokorozawa, Saitama 359-8513, Japan.
E-mail address: matumoto@ndmc.ac.jp

0887-8994/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pediatrneurol.2014.03.013

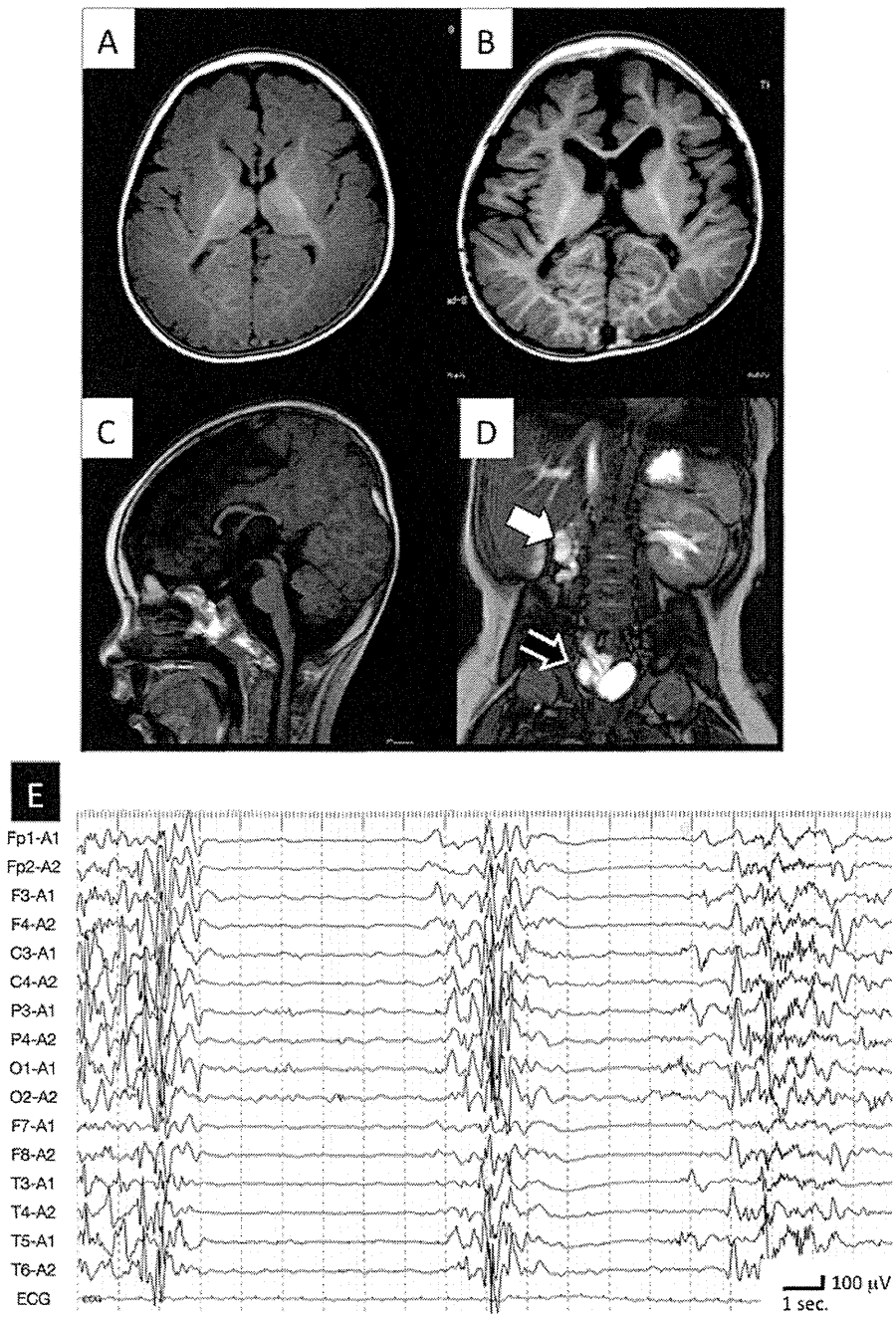


FIGURE 1.
MRI of the brain and abdomen at 4 months of age (A, D) and at 5 years of age (B, C). (A) No abnormal findings were evident on this T_1-weighted image. (B, C) Diffuse cortical atrophy, dilated lateral ventricles, and a thin corpus callosum were evident on the T_1-weighted images. (D) A highly dysplastic right kidney (arrow) and right ureterocele (open arrow) were observed on this T_2-weighted image. (E) An asleep EEG obtained when the patient was 4 months old revealed diffuse high-voltage spikes and slow bursts with intermittent suppressed activity, comprising a suppression-burst pattern. ECG, electrocardiogram; EEG, electroencephalogram; MRI, magnetic resonance imaging.

#613477). Both *STXBP1* and *SPTAN1* are located within the same 9q33.3q34.11 genomic region. More recently, Campbell et al.[1] reported novel 9q34.11 deletions encompassing combinations of four Mendelian disease genes (*STXBP1*, *SPTAN1*, endoglin [*ENG*], and torsin family 1, member A [*TOR1A*]) in 10 patients using array comparative genomic hybridization, revealing *cis*-genetic effects leading to complex phenotypes including multisystemic vascular dysplasia, early-onset primary dystonia, epilepsy, and intellectual disability in various combinations. We describe a patient with EIEE with a 9q33q34 microdeletion, in which the patient also showed unique neurological and physical abnormalities that have not yet been described with a 9q33q34 microdeletion.

TABLE.
Summary of Clinical Features of Individuals With *STXBP1* Deletions

Clinical Finding	Present Patient	Mignot et al.[5]	Campbell et al.[1] P1	Saitsu et al.[6] P2231	Campbell et al.[1] P3	Saitsu et al.[2,3]
Deletion size (Mb)	2.0	3-3.5	2.9	2.85	2.6	2.25
Mendelian disease genes involved	*STXBP1, ENG, SPTAN1*	*LMX1B, STXBP1*	*STXBP1* (exon 16-20), *ENG, SPTAN1, TOR1A*	*STXBP1, SPTAN1, ENG, TOR1A*	*STXBP1, ENG, SPTAN1*	*STXBP1, ENG, SPTAN1*
Epilepsy type	Epileptic spasms	Infantile spasms	None	Epileptic spasms	Tonic seizure, localized related epilepsy	Tonic seizure, oral automatism
Age at onset of epilepsy	4 mo	6 mo	NA	1 wk	6 yr	2 mo
EEG	Suppression-burst	Multifocal spikes	NA	Suppression-burst	Right temporal spike	Suppression-burst
Response to therapy	VitB6, ZNS, ACTH, VPA, NZP, ketogenic diet; refractory, IVIG, CLB; effective	VGB, VPA, steroid; effective	NA	Refractory to antiepileptic drug. Ketogenic diet slightly effective	LEV; not effective	Thyrotropin-releasing hormone; effective
MRI/CT	Global atrophy, thin corpus callosum	Global atrophy	Normal	Thin corpus callosum, small cerebellum	Chiari type I malformation	Cortical atrophy, diffuse hypomyelination, thin corpus callosum
Cognition	Profound MR	Severe MR, a few words	Profound MR	Profound MR	Severe MR	Profound MR
Neurological examination	Axial hypotonia, limb hypertonia, status dystonicus in infancy	Joint hyperlaxity	Axial hypotonia, hand hypertonia	Spastic quadriplegia	Hypotonia	Hypotonic quadriplegia
Ophthalmologic abnormalities	Rotatory nystagmus	NA	None	NA	Strabismus	Normal
Other manifestations	GER, dysplastic right kidney, ureterocele, umbilical hernia	Nail malformation, generalized tremor	Mild dysmorphic features	Multiple anomalies: cleft lip/palate, VSD, overlapping fingers, small penis	Microcephaly, plagiocephaly, dysmorphic features	Tremulous arm movements

Abbreviations:
ACTH = Adrenocorticotropic hormone
CLB = Clobazam
CT = Computed tomography
EEG = Electroencephalography
GER = Gastroesophageal reflux
Hyps. = Hypsarrhythmia
IVIG = Intravenous immunoglobulin
LEV = Levetiracetam
MR = Mental retardation
MRI = Magnetic resonance imaging
NA = Not available
NZP = Nitrazepam
P = Patient
PB = Phenobarbital
VGB = Vigabatrin
VitB6 = Vitamin B6
VPA = Valproic acid
VSD = Ventricular septal defect
ZNS = Zonisamide

Patient Description

The patient was born after a 42 week gestation weighing 2966 g. He was the first child of nonconsanguineous Japanese parents. He was brought to our hospital when he was 4 months old because of repetitive short seizures or spasms. His examination revealed a flat nasal bridge and a large umbilical hernia. Head control had not been acquired, and no visual pursuit or social smile was evident. Truncal hypotonia was marked, but the extremities were hypertonic, showing tightly clenched fists. Deep tendon reflexes were not exaggerated. Complete blood cell count and blood chemistry, including lactate, pyruvate, ammonia, copper, and amino acid analysis, revealed normal findings. Cerebrospinal fluid was normal. Chromosome analysis (G-band) revealed normal karyotype of 46,XY. Brain magnetic resonance

TABLE.
Summary of Clinical Features of Individuals With *STXBP1* Deletions *(Continued)*

Campbell et al.[1] P2	Campbell et al.[1] P10	Campbell et al.[1] P4	Campbell et al.[1] P5	Deprez et al.[7] P5	Deprez et al.[7] P4	Milh et al.[8]	Saitsu et al.[6] P1506
1.8 *STXBP1, ENG, SPTAN1*	0.81 *STXBP1, ENG*	0.25 *STXBP1*	0.067 *STXBP1* (exon 1-4)	0.0423-0.116 *STXBP1* (exon 1)	0.023-0.0354 *STXBP1* (exon 12-20)	0.01? *STXBP1* (exon 8-14)	0.0046 *STXBP1* (exon 4)
Infantile spasms, myoclonus	None	Partial seizures and nocturnal myoclonic-atonic	Neonatal seizure	Tonic seizure, clonic seizure	Epileptic spasms	Epileptic spasms	Tonic and myoclonic seizure
3 mo	NA	5 wk	Neonate	10 wk	4.5 mo	3 days	1 mo
Hyps.	NA	Bifrontal temporal spikes	Multifocal seizure discharge	Synchronous epileptiform activity	Hyps.	Suppression-burst	Suppression-burst
ACTH for infantile spasms, ZNS for myoclonus epilepsy; effective	NA	Refractory to antiepileptic drug	NA	PB, VGB; effective	VGB, steroid, ACTH; effective	VGB, steroid, ACTH; effective	High-dose PB; effective
Normal	Normal	Chiari type I malformation	Normal	Slightly dilated temporal horns	Normal	Normal?	Normal
Severe MR	Moderate MR, 20 words	Severe MR, autism, a few words	Severe MR, autism	Severe MR	Severe MR	No speech	NA
Hypotonia, motor dyspraxia	Ataxia, hypotonia, walk, run	Hypotonia, ataxia, tremulousness, awkward gait	Hypotonia, ataxia, dyskinesia, pyramidal signs, independent gait	Hypotonia, ataxia	Subtle hypertonia, ataxia	No walk	NA
Strabismus	None	None	None	NA	NA	NA	NA
Nephrocalcinosis, GER, pulmonary arteriovenous malformation, mild dysmorphic features	Mild dysmorphic features	None	GER	None	Stereotypic behavior	Jerks (nonepileptic)	NA

imaging (MRI) was normal (Fig 1A). Abdominal MRI revealed a highly dysplastic right kidney and a right ureterocele (Fig 1D). EEG revealed a suppression-burst pattern (Fig 1E).

A diagnosis of EIEE was made, and treatment with vitamin B6 and zonisamide was initiated. However, the spasms and EEG abnormality were sustained. Subsequently, adrenocorticotropic hormone, valproic acid, nitrazepam, and a ketogenic diet were administered in that order. The epileptic spasms sometimes disappeared in response to these drugs but soon relapsed. Furthermore, abnormal eye movements (rotatory nystagmus, Supplementary Video S1), irritability, and greatly increased muscle tone of the extremities and trunk appeared. When he was awake, tightly clenched fists, stretched and crossed legs, and an extended neck and trunk were observed frequently, sometimes showing status dystonicus. Treatment with intravenous immunoglobulin and clobazam,

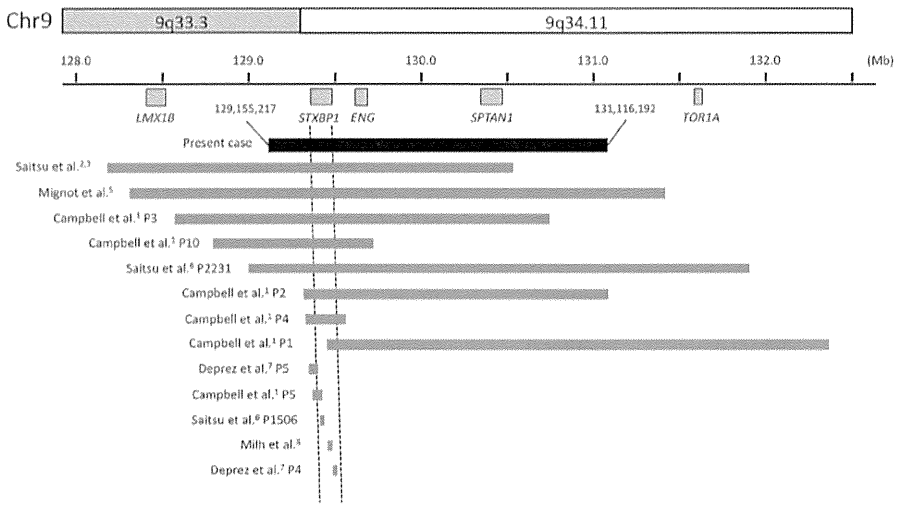


FIGURE 2.
The microdeletion at 9q33.3q34.11 involving *STXBP1* in the present case and other cases reported to date. This genomic region includes five dose-sensitive disease genes (*LMX1B*, *STXBP1*, *ENG*, *SPTAN1*, and *TOR1A*). Chr, chromosome; P, Patient.

administered at 11 months of age, decreased his seizures. The immunoglobulin therapy was repeated a total of six times. He is now 5 years old, and his developmental milestones are markedly delayed: he is bedridden, requires enteral nutrition via gastrostomy, and is unable to speak or smile. However, seizures are fairly controlled with valproic acid, clobazam, and topiramate. Abnormal eye movements are still observed frequently. The findings of the EEGs have changed with time but still indicate high epileptic activities.

Molecular and cytogenetic analysis

A whole-genome array analysis that covers all human chromosomes with 4523 bacterial artificial chromosomes (BACs) at intervals of approximately 0.7 Mb[4] was performed after informed consent was obtained from the patient's parents, and it revealed a copy number loss on chromosome 9q33.3q34.11. Furthermore, an oligonucleotide array (NimbleGen Human CGH 2.1M Whole-Genome Tiling Array, Roche NimbleGen, Madison, WI) analysis confirmed the boundaries of the abbreviation: arr 9q33.3q34.11 (129,155,217-131,116,192)x1. The region of genomic copy number loss was 1961 kb in size and encompasses *STXBP1*, *ENG*, *SPTAN1*, and 52 other genes (Fig 2). The data are presented according to the NCBI36/hg18 (March 2006) assembly.

A metaphase fluorescence in situ hybridization analysis using BAC clones (RP11-29B9 [9q24.1] and RP11-456D9 [9q34.11]) confirmed the heterozygous deletion of RP11-456D9 in the lymphocytes obtained from the patient. Fluorescence in situ hybridization analysis was also performed using lymphocytes obtained from the patient's parents, the results of which revealed a de novo deletion of 9q34.11 in the patient.

Discussion

Saitsu et al.[2] first reported a *de novo* 2.0-Mb microdeletion at 9q33.3-q34.11 in a girl with EIEE. Campbell et al.[1] reported 10 patients with a microdeletion in 9q34 who manifested a phenotype of multisystemic vascular dysplasia, early-onset primary dystonia, epilepsy, and intellectual disability in various combinations. All four patients who had epilepsy had *STXBP1* deletions, so haploinsufficiency of *STXBP1* was suggested as a definite cause of epilepsy in these cases. To date, 14 patients (including ours) with deletion mutations of *STXBP1* have been reported (Table, Fig 2). The deletion sizes ranged from 4.6 kb to 3.5 Mb. Five of the 14 patients had EIEE with suppression-burst, and six had early-onset epilepsy but not EIEE. One patient first developed epilepsy at 6 years of age, and two patients had no epilepsy as of the publication.

Our patient's epilepsy was resistant to multiple antiepileptic drugs and a ketogenic diet but responded to immunoglobulin and clobazam initiated at 11 months of age. Most patients with a deletion mutation of *STXBP1* demonstrate responses to antiepileptic treatments (Table). A previous study reported that approximately half of patients with *STXBP1* mutations exhibit good seizure control in response to antiepileptic drugs.[5] The normal findings of brain MRI, except for brain atrophy in some instances, may contribute to this observation.

Our patient reported here exhibited different signs and symptoms than the other 13 patients who had deletion mutations in *STXBP1*; these included abnormal eye movements, status dystonicus in infancy, and nephro-ureteral malformations. Abnormal eye movements are observed both at rest and during voluntary eye movements, so dysfunction of vestibular system is suspected as the cause. Previous reports of patients with *STXBP1* mutation or microdeletion of 9q33q34 did not describe any ophthalmologic abnormalities except for two patients with strabismus.[1] This is the first report to describe abnormal eye movements caused by neurological, rather than ophthalmologic, abnormalities with the 9q33q34 microdeletion. Further investigations are required to determine whether the abnormal eye movements observed in this patient are characteristic of 9q33q34 microdeletion.

Our patient had a deletion of *SPTAN1*, but it is difficult to assess the effects of the deletion on his phenotype. Because two patients with only a *SPTAN1* deletion did not develop epilepsy,[1] *SPTAN1* haploinsufficiency alone may not lead to an epileptic phenotype. Furthermore, it has been postulated that in-frame *SPTAN1* mutations may cause the early infantile epileptic encephalopathy phenotype through a dominant negative effect.[3] These findings suggest that the

infantile spasms observed in our patient may have been mainly caused by the haploinsufficiency of *STXBP1*.

Hereditary hemorrhagic telangiectasia type 1 (HHT1) is an autosomal dominant disorder characterized by epistaxis, telangiectasia, and multiorgan vascular dysplasia. HHT1 is caused by mutations of *ENG*. The genomic deletion of *ENG* was observed in some individuals manifesting a phenotype of HHT1 or no manifestations of vascular symptoms.[1,9] Our patient has no vascular symptoms, although there is a possibility that these may develop in the future. The urogenital malformations of highly dysplastic kidney and ureterocele observed in our patient might have been caused by vascular dysplasia during fetal kidney and ureter development. However, no urogenital anomaly in HHT1 has been reported, although renal arteriovenous malformations or telangiectasia of bladder have been described.[10,11]

TOR1A mutations cause early-onset torsion dystonia.[12] It has been suggested that the torsion dystonia is caused by a loss of function of the mutated *TOR1A*, and the penetrance of symptoms is as low as 30-40% among mutation-carrying subjects.[12] Only one out of four patients was reported to exhibit dystonia even with entire *TOR1A* deletion.[1] Our patient presented with severe dystonia in infancy, but it seems unlikely that *TOR1A* was the cause of his dystonia. One reason is that the microdeletion found in the present case did not include *TOR1A* (Fig 2), although the positional effect of microdeletion in 9q33q34 should be considered. Another reason is that almost all patients with early-onset torsion dystonia with a *TOR1A* mutation had mutations confined to c.907_909delGAG.

Microdeletions in 9q33q34, including *STXBP1*, are associated with epileptic disorders in early infancy and intellectual disability, and this report adds complications including abnormal eye movements, generalized dystonia, and nephroureteral malformation. None of the other genes included in the deleted chromosomal region explained the phenotype observed in the present patient due to haploinsufficiency. The pathogenic mechanism involving defective genes has not been completely elucidated, so future clinical surveys combined with analyses to determine the exact range of gene deletion would be helpful to clarify the variable phenotype of a 9q33q34 microdeletion.

The authors thank the patient and his family for their participation in this study and Drs. S. Kajiwara, MD, Y. Ogura, MD, and T. Asano, MD, PhD for clinical support.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.pediatrneurol.2014.03.013

References

1. Campbell IM, Yatsenko SA, Hixson P, et al. Novel 9q34.11 gene deletions encompassing combinations of four Mendelian disease genes: STXBP1, SPTAN1, ENG, and TOR1A. *Genet Med.* 2012;14: 868-876.
2. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet.* 2008;40:782-788.
3. Saitsu H, Tohyama J, Kumada T, et al. Dominant-negative mutations in alpha-II spectrin cause West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. *Am J Hum Genet.* 2010;86:881-891.
4. Hayashi S, Imoto I, Aizu Y, et al. Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies. *J Hum Genet.* 2011;56:110-124.
5. Mignot C, Moutard ML, Trouillard O, et al. STXBP1-related encephalopathy presenting as infantile spasms and generalized tremor in three patients. *Epilepsia.* 2011;52:1820-1827.
6. Saitsu H, Kato M, Shimono M, et al. Association of genomic deletions in the STXBP1 gene with Ohtahara syndrome. *Clin Genet.* 2012;81: 399-402.
7. Deprez L, Weckhuysen S, Holmgren P, et al. Clinical spectrum of early-onset epileptic encephalopathies associated with STXBP1 mutations. *Neurology.* 2010;75:1159-1165.
8. Milh M, Villeneuve N, Chouchane M, et al. Epileptic and nonepileptic features in patients with early onset epileptic encephalopathy and STXBP1 mutations. *Epilepsia.* 2011;52:1828-1834.
9. Shoukier M, Teske U, Weise A, Engel W, Argyriou L. Characterization of five novel large deletions causing hereditary haemorrhagic telangiectasia. *Clin Genet.* 2008;73:320-330.
10. Cooke DA. Renal arteriovenous malformation demonstrated angiographically in hereditary haemorrhagic telangiectasia (Rendu-Osler-Weber disease). *J R Soc Med.* 1986;79:744-746.
11. Plauchu H, de Chadarévian JP, Bideau A, Robert JM. Age-related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. *Am J Med Genet.* 1989;32: 291-297.
12. Ozelius LJ, Hewett JW, Page CE, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. *Nat Genet.* 1997;17:40-48.

BCG vaccination in patients with severe combined immunodeficiency: Complications, risks, and vaccination policies

Beatriz E. Marciano, MD,[a] Chiung-Yu Huang, PhD,[b] Gyan Joshi, PhD,[b] Nima Rezaei, MD,[c] Beatriz Costa Carvalho, MD,[d] Zoe Allwood, MD,[e] Aydan Ikinciogullari, MD,[f] Shereen M. Reda, MD,[g] Andrew Gennery, MD,[h] Vojtech Thon, MD,[i] Francisco Espinosa-Rosales, MD,[j] Waleed Al-Herz, MD,[k] Oscar Porras, MD,[l] Anna Shcherbina, MD,[m] Anna Szaflarska, MD,[n] Şebnem Kiliç, MD,[o] Jose L. Franco, MD, PhD,[p] Andrea C. Gómez Raccio, MD,[q] Persio Roxo, Jr, MD,[r] Isabel Esteves, MD,[s] Nermeen Galal, MD,[t] Anete Sevciovic Grumach, MD, PhD,[u] Salem Al-Tamemi, MD,[v] Alisan Yildiran, MD,[w] Julio C. Orellana, MD,[x] Masafumi Yamada, MD,[y] Tomohiro Morio, MD,[z] Diana Liberatore, MD,[aa] Yoshitoshi Ohtsuka, MD,[bb] Yu-Lung Lau, MD,[cc] Ryuta Nishikomori, MD,[dd] Carlos Torres-Lozano, MD,[ee] Juliana T. L. Mazzucchelli, MD,[d] Maria M. S. Vilela, MD,[d] Fabiola S. Tavares, MD,[d] Luciana Cunha, MD,[ff] Jorge A. Pinto, MD,[ff] Sara E. Espinosa-Padilla, MD,[j] Leticia Hernandez-Nieto, MD,[j] Reem A. Elfeky, MD,[g] Tadashi Ariga, MD,[y] Heike Toshio, MD,[dd] Figen Dogu, MD,[f] Funda Cipe, MD,[f] Renata Formankova, MD,[gg] M. Enriqueta Nuñez-Nuñez, MD,[ee] Liliana Bezrodnik, MD,[q] Jose Gonçalo Marques, MD,[s] María I. Pereira, PhD,[x] Viviana Listello, MD,[x] Mary A. Slatter, MD,[h] Zohreh Nademi, MD,[h] Danuta Kowalczyk, MD,[n] Thomas A. Fleisher, MD,[hh] Graham Davies, MD,[e] Bénédicte Neven, MD,[ii] and Sergio D. Rosenzweig, MD, PhD[jj] *Bethesda, Md, Tehran, Iran, São Paulo and Minas Gerais, Brazil, London and Newcastle upon Tyne, United Kingdom, Ankara, Bursa, and Atakum-Samsun, Turkey, Cairo, Egypt, Brno and Prague, Czech Republic, Mexico City and Guadalajara, Mexico, Kuwait City, Kuwait, San Jose, Costa Rica, Moscow, Russia, Krakow, Poland, Medellin, Colombia, Buenos Aires and Córdoba, Argentina, Lisbon, Portugal, Muscat, Oman, Sapporo, Tokyo, Hyogo, and Kyoto, Japan, Hong Kong, China, and Paris, France*

Background: Severe combined immunodeficiency (SCID) is a syndrome characterized by profound T-cell deficiency. BCG vaccine is contraindicated in patients with SCID. Because most countries encourage BCG vaccination at birth, a high percentage of patients with SCID are vaccinated before their immune defect is detected.

From [a]the Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; [b]the Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; [c]the Pediatric Center of Excellence, Children's Medical Center Hospital, Tehran University of Medical Sciences; [d]the Department of Pediatrics, Federal University of São Paulo; [e]the Immunology Department, Great Ormond Street Hospital for Children, London; [f]the Department of Pediatric Immunology and Allergy, Ankara University Medical School; [g]the Department of Pediatric Allergy and Immunology, Children's Hospital, Ain Shams University, Cairo; [h]the Paediatric Immunology Department, Great North Children's Hospital, Newcastle upon Tyne; [i]the Department of Clinical Immunology and Allergy, Medical Faculty of Masaryk University, St Anne's University Hospital, Brno; [j]the National Institute of Pediatrics, Mexico City Federal District; [k]the Department of Pediatrics, Faculty of Medicine, Kuwait University, Al-Sabah Hospital, Kuwait City; [l]the Department of Immunology and Rheumatology, National Children's Hospital "Dr. Carlos Sáenz Herrera," San Jose; [m]the Department of Clinical Immunology, Center for Pediatric Hematology, Oncology and Immunology, Moscow; [n]the Department of Clinical Immunology and Transplantology, Children's University Hospital, Krakow; [o]the Pediatric Immunology Division, Uludag University Medical Faculty, Bursa; [p]the Primary Immunodeficiency Group, University of Antioquia, Medellin; [q]the Immunology Unit, Children's Hospital Ricardo Gutierrez, Buenos Aires; [r]the Faculty of Medicine of Ribeirão Preto, University of São Paulo; [s]the Primary Immunodeficiency Center, Lisbon Academic Center, Santa María Hospital, Lisbon; [t]Cairo University; [u]Faculty of Medicine of ABC, Santo André, São Paulo; [v]the Department of Child Health, Sultan Qaboos University Hospital, Muscat; [w]the Department of Pediatric Immunology-Allergy, Ondokuz Mayis University, Atakum-Samsun; [x]the Division of Allergy and Clinical Immunology, Santísima Trinidad Children's Hospital, Córdoba; [y]the Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo; [z]Tokyo Medical and Dental University; [aa]Pediatric Immunology, Italian Hospital, Buenos Aires; [bb]Hyogo College of Medicine; [cc]the Department of Pediatrics and Adolescent Medicine, LKS Faculty of Medicine, University of Hong Kong; [dd]the Department of Pediatrics, Kyoto University Hospital; [ee]the Department of Clinical Immunology and Allergology, Western National Medical Center, Guadalajara; [ff]the Federal University of Minas Gerais; [gg]Charles University, and University Hospital Motol, Prague; [hh]the Immunology Service, DLM, CC, National Institutes of Health, Bethesda; [ii]the Immunology-Hematology and Rheumatology Service, Necker Hospital, Paris; and [jj]the Primary Immunodeficiency Clinic and Infectious Diseases Susceptibility Unit, LHD, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda.

Supported by the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Disease. V.T. was supported by the European Community's Seventh Framework Program FP7/2007-2013 under grant agreement no. 201549 (EURO-PADnet HEALTH-F2-2008-201549).

Disclosure of potential conflict of interest: N. Rezaei is employed by and has received research support from Tehran University Med Sci, has received royalties from Springer, and has been supported by an American Academy of Allergy, Asthma & Immunology (AAAAI) Young Investigator Award. B. Costa Carvalho has received payment for development of educational presentations from the Federal University of Sao Paulo (FAPESP) and has received travel support from Octapharma. V. Thon has received research support from EURO-PADnet HEALTH (F2-2008-201549). J. L. Franco has received consultancy fees from Baxter and Kedrion and has received lecture fees from Grifols. A. Sevciovic Grumach is a board member for Latin American Society for Immunodeficiencies and has received consultancy fees and lecture fees from SHIRE and CSL. T. Morio has received grant-in-aids for scientific research from the Japan Science and Technology Agency; the Ministry of Education, Culture, and Sport; and the Ministry of Health, Labour, and Welfare in Japan and has received lecture fees from Abbvie, CSL Behring, Chugai Pharmaceutical, Meiji Pharmaceuticals, Teijin Pharma, and Toray Medical. S. D. Rosenzweig has received consultancy fees from InPractice and UpToDate. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication July 5, 2013; revised February 12, 2014; accepted for publication February 17, 2014.

Corresponding author: Sergio D. Rosenzweig, MD, PhD, Immunology Service, DLM, CC, NIH, 10 Center Dr, Bldg 10, 2C410F, Bethesda, MD 20892. E-mail: srosenzweig@cc.nih.gov.

0091-6749

http://dx.doi.org/10.1016/j.jaci.2014.02.028

Objectives: We sought to describe the complications and risks associated with BCG vaccination in patients with SCID.
Methods: An extensive standardized questionnaire evaluating complications, therapeutics, and outcomes regarding BCG vaccination in patients given a diagnosis of SCID was widely distributed. Summary statistics and association analysis was performed.
Results: Data on 349 BCG-vaccinated patients with SCID from 28 centers in 17 countries were analyzed. Fifty-one percent of the patients had BCG-associated complications, 34% disseminated and 17% localized (a 33,000- and 400-fold increase, respectively, over the general population). Patients receiving early vaccination (≤1 month) showed an increased prevalence of complications ($P = .006$) and death caused by BCG-associated complications ($P < .0001$). The odds of experiencing complications among patients with T-cell numbers of 250/μL or less at diagnosis was 2.1 times higher (95% CI, 1.4-3.4 times higher; $P = .001$) than among those with T-cell numbers of greater than 250/μL. BCG-associated complications were reported in 2 of 78 patients who received antimycobacterial therapy while asymptomatic, and no deaths caused by BCG-associated complications occurred in this group. In contrast, 46 BCG-associated deaths were reported among 160 patients treated with antimycobacterial therapy for a symptomatic BCG infection ($P < .0001$).
Conclusions: BCG vaccine has a very high rate of complications in patients with SCID, which increase morbidity and mortality rates. Until safer and more efficient antituberculosis vaccines become available, delay in BCG vaccination should be considered to protect highly vulnerable populations from preventable complications. (J Allergy Clin Immunol 2014;133:1134-41.)

Key words: *Primary immunodeficiency, severe combined immunodeficiency, vaccine, BCG, mycobacteria, newborn screening, hematopoietic stem cell transplant, immune reconstitution syndrome*

Tuberculosis is a major global health problem. In 1993, the World Health Organization (WHO) declared the disease a global public health emergency, and in 2011, one third of the world's population was thought to be infected with *Mycobacterium tuberculosis*, with almost 9 million new cases diagnosed and 1.4 million deaths attributed to this organism. In recent years, most technologically advanced countries have managed to control, although not eradicate, tuberculosis. With more than 4 billion doses applied, the live attenuated *Mycobacterium bovis* BCG vaccine has been a part of efforts to control tuberculosis and remains one of the most widely used of all current vaccines worldwide. Since the 1960s, it has been administered routinely in the majority of countries, and currently, approximately 120 million persons, mostly newborns, are vaccinated every year through national childhood immunization programs. The BCG vaccine has a documented protective effect against meningitis and disseminated tuberculosis in children; however, it does not prevent primary infection and, more importantly, does not prevent reactivation of latent pulmonary infection, the principal source of bacillary spread in the community. The effect of BCG vaccination on transmission of *M tuberculosis* is therefore limited (reviewed in Plotkin et al[1] and the Global Tuberculosis Report, 2012, WHO, http://www.who.int/tb/publications/global_report/gtbr12_main.pdf).

Abbreviations used
HSCT: Hematopoietic stem cell transplantation
IL2RG: IL-2 receptor γ
IRS: Immune reconstitution syndrome
MAT: Multidrug antimycobacterial therapy
RAG: Recombination-activating gene
SCID: Severe combined immunodeficiency
WHO: World Health Organization

Despite its long history and extensive use, there appears to be no other vaccine as controversial as BCG, and its history contains aspects of folklore and superstition that often supersede facts in public health discussions and policy.[1-3]

Severe combined immunodeficiency (SCID) includes a heterogeneous group of genetic conditions characterized by profound deficiencies in T-cell (and in some types B-cell, natural killer cell, or both) numbers and function. If untreated, infants with typical SCID succumb early in life from severe and recurrent infections. Mutations in different genes affecting cytokine signaling (eg, IL-2 receptor γ *[IL2RG]* and *IL7RA*), antigen receptor processing (eg, recombination-activating gene 1 *[RAG1]*, *RAG2*, and *CD3D*), or nucleotide processing (eg, adenosine deaminase *[ADA]*) cause this fatal childhood condition, unless immune reconstitution can be accomplished.[4] However, it should be noted that patients with severe manifestations of other syndromic conditions might have clinical signs and symptoms consistent with SCID.[5] BCG, as other live attenuated vaccines, is absolutely contraindicated in patients with SCID (as reviewed by Plotkin et al[1] and the Centers for Disease Control and Prevention[6] and the Global Tuberculosis Report, 2012, World Health Organization, http://www.who.int/tb/publications/global_report/gtbr12_main.pdf). However, because it is usually administered at birth, patients with SCID in most countries using BCG are vaccinated before their immune deficiency is diagnosed.

The aim of this study was to describe the complications and risks associated with BCG vaccination in patients given a diagnosis of SCID, the most severe form of primary immunodeficiency diseases.

METHODS

An extensive standardized questionnaire evaluating diagnostics, therapeutics, and outcomes concerning BCG-vaccinated patients with SCID was developed by an *ad hoc* scientific interest group (the "BCG infection in SCID patients interest group"; N.R., G.D., B.N., and S.D.R.; see Table E1 in this article's Online Repository at www.jacionline.org). The questionnaire was widely distributed to primary immunodeficiency patients/caregivers through professional organizations (the European Society for Immunodeficiencies, Latin American Society for Immunodeficiencies, and Clinical Immunology Society), patient advocacy groups (the Jeffrey Modell Foundation), and individually to other colleagues by members of the scientific interest group. All data for this retrospective study represented a 10-year cumulative experience for each reporting institution and were collected between April 2010 and March 2012.

Data relevant to (1) SCID diagnosis, treatment, immune reconstitution, and outcome, as well as (2) BCG vaccination and (3) BCG-associated complication diagnosis, treatment, and outcome was analyzed. For the purposes of this multicenter international retrospective study, we analyzed patients given diagnoses of SCID at the participating centers based on the clinical and laboratory findings of recurrent/severe infections and/or failure

TABLE I. BCG-vaccinated patients with SCID: distribution and HSCT

Country (centers)*	Universal BCG vaccination at birth†	BCG-vaccinated patients with SCID (n = 349)	HSCT‡ (n = 190)
Argentina (3)	Yes	10	6
Brazil (3)	Yes	58	24
Colombia (1)	Yes	6	1
Costa Rica (1)	Yes	10	6
Czech Republic (1)§	Yes	15	8
Egypt (1)	Yes	26	1
France (1)	No	44	44
Iran (1)	Yes	31	0
Japan (4)	No	6	6
Kuwait (1)	No	10	4
Mexico (2)	Yes	14	5
Oman (1)	Yes	4	2
Poland (1)	Yes	8	5
Portugal (1)	Yes	5	5
Russia (1)	Yes	8	0
Turkey (3)	No	40	27
United Kingdom (2)	No	54	46

*A total of 821 patients were given diagnoses of SCID in these centers, including 349 who were BCG vaccinated and reported for the current study.
†For recent changes or individualized BCG vaccination policies in different countries, please refer to http://www.bcgatlas.org/.
‡Other forms of SCID treatment (eg, gene therapy, 3 patients; enzyme replacement, 2 patients; or thymus transplantation, 1 patient) are also included in this category.
§National Center Database of Primary Immunodeficiencies, which collects data from 13 centers in the Czech Republic.

to thrive, severe T-cell lymphopenia (in the absence of a condition consistent with Omenn syndrome or maternal engraftment), and/or severe functional T-cell defects. BCG-associated complications were defined based on clinical, microbiological, and/or histopathologic findings and were classified as localized (persistent lesions [ulcer, abscess, fistula, or lymphadenopathy] limited to the region of inoculation) or disseminated (evidence of infection distal to injection-site lesions, including positive blood or bone marrow cultures).[7] Data entered by the referring centers detailing pathologic manifestations that were attributed to an excessive and/or dysregulated immune response to BCG as a consequence of improvement in immune status were associated with a diagnosis of immune reconstitution syndrome (IRS).[8] Deaths caused by BCG-associated complications, as well as all-cause mortality, were analyzed as outcome variables. BCG-associated deaths were defined as cases in which the primary cause of death was strongly associated with BCG-associated complications, as determined by the clinical care team. Continuous variables were compared by using the Kruskal-Wallis test. The Fisher exact test was used to compare proportions. Logistic regression was used to evaluate the effects of covariates on a binary outcome variable. Kaplan-Meier curves were plotted and compared by using the log-rank test. Cox regression was used to evaluate the effects of covariates in a time-to-event analysis. All P values are 2-sided, and P values of less than .05 were considered statistically significant. Data analyses were performed with SAS software (version 9.3; SAS Institute, Cary, NC).

As with any retrospective observational study of this nature, there are limitations that should be considered when interpreting the results. We acknowledge the possibility of diagnostic criteria discrepancies among the participating centers. Our analysis only included children who received BCG vaccinations, and these children might not be representative of the entire SCID population. Because of the limitations of data collection, we used the midpoint of the reported time interval of BCG vaccination and hematopoietic stem cell transplantation (HSCT) in the time-to-event analysis. Additional variability and bias might be introduced by using this *ad hoc* method.

RESULTS

Population demographics

A total of 821 patients were given diagnoses of SCID in the 28 participating centers from 17 different countries, 349 of whom were BCG vaccinated (42%) and analyzed in this retrospective study (Table I). When the analysis was restricted to countries with mandatory at-birth BCG vaccination policies, the rate of BCG-vaccinated patients with SCID increased to 88%.

SCID diagnosis

SCID diagnosis was established in 9% of the patients before the age of 1 month, in 29% before 3 months, in 63% before 6 months, and in 90% before 1 year (Fig 1, *A*). The specific type of SCID diagnosis was determined in 159 (46%) patients and not defined in the remainder of the cohort. *IL2RG* deficiency was the most frequently reported, followed by defects in *RAG1/RAG2*, *ADA*, MHC class II deficiency, *IL7RA*, Artemis *(DCLRE1C)*, Janus kinase 3 *(JAK3)*, purine nucleoside phosphorylase *(PNP)*, zeta chain–associated protein of 70 kDa *(ZAP70)*, and Cernunnos *(NHEJ1)*. We cannot formally exclude that among the patients with no specific SCID type defined, some could have been affected by other known primary immunodeficiency diseases presenting with an SCID-like phenotype of severe T-cell lymphopenia and/or severe functional T-cell defects and increased susceptibility to mycobacterial diseases (eg, Mendelian susceptibility to mycobacterial disease–associated genetic defects).

BCG vaccination

Age at vaccination was determined in 345 of 349 patients with SCID. The majority (258/345 [75%]) were vaccinated within the first month of life (<1 week, 204 patients; 1-2 weeks, 6 patients; and 3-4 weeks, 48 patients), whereas the remainder (87/345) were vaccinated later (1-3 months, 74 patients; 4-6 months, 8 patients; 7-12 months, 3 patients; and >12 months, 2 patients). BCG vaccine was administrated on the deltoid area in all patients: 301 intradermally, 38 subcutaneously, and 10 in an undetermined manner. The vaccine strain was reported in 252 patients: Danish, 88 patients; Moreau, 66 patients; Pasteur, 32 patients; Glaxo, 29 patients; Tokyo, 19 patients; and Russia, 18 patients.

BCG-associated complications

BCG-associated complications are described in Fig 1, *B* to *F*. After BCG vaccination, 177 (51%) patients with SCID had complications: 59 (17%) localized and 118 (34%) disseminated, a 400- and 33,000-fold increase, respectively, over the general population. Age at onset of BCG-associated complications was determined in 158 patients: less than 1 month in 8 patients, 1 to 3 months in 33 patients, 4 to 6 months in 67 patients, 7 to 12 months in 34 patients, and greater than 12 months in 16 patients. Among patients presenting with disseminated complications, involvement of the extraregional lymph nodes (n = 67 [57%]), skin (n = 66 [56%]), or lungs (n = 55 [47%]) was the most common clinical presentation; BCG infections compromising the liver (n = 18 [15%]), spleen, and bones (n = 15 [13% each]) were reported less frequently. Isolation of *M bovis* BCG from bone marrow was described in 14% (n = 17) of patients with disseminated complications, whereas positive blood culture results were even more uncommon (n = 1 [1% of patients with disseminated complications]).

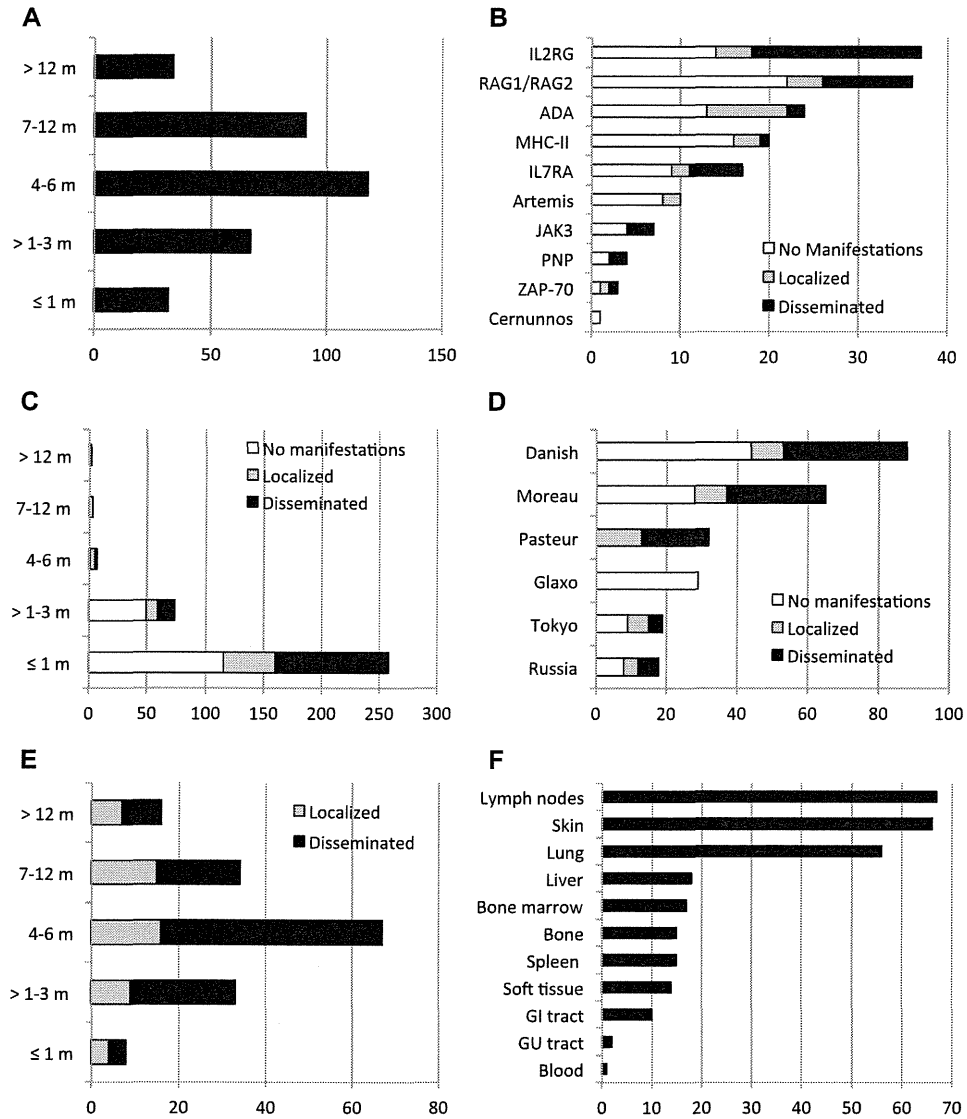


FIG 1. BCG-vaccinated patients with SCID: epidemiologic characteristics. **A,** Age at SCID diagnosis. **B,** SCID diagnosis and BCG-associated complications (no manifestations or localized or disseminated complications). **C,** Age at BCG vaccination and BCG-associated complications (no manifestations or localized or disseminated complications) distribution. **D,** BCG vaccine strain and BCG-associated complications (no manifestations or localized or disseminated complications). **E,** Age at onset of BCG-associated complications (localized or disseminated complications). **F,** Site of involvement of disseminated BCG-associated complications. *GI,* Gastrointestinal; *GU,* genitourinary. *X-axis,* number of patients.

The median absolute T-cell number at the time of SCID diagnosis in patients with localized or disseminated BCG-associated complications was significantly lower than that in patients without BCG-associated complications (P = .003, Table II). Logistic regression analysis showed that the odds of experiencing BCG-associated complications among patients with SCID with T-cell numbers of 250/μL or less at diagnosis was 2.1 times higher (95% CI, 1.4-3.4 time higher; P = .001) than that among those with T-cell numbers of greater than 250/μL, and the difference remained significant after adjusting for the age at BCG vaccination. Patients with and without BCG-associated complications were not significantly different in either B-cell or natural killer cell numbers.

Two hundred thirty-eight (68%) patients received antimycobacterial treatment after receiving a diagnosis of SCID. At the time of treatment initiation, 78 (22%) were asymptomatic in terms of BCG-associated complications, and 160 (46%) were symptomatic (53 with localized and 107 with disseminated manifestations).

Among asymptomatic antimycobacterial agent–treated patients who underwent HSCT (n = 64), 49 (77%) received multidrug antimycobacterial therapy (MAT), whereas 10 (16%) were treated with isoniazid monotherapy (no information on 5 patients). MAT included isoniazid plus rifampicin–based treatment in 49 (77%) patients, 18 of them (28%) having 1 or more additional drugs. The enteral route was preferred in 94% of these

TABLE II. BCG-vaccinated patients with SCID: statistical analysis

Age at BCG vaccination			
	BCG vaccination at ≤1 mo	BCG vaccination at >1 mo	*P* value
Sex, no. (%)			
Female	88 (34.8)	40 (46)	NS
Male	165 (65.2)	47 (54	
Age at SCID diagnosis (mo), median (range)	5 (0.5-48)	6 (0.5-100)	NS
BCG-associated complications, no. (%)			
No manifestations	115 (44.6)	54 (62.1)	.006
Loc/Diss manifest	143 (55.4)	33 (37.9)	
Age at HSCT (mo), median (range)	7 (0.5-75)	8 (0.5-107)	NS
Mortality in BCG-SCID			
BCG-rel, no. (%)	45 (18)	0 (0)	<.0001
Overall, no. (%)	132 (52.8)	38 (43.7)	NS

Median lymphocytes at SCID diagnosis			
	No manifestations	Localized or disseminated	
T cells/μL (25th-75th percentile)	197 (14-942)	49 (5-343)	.003
B cells/μL (25th-75th percentile)	103 (5-640)	140 (11-710)	NS
NK cells/μL (25th-75th percentile)	160 (38-410)	100 (19-366)	NS

BCG-rel, Death related to BCG-associated complications; *Loc/Diss manifest*, localized or disseminated manifestations of BCG-associated complications; *NK*, natural killer; *No manifestations*, no manifestations of BCG-associated complications; *NS*, not significant.

patients. No significant differences between monotherapy and MAT were detected when death caused by BCG-associated complications was compared ($P = .99$). By the time of data analysis, 63% of these patients were alive (median follow-up, 57 months; range, 4-126 months). Among symptomatic patients receiving antimycobacterial treatment and undergoing HSCT (n = 76), 64 (82%) were treated with MAT, whereas 4 (5%) were treated with isoniazid monotherapy (no information on 8 patients). MAT included isoniazid plus rifampicin–based treatment in 61 (80%) patients, 47 (62%) of them having 1 or more drugs added to the scheme. Eighty-four percent of these patients were treated through the enteral route, and 11% were treated through a mixed (enteral and parenteral) route. By the time of data analysis, 70% of these patients were alive (median follow-up, 45 months; range, 0-158 months).

BCG-associated complications were reported in 3% (2/64) of asymptomatic patients receiving antimycobacterial treatment and undergoing HSCT. Antimycobacterial treatment of already symptomatic patients undergoing HSCT resulted in complete clinical resolution of the infection in 30%, partial resolution in 46%, and no resolution in 24%. After HSCT, 59% of the patients were kept on antimycobacterial treatment: 32% for less than 3 months, 15% for 4 to 6 months, 21% for 7 to 12 months, and 32% for more than a year.

No deaths related to BCG-associated complications were reported among BCG-asymptomatic treated patients with SCID, whereas 46 deaths caused by BCG occurred among BCG-symptomatic treated patients (7 in patients who underwent HSCT and 39 in patients who did not, including 45 patients with disseminated complications and 1 patient with localized disease; $P < .0001$). The median age of death for these patients (38 with reported data) was 6.8 months. When the analysis was restricted to patients undergoing HSCT, no deaths were reported among the asymptomatic treated group (0/64), and 7 deaths occurred among the 120 symptomatic treated patients ($P = .09$).

One hundred eleven BCG-vaccinated patients with SCID (32%; 96 of them presenting with no manifestations and 15 symptomatic, including 9 with disseminated and 6 with localized complications) did not receive antimycobacterial treatment after SCID diagnosis. Forty-five (40%) of these patients underwent HSCT (32 asymptomatic and 13 symptomatic, including 8 with disseminated and 5 with localized complications), 15 of them received antimycobacterial treatment after HSCT (3 asymptomatic and 8 with disseminated and 4 with localized manifestations), 28 of them (63%) are alive, and no deaths caused by BCG-associated complications were reported (median follow-up, 46 months; range, 0-187 months). Of the remaining 66 patients (60%, 64 were asymptomatic and 2 were symptomatic, including 1 with disseminated and 1 with localized complications) who did not undergo HSCT by the time of data analysis, 22 (33%) were alive, and only 1 BCG-associated death was reported in this group (presenting with disseminated disease). Interestingly, survival rates for patients who did not receive pre-HSCT antimycobacterial treatment (27/45) was not statistically different from those in patients who received antimycobacterial treatment and underwent HSCT (94/139, $P = .47$).

Age at BCG vaccination showed a significant association with BCG-associated complications independently of the type of SCID, the vaccine strain, or the route of vaccination. Patients vaccinated within the first month of life showed an increased prevalence of BCG-associated complications (disseminated or localized) compared with patients vaccinated after 1 month of age ($P = .006$). Moreover, the odds of having BCG-associated complications among those vaccinated within the first month of life were 2.03 times higher than those vaccinated after the age of 1 month (odds ratio, 2.03; 95% CI, 1.24-3.35). A log-rank test comparing time to death caused by BCG-associated complications in patients vaccinated within or after 1 month of age also identified significant differences between these 2 groups ($P < .0001$, Fig 2). Moreover, survival analysis comparing time to death within 24 months of age before HSCT for patients vaccinated early versus late showed that the hazard of death was 2.12 times higher for those receiving early vaccination (95% CI, 1.12-3.89; Fig 2). These results strongly suggested that early BCG vaccination (≤1 month) is associated with increased BCG-associated complications and subsequent death associated with those complications.

SCID treatment

Of the 349 BCG-vaccinated patients with SCID, 190 (54%) underwent HSCT (n = 184) or another form of SCID-specific treatment (eg, gene therapy [n = 3], enzyme replacement [n = 2], or thymus transplantation [n = 1]). The median age at HSCT was 7.5 months (range, 0.5-107 months). No significant differences in T-cell engraftment were detected between patients receiving early (≤1 month) versus late (>1 month) BCG vaccination or among patients undergoing transplantations without or with BCG-associated complications (localized or disseminated). No significant differences in the proportion of death caused by