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Successful reduced-intensity SCT from unrelated cord blood in three patients with X-linked SCID

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We describe three males with X-linked SCID (X-SCID) who were successfully treated by reduced-intensity SCT from unrelated cord blood (CB). Mean age at transplant was 5.7 months (range, 3–9 months). Pre-transplant conditioning for all patients consisted of fludarabine (FLU) (30 mg/m^2 per day) from day −7 to day −2 (total dose 180 mg/m^2) and BU 4 mg/kg per day from day −3 to day −2 (total dose 8 mg/kg). All CB units were serologically matched at HLA-A, B and DR loci. Although two patients had suffered from fungal or bacterial pneumonia before transplantation, there were no other infectious complications during transplantation. All patients engrafted and achieved 100% donor chimerism. We also confirmed full donor chimerism of both T and B cells. Only one patient developed acute GVHD grade III, which was resolved by increasing the dose of oral corticosteroid. None of the patients has developed chronic GVHD during follow up for 21–77 months. None of the patient received i.v. Ig replacement post transplant, or showed delay in psychomotor development. Reduced-intensity conditioning consisting of FLU and BU and transplantation from unrelated CB was an effective and safe treatment for these patients with X-SCID.

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Introduction

SCID is the most severe form of primary immunodeficiency. About half of all cases are X-linked SCID (X-SCID; T-B + NK-SCID), caused by deficits of cytokine receptor common gamma chain.[1] Haematopoietic SCT is the only curative treatment for these high-risk patients.[2,3] In the early series, HLA-identical sibling BMT led to complete immunological reconstitution with no conditioning regimen.[4,5] Thereafter, transplants from closely matched unrelated volunteer donor (MUD) have been performed with better outcomes in terms of both survival and immunological reconstitution than following those using haploidentical donors; the majority of patients with myeloablative conditioning no longer required i.v. Ig replacement after MUD transplantation.[2,5,6] However, MUD transplantation can require a lengthy search for a suitable donor, often over 3 months, and is associated with both high frequency and intensity of GVHD. Moreover, immunological reconstitution is often incomplete especially with X-SCID after MUD transplant with no conditioning.[4] Most of these problems are resolvable by transplanting with umbilical cord blood (CB) already typed with a known number of CD34 cells supplied from CB bank, when a lower incidence of GVHD is seen.[7–9] Following the first report of CB SCT (CBT) for primary immunodeficiency patients using their sibling donors,[10] successful unrelated CBTs have been described.[11–16] Because the OS rates are over 70%, CB could be a promising source of stem cells when an HLA-identical sibling donor is not available.

X-SCID is fatal and requires SCT in the first year of life despite possible late complications such as mental and physical retardation after myeloablative transplantation received in infancy.[17–19] On the other hand, most have already suffered from bacterial and/or fungal infection at diagnosis of X-SCID. Recently developed reduced-intensity conditioning (RIC) regimens have been used in unrelated SCT for primary immunodeficiency patients, because of their intense immune suppressive qualities and reduced myelotoxicity.[20] Thus, reduced-intensity SCT (RIST) from CB could be a choice for patients with X-SCID but is not fully established to date. We report our single centre experience of successful allogeneic RIST from unrelated CB for treatment of X-SCID.

Patients and methods

Patients

Three patients with X-SCID received unrelated CBT because they had no HLA-matched sibling donors. As

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shown in Table 1, mutations in the common gamma chain gene were detected in all patients. Patients 1 and 2 had suffered from pneumonia caused by aspergillus and bacteria, respectively, at the diagnosis of X-SCID. Patient 3 was diagnosed as having X-SCID at birth because his brother had the same disease (Table 1).

Conditioning regimen and GVHD prophylaxis

Pre-transplant conditioning for all patients consisted of fludarabine (FLU) (30 mg/m^2 per day) from day −7 to day −2 (total dose 180 mg/m^2) and BU 4 mg/kg per day (oral in patient 1 and 2; i.v. in patient 3) from day −3 to day −2 (total dose 8 mg/kg). Neither ATG nor Campath was included in the conditioning regimen.

Prophylaxis for aGVHD included cyclosporine A (3 mg/kg) from day −1 to day +180, and methylprednisolone 0.5 mg/kg per day (day + 7 to +13), 1 mg/kg per day (day + 14 to +28), 0.5 mg/kg per day (day + 29 to +42), 0.3 mg/kg per day (day + 43 to +56) and 0.2 mg/kg per day (day + 57 to +72). Cyclosporine A was discontinued by day + 180, after confirming the absence of clinical GVHD.

Graft characteristics

As shown in Table 1, all CBs were collected from female donors. All of these CB units were serologically well matched at 6/6 (A, B, DR) HLA loci. Infused nucleated cell doses were 11–20 $\times 10^7$/kg (mean, 15×10^7/kg), which contained CD34+ stem cells, ranging from 3.2 to 6.7×10^5/kg (mean, 5.1×10^5/kg).

Supportive care

Supportive care for transplantation in our institution has been previously described.[21] Briefly, all patients received a continuous infusion of low molecular weight heparin, 100 unit/kg per day, as prophylaxis against hepatic veno-occlusive disease (VOD), from day −7 to day +30. Oral polymixin B and amphotericin B, inhaled vancomycin, tobramycin and amphtericin B were given as antibacterial and antifungal prophylaxis. Antiviral prophylaxis consisted of oral acyclovir (600 mg/m^2 per day) from day −7 to day +35 and weekly i.v. γ-globulin (200 mg/kg per 2 weeks) from day −6 to day +90. G-CSF was given i.v. at 5 μg/kg from day +5 until the ANC reached 500/μL or more for 3 consecutive days. Oral mucositis was treated with i.v. pentazocine and parental nutrition.

aGVHD was diagnosed and graded according to the Seattle criteria,[22] and treated with prednisolone. CMV infection was diagnosed on the basis of CMV antigenemia, and treated with ganciclovir in combination with anti-CMV high titer γ-globulin.

Chimerism studies

Hematological recovery was defined as achievement of both an ANC > 500/μL for 3 consecutive days and a platelet count $> 50 \times 10^9$/L for 7 consecutive days without the need for transfusion. Chimerism was tested by FISH of peripheral mononuclear cells using X and Y chromosome probes. Chimerism of T or B cell lineage was assessed by flow cytometry using intracytoplasmic staining of common gamma chain together with fluorescein-labelled antibodies against CD3 and CD19.

Table 1 Patient characteristics

Patient	*1*	*2*	*3*
Age at diagnosis (months)	8	4	0
Age at CBT (months)	9	5	3
Mutations in common gamma chain	682T > G in exon 5	9_10insA in exon 1	216G > A in exon 2
HLA identity	6/6	6/6	6/6
Nucleated cell dose ($\times 10^7$/kg)	11.2	20.4	15.6
CD34+ cell dose ($\times 10^5$/kg)	6.7	5.3	3.2
Cytomegarovirus serology	Positive	Positive	Positive
Haematological recovery			
Neutrophil > 500/μL	day + 19	day + 22	Day + 27
Platelet > 50×10^9/L	day + 28	day + 43	Day + 31
Complications at CBT	aspergillus pneumonia	bacterial pneumonia	None
Additional infections during CBT	None	None	None
GVHD			
Acute(grade)	0	III	0
Chronic	—	—	—
Chimerism (donor%)	100	100	100
B-cell engrafted(donor % (day))	100 (day + 120)	100 (day + 89)	100 (day + 83)
i.v.Ig replace at present	None	None	None
Performance Status (ECOG, Oken MM *et al*[35])	PS-0	PS-0	PS-0
Outcome	Alive +77mo	Alive +69mo	Alive +21 mo
Height	−0.53 s.d.	−0.49 s.d.	−2.4 s.d.
Body mass index	15.0	16.1	15.6
Mental status	Normal	Normal	Normal

Abbreviations: CBT = cord blood transplantation; ECOG = Eastern Cooperative Oncology Group.

Immunological reconstitution studies
Immunological reconstitution status after transplantation was monitored by serum immunoglobulin levels (IgG, IgA and IgM) and flow cytometry analyses of peripheral mononuclear cells for CD3, CD4, CD8, CD16, CD19 and CD56.

Results

Engraftment and chimerism
As shown in Figures 1a–c, all patients achieved engraftment of ANC $>500/\mu L$ at a mean of 22 days (range, 19–27 days). Mean time to platelet engraftment (platelets $>50\times10^9/L$) was 34 days (range, 28–43 days). Donor chimerism (100%) was demonstrated by FISH with X and Y chromosome probes at 1-year post transplantation. We also confirmed full donor chimerism of both T and B cells. Chimerism has been stable to date with a mean follow-up of 53 months (range, 21–77 months). Frequencies of transfusion were similar to the other patients with non-malignant conditions, such as congenital metabolic disorders, receiving RIST in our institute.

Regimen related toxicity
Patient 1 received anti-fungal treatment with i.v. micafungin 3 mg/kg before conditioning until day + 90 for pre-existing lung aspergillosis, which showed no exacerbation during CBT. Clinical and radiological improvement were achieved after CB engraftment. Patient 2 was already suffering from bacterial pneumonia on admission; he received appropriate antibiotic treatment and recovered at the beginning of conditioning. He had no additional severe infectious complications up to engraftment. Patient 3 received CBT at 3 months of age with no previous infections. He also had no severe infectious complications up to engraftment. Although mild mucositis and increase of serum transaminase occurred, none of the patients experienced complicated severe regimen related toxicities such as VOD.

GVHD disease
Only patient 2 developed acute GVHD grade III, consisting of mild skin rash and diarrhea that resolved by increasing the oral corticosteroid dose. None of the patients have developed chronic GVHD or associated complications during follow-up.

Immunological reconstitution
PBL sub-populations gradually reconstituted in all patients after UCB transplantations. As shown in the figures, peripheral blood CD3-, CD19-, CD56-positive cells gradually increased up to almost normal age-related levels by 1-year post transplantation. We confirmed common gamma chain expression in all of these lymphocyte sub-populations. None of the patient had received further i.v. Ig replacement by 5–6 months after transplantation. Moreover, in all the patients, specific antibodies were produced against influenza, pertussis and measles following vaccination.

Growth and psychomotor development
As shown in Table 1, all three patients have shown normal psychomotor development and performance status to date. Only patient 3 had short stature of −2.4 s.d. at 21 months follow-up at post transplant, although the other two patients had normal growth development.

Discussion

We describe three patients with X-SCID successfully treated by CBT with a RIC regimen using FLU/BU. Both FLU/melphalan (LPAM) and FLU/BU regimens with or without modifications such as additional serotherapy are widely used for RIC regimen.[23,24] Although the FLU/LPAM regimen results in satisfactory engraftment,[20] LPAM is toxic to both primitive and committed stem cells resulting in early onset and prolonged duration of neutropenia.[25] In addition, LPAM-containing conditioning has been identified as a risk factor for VOD,[26] possibly because LPAM induces more severe mucosal injury in the oral cavity and gastrointestinal tract compared with other agents.[27–29] On the other hand, BU is preferentially toxic to committed stem cells.[25] Although conditioning regimens including standard-dose BU are associated with a high rate of treatment-related complications due to organ toxicity, reduced-dose BU in combination with FLU is less myelosuppressive and toxic than FLU/LPAM or the standard-dose of BU regimen.[30] Our patients achieved full donor T- and B-cell chimerism and clinical cure with minimum complications related to myelotoxicity. To date, none of our patients has shown mental or growth delay with the exception of patient 3, who had short stature of −2.4 s.d. at 21 months after CBT. Growth and endocrine function are commonly affected by standard myeloablative conditioning but this has not been reported with BU-based RIC for SCT during infancy and childhood.[31] Patient 3 possibly has growth retardation following the BU-based RIC regimen because he underwent SCT at 3 months old. Further studies are necessary to determine the appropriate time for SCT to prevent late complications such as growth retardation.

Although CBT is more tolerant to HLA disparity, HLA incompatibility increases the incidence and intensity of GVHD and transplantation-related mortality.[16,32] The low incidence of severe GVHD in our series of patients possibly reflects the good match of the CB units. As unrelated CB is immediately available from CB banks, it is advantageous for patients with X-SCID who need urgent transplantation.

Pre-existing infections are the principal risk factors for a poor outcome after SCT. Patients 1 and 2 overcame pre-existing fungal and bacterial pneumonia, respectively, and recovered after transplantation. This suggests that, in addition to the appropriate antimicrobial therapy, early immunological reconstitution with minimum immune suppression after SCT using a RIC regimen contributes to the freedom from infection. Detection of fungal Ags or viral nucleotides by PCR-based techniques is critical for the early diagnosis and appropriate treatment of infections. On the other hand, early diagnosis of X-SCID before any infectious episodes, as shown in patient 3, is necessary for

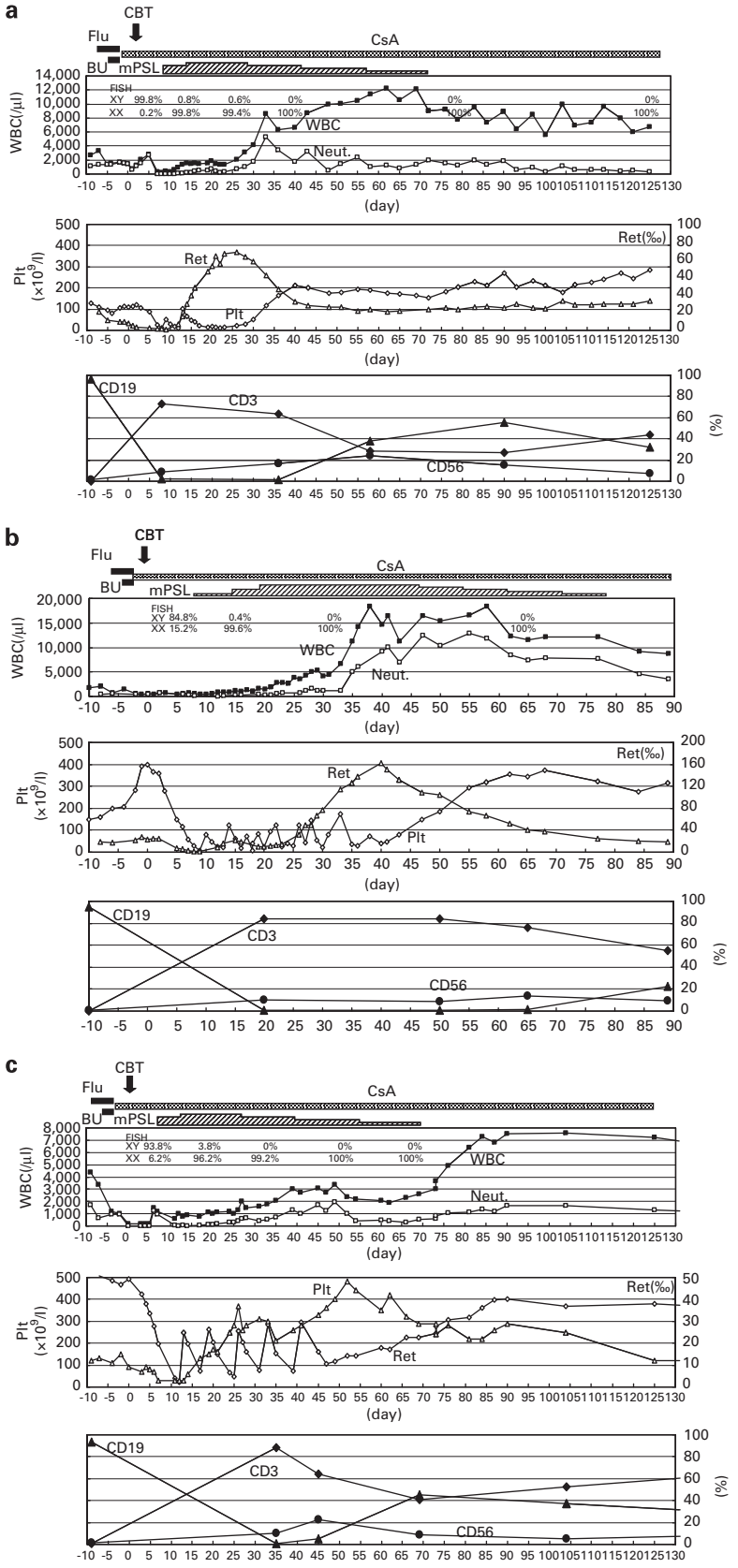

Figure 1 Clinical course of the three patients with X-SCID receiving RIST from CB. **a**, Patient 1; **b**, Patient 2; **c**, Patient 3; ■, WBC; □, Neutrophils (Neut); △, Reticulocyte (Ret); ◇, Platelet (Plt); ◆, CD3; ▲, CD19; •, CD56; respectively. Note that all patients have engrafted and reached $>500/\mu L$ neutrophils with day + 19, + 22, + 27, respectively (mean day + 22). Platelet engraftment (platelets $>50 \times 10^9/L$) was day + 28, + 43, + 31, respectively (mean day + 34).

safe SCT, particularly in the patients with SCID. Recently developed neonatal mass-screening by quantitative assay for T-cell receptor excision circles might be useful for early diagnosis of patients with a wide variety of SCID genotypes.[33,34]

In conclusion, CBT is a suitable alternative to BMT in X-SCID patients requiring SCT who have no matched sibling donors. RIC consisting of FLU and BU is an effective and safe treatment for such cases. Further studies are necessary to determine the appropriate time for SCT to minimize late complications such as growth retardation.

Conflict of interest

The authors declare no conflict of interest.

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