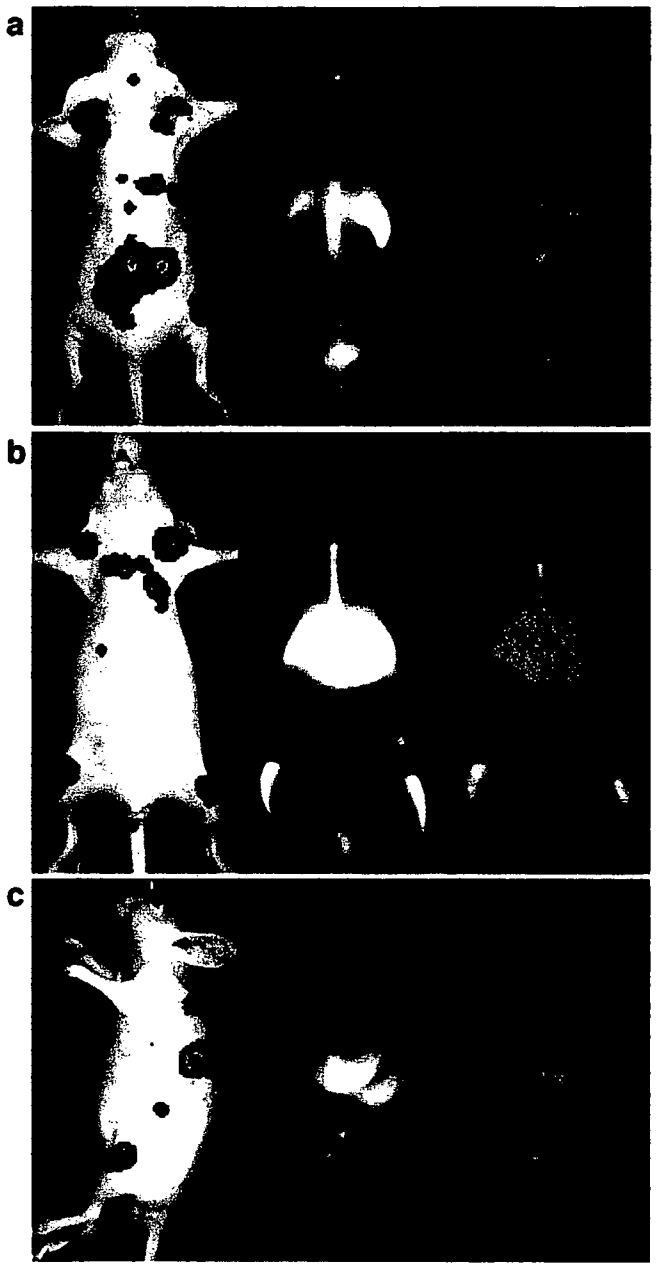

Fig. 4 Bioluminescent (*left*), fluorescent (*middle*), and fusion images (*right*) of mice inoculated with Ba/F3–Luc/Wt cells and injected with amino-PEG–QDs. **a** Dorsal images obtained 7 days after cell inoculation. Bioluminescent foci are shown in the lumbar spine, left iliac bone, right ovary, knees, thoracic spine, liver, scapulas, humeri, and interparietal bone. **b** Ventral images of the mouse presented in **a**. Bioluminescent foci are shown in proximal humeri, anterior tips of the ribs, sternum, liver, femurs, and tibias. **c** Left-lateral images obtained 9 days after cell inoculation. Bioluminescent foci are shown in the spleen, left femur, and left tibia. The focus on the dorsal portion of the chest was judged to be in the left scapula based on the dorsal images

assessed. The signals were shown to originate predominantly from the femur for four knees, predominantly from the tibia for one knee, and similarly from the femur and tibia for four knees. In the skeleton, bioluminescent foci were frequently located in areas showing strong fluorescent signals. Most bioluminescent foci in the neck were detected in proximal humeri, and most bioluminescent foci in the head were detected in the interparietal bone or frontal bone. Strong fluorescent signals were also seen in these areas. Fluorescent signals were relatively high at the anterior tips of the ribs, where bioluminescent foci were often found.

Discussion

It has been reported that the reticuloendothelial system of a mouse can be visualised non-invasively after QD injection [8]. In this study, we performed in vivo FLI on live mice after intravenous injection of amino-PEG–QD without targeting ligands and investigated the temporal patterns of QD distribution and the structures delineated on the acquired fluorescent images. Long-term, repeated imaging of the reticuloendothelial system was feasible after a single injection, and various bony structures and superficial lymph nodes were visualised in addition to the liver and spleen. Although a detailed assessment of toxicity was not performed, the appearances and body weights of the mice did not suggest any toxicity despite the prolonged retention of QD. These results indicate the utility of amino-PEG–QD for imaging of the reticuloendothelial system. Jackson et al. reported the lack of apparent acute toxicity in rats injected with higher doses of amino-PEG–QD (up to 50 pmol/g), supporting the safety of the agent [23]. Image contrast tended to be better at the earlier time points, and quantitative analysis also demonstrated stronger signals at these time points. However, 1 h after injection, patchy fluorescent signals were observed outside the reticuloendothelial structures, which may have been caused by extravasation of QD; in addition, the visualisation of lymph nodes was relatively obscure. The optimal time point for scanning was, therefore, found to be approximately 3–6 h after injection.

Selective accumulation of amino-PEG–QD in the reticuloendothelial system was confirmed by ex vivo FLI. The measurement was performed 24 h after injection, and contrast ratios would be higher at earlier time points. Superficial and deep lymph nodes were delineated as intense fluorescent spots. FLI using QD may serve as a guide when searching for lymph nodes during dissection. Although the upper thoracic spine was not visible on in vivo dorsal images, it was delineated on the ventral image acquired after removal of the internal organs and anterior thoracic wall. The presence of thick soft-tissues on the dorsal side of the upper thoracic spine appears to be responsible for the non-visualisation on in vivo images. Difficulty in assessing deep structures is a major limitation of optical imaging compared to X-ray imaging or MRI.

We attempted to use fluorescent reticuloendothelial imaging for the localisation of bioluminescent signals. In these experiments, mice were inoculated with model cells of a haematological malignancy that stably expresses firefly luciferase. First, we examined whether the administration of amino-PEG–QD affects the quantitative results of bioluminescent tumour monitoring. Alteration in bioluminescence intensity may be inferred to occur because of inhibition of the luciferase-dependent bioluminescent reaction, enhanced light attenuation in QD-containing tissues, changes in luciferase expression per cell, or variations in proliferative activity of the inoculated cells. However, whole-body bioluminescent signals were comparable between QD-injected mice and controls from 3 h to 3 weeks after QD injection, justifying the use of the FLI technique in quantitative bioluminescent tumour monitoring.

The intravenous administration of amino-PEG–QD enabled repeated localisation of bioluminescent foci on maps of the reticuloendothelial system and facilitated the identification of the structures harbouring the inoculated cells. Although even a single dose of QD offered useful anatomical information longitudinally, additional injection would improve the assessment of the relationship between bioluminescent foci and reticuloendothelial structures, especially spleen, in follow-up experiments. We determined the involved structures using ex vivo BLI [14, 20, 21] to evaluate the utility of combining FLI with bioluminescent tumour monitoring. In vivo BLI/FLI, accompanied by fusion of both images, improved the accuracy and confidence level of the localisation of the bioluminescent foci compared to BLI alone. Both BLI and FLI images were acquired using an identical CCD camera system with the mouse position unchanged, which made comparison between them easy and reliable. Fusion of the fluorescent and bioluminescent images was achieved without special data processing, and aided detailed interpretation of the relationship between bioluminescent foci and reticuloendothelial structures. An experienced investigator could usually predict the involved organ based on the photographic surface images. However, when a bioluminescent focus is present near the border between two structures, it is impossible to define the involved organ confidently. Alteration of organ morphology induced by disease progression may make the localisation more difficult in disease model animals. In addition, sub-optimal positioning of mice in the imaging chamber may prevent accurate localisation. Although a spinal lesion should be identified as a central focus on a dorsal image, it may be displaced because of distortion of the posture and may mimic a lesion in the liver, spleen or iliac bone. When two or more mice are examined simultaneously, mice placed in the peripheral area of the FOV are imaged in a somewhat oblique projection, and thus, it is difficult to determine the precise location of the spine. Fluorescent reticuloendothelial imaging using amino-PEG–QD would serve as a useful, convenient adjunct to BLI when the localisation of bioluminescent foci is desired.

In vivo BLI/FLI enabled the identification of bones containing inoculated cells. Determining the distribution of the inoculated cells among the bones may be beneficial for harvesting the cells for in vitro analysis. Skeletal bioluminescent foci were frequently demonstrated in areas showing intense fluorescent signals. It is suggested that bones with high bone marrow content accumulate amino-PEG–QD avidly and, at the same time, are susceptible to tumour cell implantation. FLI using amino-PEG–QD appears to aid in defining the distribution of bone marrow.

Combining X-ray imaging, CT or MRI with BLI can also add anatomical information to BLI [11–14]. However, FLI with amino-PEG–QD presents major advantages over other anatomical imaging methods, such as the wide availability and correct registration of bioluminescent and fluorescent images, as FLI can be performed immediately after BLI using the same CCD camera system. Moreover, BLI/FLI is neither time-consuming nor technically demanding. The FLI technique visualises the liver, spleen and lymph nodes in addition to the skeleton. Ionising radiation is not used, so the possible effects of radiation exposure on inoculated cells or host animals need not be considered. Disadvantages of FLI include difficulty in discriminating between overlapping organs because most in vivo FLI studies provide projectional images unlike CT or MRI. One hepatic focus was judged as a costal focus using in vivo BLI/FLI in this study, a misinterpretation that appears to be related to the overlap between the ribs and liver. Non-invasive FLI usually offers relatively low spatial resolution because of light scattering in the tissues. FLI with amino-PEG–QD visualises the reticuloendothelial organs only and cannot define other organs or tumour lesions directly. In animal models of haematological malignancies, lesions usually develop in the reticuloendothelial system, so the utility of FLI with amino-PEG–QD as anatomical landmarks appears to be high. The optimal anatomical imaging method should be selected for each experiment by considering the benefits of each method and the requirements of the experiment.

Although we injected 5 pmol/g amino-PEG–QD in this study, a dose of 2.5 pmol/g provided almost the same information soon after injection (data not shown). The appropriate injection dose remains to be defined, and it appears to depend on the CCD camera system, the fluorescent filters and the emission peak and surface coating of QD. We fused fluorescent and bioluminescent images using general-purpose software, and the use of dedicated software integrated into the data acquisition

system would improve the method in convenience. Intake of an alfalfa-free diet has been reported to reduce autofluorescence from the intestine [18]. Although we confirmed the beneficial effect of the alfalfa-free diet (data not shown) and provided the diet for at least 7 days before FLI, autofluorescence originating from intestinal contents remained relatively strong. Further modification of the diet may lead to a reduction in the required injection dose and improvement of the image quality.

In conclusion, we characterised in vivo fluorescent reticuloendothelial imaging using QD without targeting ligands and investigated its use in combination with in vivo BLI. The QD injected intravenously selectively accumulated in the reticuloendothelial system. Various structures were visualised soon after injection, and long-term repeated observation was possible without additional injections, although image contrast gradually decreased. No toxicity was indicated, and the intravenous administration did not affect quantitative results of bioluminescent tumour monitoring. Combining FLI using QD with BLI demonstrated the relationship between bioluminescent foci and reticuloendothelial structures and aided the localisation of bioluminescent foci. This combined imaging approach is practical and expected to allow bioluminescent tumour monitoring to yield more benefits.

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ORIGINAL ARTICLE

Preemptive therapy with ganciclovir 5 mg/kg once daily for cytomegalovirus infection after unrelated cord blood transplantation

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The efficacy and safety of preemptive therapy using ganciclovir (GCV) 5 mg/kg once daily for CMV infection after unrelated cord blood transplantation (CBT) were studied. The initial preemptive therapy with GCV 5 mg/kg once daily led to resolution of CMV antigenemia in 25 of 34 patients (74%). In the remaining 9 patients (26%), antigenemia resolved after dose-escalation of GCV or change to foscarnet therapy. Recurrence of antigenemia was seen in 18 patients (53%). A total of 12 patients received the second preemptive therapy with GCV 5 mg/kg once daily, which led to resolution of antigenemia in 11 of 12 patients (92%). The remaining 1 patient (8%) required change to foscarnet therapy. None of 34 patients developed CMV disease. Neutropenia with an absolute neutrophil number of less than 1 and 0.5×10^9 per liter after GCV therapy occurred in 12 (35%) and 1 (3%) patients, respectively, after the initial therapy, and in 2 (17%) and 0 (0%) patients, respectively, after the second therapy. No patients developed neutropenic fever or secondary graft failure after GCV therapy. There were no deaths directly attributable to GCV therapy. The present study suggests that antigenemia-based preemptive strategy using GCV 5 mg/kg once daily is feasible and effective for CBT recipients.
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Keywords: preemptive therapy; CMV; ganciclovir; neutropenia; cord blood transplantation

Introduction

CMV disease is one of the major infectious complications after allogeneic hematopoietic SCT.[1,2] By applying various surveillance methods such as antigenemia and PCR assays, preemptive strategies with ganciclovir (GCV) have been widely used for preventing early CMV disease after SCT. In most preemptive strategies, GCV is administered intravenously at a dose of 5 mg/kg twice daily as an initial induction phase for 1–2 weeks, then followed by 6 mg/kg once daily as a maintenance phase.[3] One of the major adverse effects of GCV is neutropenia.[4] In an attempt to reduce the incidence of GCV-related neutropenia, preemptive therapy with GCV 5 mg/kg once daily has previously been used as initial induction therapy for BMT and peripheral blood SCT (PBSCT) recipients.[5–7] The favorable outcomes suggested that this strategy could reduce the incidence of GCV-related neutropenia but retained the efficacy for preventing CMV disease in BMT and PBSCT recipients.

Umbilical cord blood transplantation (CBT) from an unrelated donor has recently been utilized as an alternative therapy for patients who do not have suitable donors for BMT or PBSCT.[8,9] Previous studies have shown that CMV infection occurs frequently in adult patients after CBT.[10,11] In addition, patients with CMV infection after CBT have a higher probability of secondary graft failure than those without CMV infection.[11] Toxicity of GCV may be associated with secondary graft failure in CBT recipients with CMV infection.

In our previous study, we reported the results of antigenemia-based preemptive GCV therapy for CMV infection in adult patients after unrelated CBT.[12] The preemptive therapy consisted of GCV 5 mg/kg twice daily as an initial induction phase for 2 weeks and 5 mg/kg once daily as a maintenance phase for 1 week or more. Because of recent favorable outcomes in BMT and PBSCT recipients, we examined the efficacy and toxicity of preemptive therapy using GCV 5 mg/kg once daily for CMV infection after unrelated CBT in the present study.

Patients and methods

Patients

Between May 2002 and April 2006, 60 patients underwent CBT using a conditioning regimen containing 12 Gy total body irradiation. Among them, 34 patients who developed CMV antigenemia and initially received preemptive ther-

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apy with GCV 5 mg/kg once daily were analyzed in the present study. Patient characteristics are listed in Table 1. The remaining 26 patients were excluded from the analysis for the following reasons. Four patients did not achieve cord blood-derived myeloid engraftment. A total of 16 patients including 8 CMV-seronegative patients did not develop antigenemia during the first 90 days after CBT. The remaining 6 patients received GCV therapy, but the initial dose was not 5 mg/kg once daily. Two patients with a creatinine clearance rate (CCR) of less than 30 ml/min initially received GCV 2 or 3 mg/kg once daily. Two patients with pulmonary symptoms and signs initially received GCV 5 mg/kg twice daily. In both patients, organizing pneumonia but not CMV pneumonia was diagnosed with transbronchial lung biopsy specimens.[13] The remaining two patients who did not have pulmonary symptoms or renal dysfunction received GCV 5 mg/kg twice daily, but there were no specific reasons for choosing the conventional-dose GCV therapy. Therefore, preemptive therapy with GCV 5 mg/kg once daily was applied to patients who did not have pulmonary symptoms possibly due to CMV pneumonia or renal dysfunction with a CCR of less than 30 ml/min at the initiation of GCV therapy.

Table 1 Patient characteristics

	All	*LD-GCV*
No. of patients	60	34
Age, y		
Median	41	43
Range	16–55	22–55
Gender, n		
Male	34	18
Female	26	16
Disease, n		
AML	35	21
ALL	8	6
CML	3	1
MDS	8	3
NHL	6	3
Disease status[a], *n*		
Low-risk	32	18
High-risk	28	16
TNC, $\times 10^7$/kg		
Median	2.35	2.34
Range	1.65–5.13	1.77–5.13
CMV serostatus, n		
Negative	7	0
Positive	53	34
HLA matching[b], *n*		
5/6 or 4/6	34	22
3/6 or 2/6	26	12
Preparative regimen, n		
TBI + CY + AraC	49	25
TBI + CY	1	1
TBI + FLU + AraC	7	6
TBI + FLU + MEL	3	2
GVHD prophylaxis, n		
CSP + MTX	59	33
CSP	1	1
Neutrophil engraftment, d		
Median	22	21
Range	16–46	16–32

Abbreviations: CSP = cyclosporin; FLU = fludarabine; LD-GCV = LD-GCV = low-dose ganciclovir; MEL = melphalan.
LD-GCV indicates low-dose (5 mg/kg once daily) ganciclovir therapy.
[a]Low-risk diseases were defined as acute leukemia and lymphoma in the first or second complete remission, myelodysplastic syndrome in early phases and chronic myelogenous leukemia in the first chronic phase. High-risk diseases were defined as those other than the above.
[b]The matching of HLA-A and -B was confirmed by low-resolution typing methods, and the matching of HLA-DRB1 was confirmed by high-resolution typing methods.

Transplantation procedures and supportive care
Transplantation procedures and supportive care were described previously.[14] The preparative regimen and GVHD prophylaxis are described in Table 1. All patients received 1000 mg per day acyclovir orally from day −3 to day +35 to prevent herpes simplex virus infection. To facilitate neutrophil engraftment, recombinant human G-CSF was administered intravenously at a dose of 5 μg/kg per day from day +1 after CBT. G-CSF administration was discontinued when an ANC increased to more than 3×10^9 per liter irrespective of whether patients were during GCV therapy or not.

Preemptive GCV therapy
CMV infection was monitored using an antigenemia assay twice a week after engraftment. The antigenemia assay consisted of direct immunostaining of polymorphonuclear leukocytes with monoclonal antibodies C10/C11 (Clonab CMV; Biotest, Dreieich, Germany). The results are expressed as the number of antigen-positive cells per 3×10^5 cells on two slides. Preemptive GCV therapy was initiated when one or more positive cells were detected on two slides. GCV was administered intravenously at a dose of 5 mg/kg once daily. GCV therapy was discontinued when neutropenia with an ANC of less than 1×10^9 per liter developed, or when negative results on two or more consecutive tests were obtained. When antigenemia test results worsened after GCV therapy, the dose of GCV was increased to 5 mg/kg twice daily or GCV was changed to foscarnet.

Statistical methods
The frequencies of categorical variables were compared using the Fisher's exact test. The values of antigenemia test results between two groups were compared using the Mann–Whitney *U*-test.

Results

Initial preemptive therapy with GCV 5 mg/kg once daily
Preemptive therapy with GCV 5 mg/kg once daily was initiated at a median of 35 days (range 21–71) after CBT. The median value of the antigenemia test result was two cells (range 1–29) at the initiation of GCV therapy. In 23 of

34 (68%) patients, GCV therapy was completed after consecutive negative results on the antigenemia test (Table 2). In other two (6%) patients, GCV therapy was discontinued because of the development of neutropenia with an ANC of less than 1×10^9 per liter. However, the two patients achieved consecutive negative results on the antigenemia test after the discontinuation of GCV therapy. For these 25 (74%) patients, preemptive therapy with GCV 5 mg/kg once daily led to the resolution of antigenemia. The resolution of antigenemia was first achieved within 6 and 13 days after the initiation of GCV therapy in 10 (29%) and 18 (53%) patients, respectively. The median duration of GCV therapy in these 25 patients was 16 days (range 8–45). In contrast, therapy with GCV 5 mg/kg once daily was discontinued in the remaining nine (26%) patients because of increasing antigenemia values; the GCV dose was increased to 5 mg/kg twice daily in eight patients a median of 11 days (range 5–13) after the initiation of preemptive GCV therapy, and GCV was changed to foscarnet in one patient of 13 days after the initiation of GCV therapy. The reason for choosing foscarnet in this patient was that the ANC decreased to 1.35×10^9 per liter after 11 days of preemptive GCV therapy. After dose-escalation of GCV or changing to foscarnet therapy, consecutive negative results on the antigenemia test were obtained in all nine patients. In eight patients who required dose-escalation of GCV, the total duration of GCV therapy was 24 days (range 21–29).

Recurrence of antigenemia and second GCV therapy

Of the 34 patients who received initial preemptive therapy with GCV, 5 mg/kg once daily, 18 (53%) patients developed antigenemia again after completion of the therapy (Table 2). The median onset was 74 days (range 42–116) after CBT. Among them, four patients who developed antigenemia with a value of only one cell did not receive further antiviral therapy, because antigenemia resolved spontaneously. The second course of preemptive therapy for the remaining 14 (41%) patients was initiated a median of 72 days (range 43–106) after CBT. The median value of the antigenemia test result was three cells (range 1–11) on initiation of the second course of therapy. The antiviral agent was GCV in 12 patients and foscarnet in 2. The GCV dose was 5 mg/kg once daily in all 12 patients. In one patient who received the second course of GCV therapy, GCV was changed to foscarnet 13 days after the initiation of GCV therapy because of an increasing antigenemia value. Positive antigenemia test results in the patient resolved 8 days after the initiation of foscarnet therapy. In the remaining 11 patients, preemptive therapy with 5 mg/kg once daily was completed after consecutive negative results on the antigenemia test. The median duration of GCV therapy in the 11 patients was 15 days (range 11–22). In two patients who received the second course of preemptive therapy with foscarnet, antiviral therapies for 12 and 17 days were completed after consecutive negative results on the antigenemia test. However, 4 of 14 patients received one or two further courses of antiviral therapy for the recurrence of antigenemia.

Table 2 Results of GCV therapy

	No. of patients
Initial PT with LD-GCV	34
Negative antigenemia test results	25 (74%)
Discontinuation after negative results	23 (68%)
Discontinuation after neutropenia	2 (6%)
Increasing antigenemia test results	9 (26%)
Dose-escalation of GCV	8 (23%)
Change to foscarnet	1 (3%)
Recurrence of antigenemia	18 (53%)
Resolution without PT	4 (12%)
Second PT	14 (41%)
LD-GCV	12 (35%)
Foscarnet	2 (6%)
Second PT with LD-GCV	12
Negative antigenemia test results	11 (92%)
Discontinuation after negative results	11 (92%)
Discontinuation after neutropenia	0 (0%)
Increasing antigenemia test results	1 (8%)
Dose-escalation of GCV	0 (0%)
Change to foscarnet	1 (8%)
CMV disease	0 (0%)

Abbreviations: LD-GCV = low-dose (5 mg/kg once daily) ganciclovir; PT = preemptive therapy.

CMV disease

CMV disease did not occur in the 34 patients who initially received preemptive therapy with GCV 5 mg/kg once daily. However, CMV disease occurred in one patient who was excluded from the analysis. The patient had renal dysfunction with a CCR of 25 ml/min and received preemptive therapy with GCV 3 mg/kg once daily for antigenemia from day +22. Because of an increasing antigenemia value, the antiviral agent was changed to foscarnet on day +33 after CBT. Foscarnet therapy was continued after the resolution of antigenemia. CMV meningitis occurred on day +111, which did not respond to GCV therapy and directly caused her death.

Neutropenia after GCV therapy

The incidence of neutropenia after the initial GCV therapy was examined. The median ANC at the initiation of GCV therapy was 1.9×10^9 per liter (range $0.35–6.4 \times 10^9$ per liter). In 17 of 34 (50%) patients, no obvious decrease in the ANC was observed after GCV therapy (Table 3). In the

Table 3 Neutropenia after GCV therapy

	No. of patients
Initial PT with LD-GCV	34
No decrease in ANC	17 (50%)
Any decrease in ANC	17 (50%)
ANC $< 1 \times 10^9$ per liter	12 (35%)
ANC $< 0.5 \times 10^9$ per liter	1 (3%)
Second PT with LD-GCV	12
No decrease in ANC	7 (58%)
Any decrease in ANC	5 (42%)
ANC $< 1 \times 10^9$/ per liter	2 (17%)
ANC $< 0.5 \times 10^9$ per liter	0 (0%)

Abbreviations: LD-GCV = low-dose (5 mg/kg once daily) ganciclovir; PT = preemptive therapy.

remaining 17 patients, the minimum ANC was a median of 0.9×10^9 per liter (range $0.44–2.99 \times 10^9$ per liter) which occurred a median of 21 days (range 7–35) after the initiation of GCV therapy. A total of 12 (35%) patients developed neutropenia with an ANC of less than 1×10^9 per liter. Among them, four of eight (50%) patients who had required dose-escalation of GCV after increasing antigenemia values were included. The median duration of neutropenia was 5 days (range 1–30). A total of 2 μg/kg G-CSF administration was initiated in four patients with ANCs of 0.44, 0.62, 0.7 and 0.92×10^9 per liter. In these patients, the ANC increased to more than 1×10^9 per liter within 2 days after the initiation of G-CSF administration. Neutropenia with an ANC of less than 0.5×10^9 per liter occurred in only one (3%) patient.

Next, the incidence of neutropenia after the second course of GCV therapy was examined. The median ANC at the initiation of GCV therapy was 2.9×10^9 per liter (range $1.23–5.23 \times 10^9$ per liter). In 7 of 12 (58%) patients, no obvious decrease in the ANC was observed after GCV therapy. In the remaining five patients, the minimum ANC was a median of 1.27×10^9 per liter (range $0.67–3.07 \times 10^9$ per liter) which occurred a median of 14 days (range 13–30) after the initiation of GCV therapy. Two (17%) patients developed neutropenia with an ANC of less than 1×10^9 per liter (0.67 and 0.69×10^9 per liter). The durations of neutropenia were 5 and 9 days, respectively.

The association between G-CSF administration at the initiation of GCV therapy and the incidence of neutropenia after GCV therapy was examined. A total of 11 patients still received G-CSF at the initial course of GCV therapy, but none at the second course of therapy. After the initial GCV therapy, neutropenia less than 1×10^9 per liter occurred in 3 of 11 (27%) patients with G-CSF administration, and 9 of 24 (37%) patients without G-CSF administration ($P = 0.42$).

No patients developed neutropenic fever or infection after the initial and second courses of GCV therapy. Secondary graft failure did not occur. There were no deaths directly attributable to GCV therapy.

Thrombocytopenia after GCV therapy

At the initiation of GCV therapy, 24 of 34 (71%) patients had a platelet count of less than 20×10^9 per liter and still required platelet transfusions. In the remaining 10 (29%) patients, a platelet count was between 20 and 50×10^9 per liter in two patients, between 50 and 100×10^9 per liter in six patients, and more than 100×10^9 per liter in two patients. No patients showed obvious decrease in a platelet count during and after GCV therapy. In 19 of 24 (79%) patients who required platelet transfusions, a platelet count increased more than 20×10^9 per liter without transfusions within 21 days after the initiation of GCV therapy. In all 10 patients who did not require platelet transfusions, the platelet count increased to more than 100×10^9 per liter within 21 days.

During and after the second course of GCV therapy, no patients showed obvious decrease in a platelet count, either. Although only one (8%) patient still required platelet transfusions, his platelet count increased to more than 50×10^9 per liter within 21 days after the initiation of GCV therapy. In four of six (67%) patients with a platelet count between 20 and 50×10^9 per liter, a platelet count increased to more than 50×10^9 per liter within 21 days. A platelet count between 50 and 100×10^9 per liter in four patients and more than 100×10^9 per liter in the remaining one patient increased to more than 100 and 200×10^9 per liter, respectively. These results showed that the impact of preemptive therapy with GCV 5 mg/kg once daily on platelet recovery was relatively mild in patients after CBT.

Factors affecting the response of GCV therapy

The impacts of the severity of antigenemia, acute GVHD and steroid therapy were examined. Between 9 patients who had the increasing antigenemia test results during GCV therapy and 25 patients who did not, the values of an antigenemia test at the initiation of GCV therapy did not differ significantly (median, three cells (range 1–29) and median, two cells (range 1–18), respectively, $P = 0.41$). Between 18 patients who had recurrence of antigenemia after the completion of the initial GCV therapy and 16 patients who did not, the values of an antigenemia test also did not differ (median, three cells (range 1–29) and median, two cells (range 1–7), respectively, $P = 0.82$). Between 18 patients with grade II–IV acute GVHD and 16 patients without it, the probabilities of the increasing antigenemia test results did not differ significantly (28 and 25%, respectively, $P = 0.58$). The probabilities of the recurrence of antigenemia also did not differ (56 and 50%, respectively, $P = 0.51$). Between six patients who received steroid therapy with 0.5 mg/kg prednisolone or more and 28 patients who did not, the probabilities of the increasing antigenemia test results did not differ (50 and 21%, respectively, $P = 0.17$). The probability of the recurrence of antigenemia in patients with steroid therapy were 83%, which also did not differ significantly from that in patients without steroid therapy (46%, $P = 0.11$). However, the reason for the failure to detect significant differences between patients with and without steroid therapy was probably due to the small patient number in this study.

Discussion

In the present study, we examined the efficacy and toxicity of preemptive therapy using GCV 5 mg/kg once daily for CMV infection after unrelated CBT. In the entire cohort of 60 patients, CMV disease occurred in one patient who had severe renal dysfunction and was excluded from this preemptive strategy. However, CMV disease did not occur in 34 patients who received preemptive therapy with GCV 5 mg/kg once daily as the initial induction therapy. Because study patients were selected based on those who did not have possible CMV pneumonia or severe renal dysfunction, preemptive therapy with GCV 5 mg/kg once daily was suggested to be effective for such selected CBT recipients.

The efficacy of preemptive therapy with GCV 5 mg/kg once daily was compared with that in our previous study using conventional preemptive GCV therapy for CBT recipients[12] (Table 4). Within 21 days after the initiation of

Table 4 Comparison of efficacies between the present and previous[15] studies

	Low dose	*Conventional*
No. of patients	34	16
Acute GVHD		
Grade 0–I	16 (47%)	9 (56%)
Grade II–IV	18 (53%)	7 (44%)
Steroid therapy		
No	28 (88%)	10 (63%)
Yes	6 (18%)	6 (37%)
Negative antigenemia test results	25 (74%)	—
Discontinuation after negative results	23 (68%)	15 (93%)
Discontinuation after neutropenia	2 (6%)	1 (7%)
Increasing antigenemia test results	9 (26%)	—
Dose-escalation of GCV	8 (23%)	—
Change to foscarnet	1 (3%)	—
Recurrence of antigenemia	18 (53%)	8 (50%)
Spontaneous resolution	4 (12%)	2 (13%)
Second preemptive therapy	14 (41%)	6 (37%)
CMV disease	0 (0%)	0 (0%)

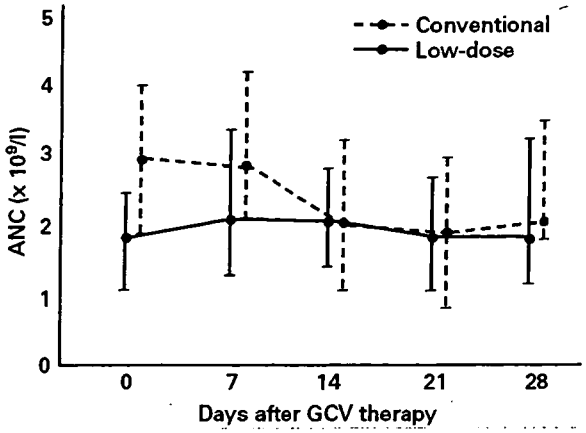

Figure 1 Serial changes in ANCs after ganciclovir (GCV) therapy. ANCs at 0, 7, 14, 21 and 28 days after the initiation of GCV therapy did not differ significantly between low-dose GCV therapy in the present study and conventional GCV therapy in the previous study[12] (Mann–Whitney *U*-test, $P=0.09$, 0.09, 0.53, 0.97 and 0.17, respectively). The dots represent a median ANC, and the horizontal lines represent 25th and 75th percentile.

GCV therapy, antigenemia resolved in 13 of 16 (81%) patients in the previous study and 31 of 34 (91%) patients in the present study. The remaining patients required prolonged administration of GCV. In the present study, nine (26%) patients developed increasing antigenemia values within 14 days after the initiation of GCV therapy and required dose-escalation of GCV or a change to foscarnet therapy. However, all patients in both studies achieved consecutive negative results on the antigenemia test without the development of CMV disease. In the previous study, 8 of 16 (50%) patients developed recurrence of antigenemia and 6 (37%) patients required one or more further courses of GCV therapy. Similarly, in the present study, 18 of 34 (53%) patients developed recurrence of antigenemia and 14 (41%) patients required one or more further courses of GCV therapy. In the entire study cohorts, none of the 28 (0%) patients in the previous study and 1 of the 60 (2%) patients in the present study developed CMV disease after CBT. These results suggest that the efficacy of a preemptive strategy using GCV 5 mg/kg once daily as initial induction therapy is largely equivalent to the conventional preemptive strategy using GCV 5 mg/kg twice daily as an initial induction phase for patients after CBT.

The incidence of neutropenia after preemptive therapy with 5 mg/kg once daily was compared with that after conventional preemptive GCV therapy for CBT patients in our previous study[15] (Figure 1). Neutropenia with an ANC of less than 1 and 0.5×10^9 per liter after initial GCV therapy occurred in 12 (35%) and 1 (3%) of 34 patients, respectively, in the present study, and 9 (53%) and 2 (12%) of 17 patients, respectively, in the previous study. Although the incidences of neutropenia tended to be lower in the present study, statistical analysis did not show significant differences ($P=0.18$ for ANC $< 1 \times 10^9$ liter and 0.25 for ANC $< 0.5 \times 10^9$ per liter). However, the proportion of patients who did not show an obvious decrease in the ANC after GCV therapy in the present study was significantly higher than in the previous study (50 vs 12%, $P<0.01$). In both previous and present studies, no patients developed neutropenic fever or secondary graft failure after GCV therapy. There were no GCV therapy-related deaths in either study. These results suggest that a preemptive strategy using GCV 5 mg/kg once daily is feasible for patients after CBT.

Favorable outcomes of preemptive therapy with GCV 5 mg/kg once daily for BMT and PBSCT recipients were previously reported.[5–7] In previous studies, dose-escalation of GCV or a change to foscarnet therapy was required in 32–39% patients when the initial response was not sufficient. Recurrence of CMV infection after the completion of initial preemptive therapy was observed in 33–43% patients. However, the incidence of CMV disease was less than 10% in all studies. In addition, GCV-related neutropenia occurred in only 3–16% patients. These results suggest that preemptive therapy with GCV 5 mg/kg once daily can reduce the incidence of GCV-related neutropenia but retain the efficacy for preventing CMV disease in BMT and PBSCT recipients.

The reported incidence of CMV infection after CBT ranges from 41 to 58% in CMV-seropositive patients.[11,16] Among 48 CMV-seropositive patients who achieved neutrophil engraftment after CBT, 40 patients (83%) showed positive test results in the present study. This incidence of CMV infection after CBT might be higher than in the previous studies. The remaining eight CMV-seropositive patients including three patients aged more than 40 years, six patients with grade II–IV acute GVHD and two patients in high-risk disease status, did not show positive antigenemia test results within 90 days after CBT. The incidence of documented CMV infection can vary depending on the monitoring strategy.[6] By using sensitive PCR methods instead of antigenemia assays, more frequent CMV infections after CBT would be identified.

The present study suggests that an antigenemia-based preemptive strategy using GCV 5 mg/kg once daily as the initial induction therapy is feasible and effective for CBT recipients. However, the study cohort included only patients who did not have possible CMV pneumonia or severe renal dysfunction. For a patient with possible CMV pneumonia, the initiation of therapy with GCV 5 mg/kg twice daily would be prudent, because of a high mortality of established CMV pneumonia.[1] For a patient with severe renal dysfunction, the GCV dose should be adjusted for the CCR of the patient.[17] Cord blood lymphocytes are functionally and phenotypically immature as compared with adult blood lymphocytes.[18] Therefore, preemptive strategy distinct from that for BMT or PBSCT recipients may be appropriate for CBT recipients. Large-scale studies are needed to determine the efficacy and safety of the preemptive strategy for preventing CMV disease in adult patients after CBT.

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Letter to the Editor

Four cases of donor cell-derived AML following unrelated cord blood transplantation for adult patients: experiences of the Tokyo Cord Blood Bank

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Keywords

cord blood bank, cord blood transplantation, donor cell leukemia.

Donor cell leukemia (DCL) is considered a rare complication following allogeneic BMT, but the actual frequency of DCL has not been specified. Cord blood (CB) is now recognized as an alternative source for stem cell transplantation (SCT), with more than 6000 CBT performed world-wide, and a few cases of DCL following CBT have been reported [1–3]. We report four cases of DCL that developed after unrelated CBT using clinical reports of 478 units available from 596 units shipped by the Tokyo Cord Blood Bank (Tokyo CBB). Two cases out of the four have been already reported elsewhere [2,3]. Tokyo CBB was informed of the development of DCL by the attending physicians of the recipients in CBT centers soon after a definite diagnosis was made. The feedback from CBT centers regarding the four DCL cases is summarized in Table 1. All the donors were well at a follow-up questionnaire of 6–12 months after birth, but further information of their health has not yet been obtained. Notification of DLC to the donors' parents has not yet been done because DLC in the recipient does not always mean occult leukemia in the donor and we do not want to create unnecessary anxiety for the parents.

The etiology of DCL is unclear and a common mechanism is unlikely according to the reported literature [4–6]. Several possibilities exist, including occult leukemia or a pre-leukemic state in the donor, a defect in immune surveillance, therapy-related stromal abnormalities, excess cytokine stimulation and DNA replication and/or repair errors associated with post-transplant expansion of stem/progenitor cells. In this regard, it should be noted that these four cases developed AML, which is relatively rare in childhood acute leukemia [7], suggesting the extrinsic influences include excessive cytokine release, infectious agents and defect immunosurveillance on leukemogenesis in the CBT setting. Nevertheless, the possibility of occult leukemia in the donor raises serious problems regarding the ethical responsibilities of the CBB to the donor. Ethical, but with a scientific background, discussion should be continued regarding the cause of DCL.

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Table 1. Four cases of DCL following CBT

Patient	1	2 [3]	3 [2]	4
Gender	Female	Female	Female	Male
Age (years)	32	32	56	30
CB gender	Male	Female	Male	Female
Disease	AML-M2	AML-M0	ATL	HD
Status at CBT	REL1	REL1	CR1	Stage IVA
Biomarker	AML1-ETO (+)	(−)	HTLV-1(+)	(−)
SCT	1st	1st	2nd	2nd
Regimen	Myeloablative	Myeloablative	Non-myeloablative	Non-myeloablative
TBI	12Gy	12Gy	(−)	2Gy
G-CSF	(+)	(+)	(+)	(+)
GvHD Prophylaxis	CsA + sMTX	CsA + sMTX	FK506 + PSL	CsA + sMTX
aGvHD	II	II	0	III-
cGvHD	(−)	(−)	(−)	Limited
DCL	AML	AML-M2	AML	AML-M5
Onset of DCL	15 months after CBT	11 months after CBT	7 months after CBT	16 months after CBT
Chimerism Diagnosis	Y-probe FISH (PB)	STR (PB)	Y-probe FISH/STR (PB)	STR (PB)
	100% (+)	100% donor type	98.3/100% donor type	100% donor type
Blast	84% in PB	13% in PB	93% in PB	50% in PB
Biomarker	AML-1/ETO (−)		HTLV-1(−)	MLL (+)

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ORIGINAL ARTICLE

Impact of ABO incompatibility on engraftment and transfusion requirement after unrelated cord blood transplantation: a single institute experience in Japan

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The impact of ABO incompatibility between donor and recipient on engraftment and transfusion requirement was studied in 95 adults who underwent unrelated cord blood transplantation (CBT). The patients included 27 ABO-identical, 29 minor, 21 major and 18 bidirectional ABO-incompatible recipients. Neutrophil engraftment did not differ between ABO-identical/minor ABO-incompatible and major/bidirectional ABO-incompatible recipients (hazard ratio (HR) 1.17, $P=0.48$). Cumulative incidence of platelet engraftment in ABO-identical/minor ABO-incompatible recipients was higher than in major/bidirectional ABO-incompatible recipients (HR 1.88, $P=0.013$). In addition, fewer platelet transfusions were required during the first 60 days after CBT in ABO-identical/minor ABO-incompatible recipients (HR 0.80, $P=0.040$). RBC engraftment did not differ between the two groups (HR 1.25, $P=0.33$). However, fewer RBC transfusions were required in ABO-identical/minor ABO-incompatible recipients than in major/bidirectional ABO-incompatible recipients (HR 0.74, $P<0.005$). No patients developed pure red-cell aplasia after CBT. These results indicate that ABO incompatibility affected platelet engraftment and transfusion requirement of RBC and platelet in CBT recipients. Further studies including larger patient numbers are required to elucidate the impact of ABO incompatibility on the clinical outcome of CBT.
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Keywords: ABO incompatibility; transfusion; pure red-cell aplasia; hemolysis; cord blood transplantation

Introduction

The presence of ABO incompatibility between donor and recipient is common in allogeneic hematopoietic SCT. The incompatibility is defined as 'major' when the recipient plasma has isohemagglutinins against donor RBC antigens, and 'minor' when the donor has isohemagglutinins against recipient RBC antigens. When combined features of major and minor incompatibility coexist, the incompatibility is defined as 'bidirectional'. Most previous studies showed no significant effect of major/bidirectional ABO incompatibility on the incidence of graft failure, GVHD, or survival after SCT.[1,2] However, many reports have described the occurrence of pure red-cell aplasia (PRCA) or delayed erythroid engraftment and increased requirement for RBC transfusion in patients after major/bidirectional ABO-incompatible allogeneic bone marrow transplantation or PBSCT.[3–5] In the present study, we investigated the impact of major/bidirectional ABO incompatibility on engraftment and transfusion requirements in adult patients who had undergone the unrelated cord blood transplantation (CBT) at a single institute.

Patients and methods

Patients

Between August 1998 and November 2005, 95 consecutive adult patients underwent unrelated CBT following a conditioning regimen including 12 Gy total body irradiation at The Institute of Medical Science, The University of Tokyo (Table 1). The study patients included 27 ABO-identical, 29 minor, 21 major and 18 bidirectional ABO-incompatible recipients of CBT. Transplantation procedures and supportive care have been described previously.[6] No patients received a conditioning regimen including antithymocyte globulin (ATG). Cyclosporine (CSP, 3 mg/kg/day) with a short course of methotrexate was administered intravenously to prevent acute GVHD in 92 patients, and CSP was given alone to the remaining three patients. To facilitate neutrophil engraftment, recombinant human G-CSF was administered intravenously at a dose of

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Table 1 Patient characteristics

	Total	Identical	Minor	Major	Bidirect
No. of patients	95	27	29	21	18
Age (years)					
Median	39	42	36	39	37
Range	16–55	18–55	16–53	16–53	20–52
Gender					
Male	50	13	18	7	12
Female	45	14	11	14	6
Disease					
AML	60	17	17	15	11
ALL	17	5	5	2	5
CML	4	1	2	0	1
MDS	7	1	3	2	1
NHL	7	3	2	2	0
Disease status					
Low-risk	22	5	10	6	0
High-risk	73	22	19	15	18
CMV serostatus					
Negative	14	4	4	3	3
Positive	81	23	25	18	15
TNC ($\times 10^7$/kg)					
Median	2.4	2.5	2.4	2.4	2.4
Range	1.2–5.3	2.0–3.7	1.6–5.3	1.8–3.4	1.2–4.0
CD34 ($\times 10^5$/kg)					
Median	0.93	1.01	0.9	0.84	1.06
Range	0.15–8.97	0.31–3.61	0.31–8.97	0.17–1.66	0.15–2.14
HLA matching					
5/6 or 4/6	69	14	23	15	17
3/6 or 2/6	26	13	6	6	1
Cryopreserved period (m)					
Median	18	17	19	21	23
Range	4–60	4–60	8–51	11–54	11–38
Preparative regimen					
TBI + CY + AraC	73	19	22	18	14
TBI + CY	11	3	4	1	3
TBI + FLU + AraC	8	3	3	2	0
TBI + FLU + MEL	3	2	0	0	1
Acute GVHD					
Grade 0–I	37	13	8	9	7
Grade II–IV	51	13	19	11	8

Abbreviations: AraC = cytarabine; FLU = fludarabine; MDS = myelodysplastic syndrome; MEL = melphalan; NHL = non-Hodgkin's lymphoma; TBI = total body irradiation; TNC = total nucleated cell.
Identical indicates ABO-identical; Minor, minor ABO-incompatible; Major, major ABO-incompatible; Bidirect, bidirectional ABO-incompatible.

5 μg/kg/day from day +1 after CBT. Anti-CMV high-titer intravenous immunoglobulin at a dose of 10 g was administered twice a month from day −2 until day +100 or longer after CBT.

Transfusion guidelines

Patients were transfused with packed RBCs to maintain a hemoglobin concentration of greater than 8 g/dl, and with packed platelets to maintain a platelet count of greater than 20×10^9/l. All platelet concentrates were prepared by single-donor apheresis. In general, a pack containing 2.0×10^{11} platelets (10 U) or more was used. All packed RBCs were derived from 400 ml blood (2 U). All packed RBCs and platelets transfused were leukocyte-filtered and irradiated to 25 Gy. The ABO type of RBCs and platelets transfused after CBT was determined by the blood groups of donor and recipient as described previously.[7,8] In minor ABO-incompatible recipients, donor-type RBCs and recipient-type platelets were transfused. In major ABO-incompatible recipients, recipient-type RBCs and donor-type platelets were transfused. In bidirectional ABO-incompatible recipients, group-O RBCs and group-AB platelets were transfused. For RBC transfusion in ABO-incompatible CBT, plasma-containing antibody to recipient ABO antigens was removed by washing and centrifugation.

Definitions

Neutrophil engraftment was defined as the day of achieving an ANC greater than 0.5×10^9/l. for 3 consecutive days. Platelet engraftment was defined as the day of achieving a platelet count greater than 20×10^9/l for 3 consecutive days without transfusion. RBC engraftment was defined as the day of achieving a reticulocyte count greater than 1% for 3 consecutive days.

High-risk diseases were defined as acute leukemia and lymphoma in more than the first complete remission, Philadelphia chromosome-positive acute lymphoblastic leukemia in any phases, myelodysplastic syndromes in advanced phase, and chronic myelogenous leukemia in more than the first chronic phase. Low-risk diseases were defined as other than the above. HLA-A and -B matching was confirmed by low-resolution typing methods and HLA-DRB1 matching was confirmed by high-resolution typing methods.

Statistical analysis

The cumulative incidence of engraftment was estimated in a competing risks setting, death and relapse being treated as competing risks.[9] In multivariate analysis, a Cox proportional hazards model was used to assess the independent effect of risk factors on engraftment and transfusion requirement after CBT. We used the stepwise variable selection procedures with a significance level of 5%. Other than ABO incompatibility, the following factors were studied: age (less than 40 years versus 40 years or more), gender (male versus female), disease status (low-risk versus high-risk), pretransplant CMV serostatus (negative versus positive), total nucleated cell (TNC) dose (less than 2.5×10^7/kg versus 2.5×10^7/kg or more), CD34-positive cell dose (less than 1.0×10^5/kg versus 1.0×10^5/kg or more), HLA-matching (5- or 4-match versus 3- or 2-match), cryopreserved period of grafts (less than 24 months versus 24 months or more), and severity of acute GVHD (grade 0–I versus II–IV).

Results

Neutrophil engraftment

Of 95 patients who underwent CBT, three developed autologous hematopoietic recovery after CBT, and other four patients died before neutrophil recovery. Successful neutrophil engraftment was achieved in the remaining

88 patients at a median of 22 (range: 16–46) days after CBT. In patients with ABO-identical, minor, major and bidirectional ABO-incompatible CBT, the cumulative incidences of neutrophil engraftment at 60 days after CBT were 93, 93, 90 and 83%, respectively (Figure 1a, Table 2). The median days to neutrophil engraftment were 22, 23, 22 and 22, respectively. The cumulative incidence of neutrophil engraftment in ABO-identical/minor ABO-incompatible CBT did not differ significantly from that in major/bidirectional ABO-incompatible CBT (median day, 22 versus 22; cumulative incidence, 95 versus 90%; hazard ratio (HR) 1.17; $P=0.48$) (Figure 1b, Table 3). The smaller CD34-positive cell dose was significantly associated with delayed neutrophil engraftment after CBT (HR 0.65, $P=0.049$) (Table 3). Instead of an ANC of 0.5×10^9/l, when the days of achieving an ANC greater than 1.0×10^9/l for 3 consecutive days were compared, the cumulative incidence in ABO-identical/minor ABO-incompatible CBT also did not differ significantly from that in major/bidirectional ABO-incompatible CBT (median day, 25 versus 24; cumulative incidence, 95 versus 90%; HR 1.15; $P=0.52$).

Platelet engraftment and transfusion

Of the 88 patients with neutrophil engraftment, three patients relapsed and two patients died without relapse, before platelet engraftment. Platelet engraftment was achieved in the remaining 83 patients at a median of 40 (range: 22–99) days after CBT. In patients with ABO-identical, minor, major and bidirectional ABO-incompatible CBT, the cumulative incidences of platelet engraftment at 90 days after CBT were 89, 90, 86 and 61%, respectively (Figure 2a, Table 2). The median days of platelet engraftment were 39, 41, 42 and 59, respectively. The cumulative incidence of platelet engraftment in ABO-identical/minor ABO-incompatible CBT was significantly higher than that in major/bidirectional ABO-incompatible CBT (median day, 40 versus 42; cumulative incidence, 91 versus 74%; HR 1.88; $P=0.013$) (Figure 2a, Table 3). Pretransplant CMV seropositivity and the smaller CD34-positive cell dose were also isolated risk factors

Table 2 Results

	Identical	*Minor*	*Major*	*Bidirect*
Engraftment[a]				
Neutrophil				
Cumulative incidence (%)	93	93	90	83
95% CI	81–100	82–100	76–100	63–100
Platelet				
Cumulative incidence (%)	89	90	86	61
95% CI	75–100	77–100	69–100	37–86
RBC				
Cumulative incidence (%)	89	90	90	72
95% CI	76–100	77–100	76–100	50–95
Transfusion requirement[b]				
Platelet				
Average (units/kg)	9.4	7.6	11.0	9.8
95% CI	7.5–11.2	6.6–8.5	8.3–13.8	7.8–11.7
RBC				
Average (units/kg)	0.54	0.40	0.64	0.61
95% CI	0.43–0.65	0.33–0.47	0.50–0.78	0.46–0.77

Abbreviation: CI = confidence interval.
[a]The cumulative incidence of neutrophil engraftment was estimated at 60 days after cord blood transplant (CBT). The cumulative incidences of platelet and RBC engraftment were estimated at 90 days after CBT.
[b]Transfusion requirement during the first 60 days after CBT.

Table 3 Multivariate analyses of risk factors for engraftment after CBT

	Hazard ratio	*95% CI*	*P-value*
Neutrophil engraftment at 60 days			
ABO identical/minor	1.17	0.75–1.82	0.48
CD34 ($<1.0 \times 10^5$/kg)	0.65	0.42–0.99	0.049
Platelet engraftment at 90 days			
ABO identical/minor	1.88	1.14–3.09	0.013
CMV seropositivity	0.38	0.20–0.72	<0.005
CD34 ($<1.0 \times 10^5$/kg)	0.58	0.36–0.92	0.021
RBC engraftment at 90 days			
ABO identical/minor	1.25	0.80–1.94	0.33
CMV seropositivity	0.47	0.25–0.87	0.017
CD34 ($<1.0 \times 10^5$/kg)	0.61	0.38–0.95	0.030

Abbreviation: CBT = cord blood transplant.

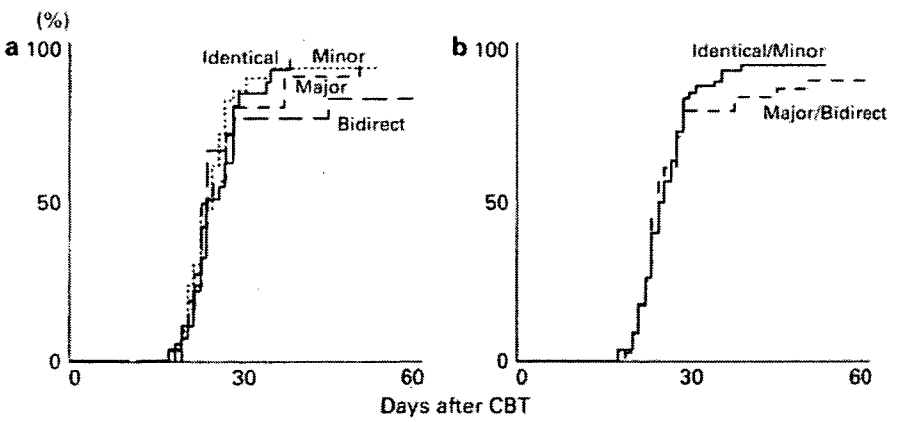

Figure 1 Cumulative incidences of neutrophil engraftment after cord-blood transplant (CBT) in patients receiving ABO-identical, minor, major, and bidirectional ABO-incompatible grafts (a), and ABO-identical/minor ABO incompatible and major/bidirectional ABO-incompatible grafts (b). Identical, ABO-identical; minor, minor ABO-incompatible; major, major ABO-incompatible; bidirect, bidirectional ABO-incompatible.

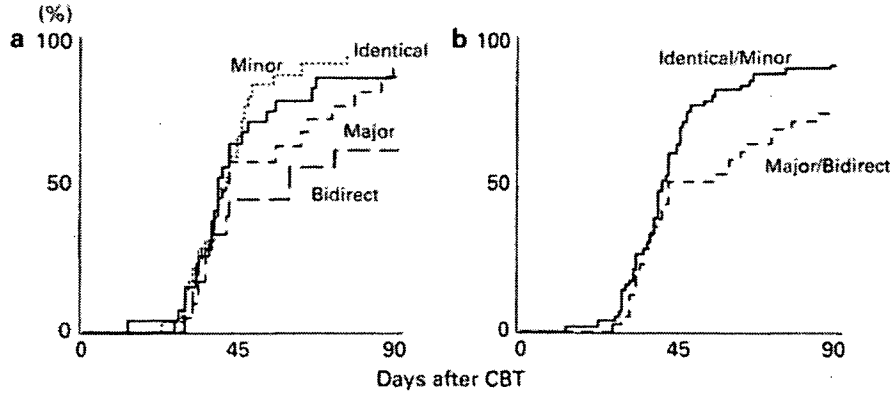

Figure 2 Cumulative incidences of platelet engraftment after CBT in patients receiving ABO-identical, minor, major, and bidirectional ABO incompatible grafts (a), and ABO-identical/minor ABO-incompatible and major/bidirectional ABO-incompatible grafts (b).

for delayed platelet engraftment after CBT (HR 0.38, $P<0.005$, and HR 0.58, $P=0.021$, respectively) (Table 3). Instead of a platelet count of 20×10^9/l, when the days of achieving a platelet count greater than 50×10^9/l for 3 consecutive days without requiring a transfusion were compared, the cumulative incidence in ABO-identical/minor ABO-incompatible CBT did not differ significantly from that in major/bidirectional ABO-incompatible CBT (median day, 48 versus 48; cumulative incidence, 87 versus 68%; HR 1.27; $P=0.31$).

Of the 88 patients with neutrophil engraftment, two relapsed and another died without relapse within 60 days after CBT. Two patients who developed massive bleeding died within 60 days after CBT, and were therefore not included in further analysis. In the remaining 85 patients, the requirement for platelet transfusion was compared after adjusting for patient body weight. In patients with ABO-identical, minor, major and bidirectional ABO-incompatible CBT, the average units of platelets transfused during the first 60 days after CBT were 9.4, 7.6, 11.0 and 9.8 U/kg, respectively (Table 2). The requirement for platelet transfusion in ABO-identical/minor ABO-incompatible recipients was significantly lower than in major/bidirectional ABO-incompatible recipients (average, 8.5 versus 10.5 U/kg; HR 0.80; $P=0.040$) (Table 4).

RBC engraftment and transfusion

Of the 88 patients with neutrophil engraftment, one patient relapsed and another died without relapse before RBC engraftment. In the remaining 86 patients, RBC engraftment was achieved at a median of 33 (range: 21–96) days after CBT. In patients with ABO-identical, minor, major and bidirectional ABO-incompatible CBT, the cumulative incidences of RBC engraftment at 90 days after CBT were 89, 90, 90 and 72%, respectively (Figure 3a, Table 2). The median days of RBC engraftment were 35, 32, 35 and 33, respectively. The cumulative incidence of RBC engraftment in ABO-identical/minor ABO-incompatible CBT did not differ significantly from that in major/bidirectional ABO-incompatible CBT (median day, 33 versus 33; cumulative incidence, 91 versus 85%; HR 1.25; $P=0.33$)

Table 4 Multivariate analyses of risk factors for transfusion requirement after CBT

	Hazard ratio	*95% CI*	P-*value*
Platelet transfusion requirement			
ABO identical/minor	0.80	0.68–0.99	0.040
RBC transfusion requirement			
ABO identical/minor	0.74	0.64–0.90	<0.005
CMV seropositivity	1.49	1.11–1.87	0.013

Abbreviation: CBT = cord blood transplant.

(Figure 3b, Table 3). Pretransplant CMV seropositivity and the smaller CD34-positive cell dose were also isolated risk factors for delayed RBC engraftment after CBT (HR 0.47, $P=0.017$ and HR 0.61, $P=0.030$, respectively) (Table 3).

The requirement of RBC transfusion was then studied in 85 patients who did not develop massive bleeding and survived more than 60 days after CBT. In patients with ABO-identical, minor, major and bidirectional ABO-incompatible CBT, the average units of RBCs transfused during the first 60 days after CBT were 0.54, 0.40, 0.64 and 0.61 U/kg, respectively (Table 2). The requirement for RBC transfusion in ABO-identical/minor ABO-incompatible CBT was significantly lower than that in major/bidirectional ABO-incompatible CBT (average, 0.47 versus 0.63 U/kg, HR 0.74, $P<0.005$) (Table 3). Pretransplant CMV seropositivity was also an isolated risk factor for increased RBC transfusion after CBT (HR 1.49, $P=0.013$) (Table 4).

In seven patients, more than 50 days were required for RBC engraftment after CBT. These patients included one ABO-identical, two minor, three major and one bidirectional ABO-incompatible recipient. All seven patients had not achieved platelet engraftment on the day of RBC engraftment. In six of the seven patients, neutrophil engraftment was also delayed to more than 30 days after CBT. Therefore, no patients were considered as having developed PRCA after CBT. In addition, no patients developed delayed massive immune hemolysis after CBT.

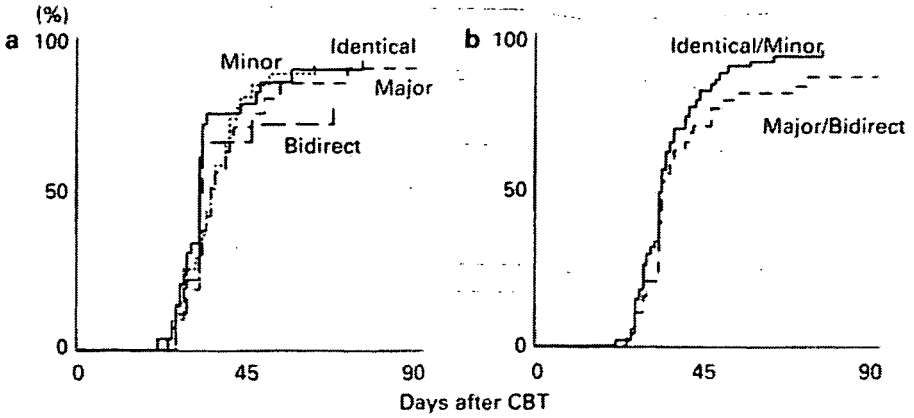

Figure 3 Cumulative incidences of RBC engraftment after CBT in patients receiving ABO-identical, minor, major, and bidirectional ABO-incompatible grafts (**a**), and ABO-identical/minor ABO-incompatible and major/bidirectional ABO-incompatible grafts (**b**).

Discussion

In the present study, we investigated the impact of ABO incompatibility on engraftment and transfusion requirement after CBT. Platelet engraftment was significantly delayed in major/bidirectional ABO-incompatible CBT, with a significantly increased requirement for platelet transfusion. In addition, although RBC engraftment in major/bidirectional ABO-incompatible CBT was not significantly delayed, the requirement of RBC transfusion was more significantly increased than with ABO-identical/minor ABO-incompatible CBT. However, no patients developed PRCA after CBT.

Many previous studies have reported the occurrence of PRCA or delayed RBC engraftment and the increased requirement of RBC transfusion in patients with major/bidirectional ABO-incompatible SCT.[1,2] This phenomenon is due to the presence of recipient isoagglutinins directed at ABH antigens on donor-derived erythroid precursor cells.[3,10] Isoagglutinins are also infused with the administration of anti-CMV high-titer immunoglobulin. In our study of patients receiving ABO-identical grafts, the requirement for RBC transfusion in blood group-O recipients was not significantly different from that in blood group-A recipients ($P=0.79$), suggesting that the influence of isoagglutinins infused with anti-CMV high-titer immunoglobulin products on transfusion requirement might be minimal. The incidence of PRCA in patients with major/bidirectional ABO-incompatible SCT may vary depending on stem cell source (bone marrow cells or G-CSF-mobilized peripheral blood stem cells), conditioning regimen (myeloablative or nonmyeloablative) and type of GVHD prophylaxis (cyclosporine, with or without methotrexate or T-cell depletion).[1,11] For prolonged PRCA after SCT, the efficacy of various therapies such as erythropoietin,[12] ATG,[13] donor lymphocyte infusion[14] and anti-CD20 monoclonal antibody[15] was reported. However, our CBT recipients with a myeloablative conditioning regimen did not develop PRCA or require such specific therapies.

Our results showed that platelet engraftment was significantly delayed in major/bidirectional ABO-incompatible CBT, with a significantly increased requirement of platelet transfusion. Badros *et al.*[16] also showed that major/bidirectional ABO incompatibility led to delayed platelet engraftment and an increased requirement for platelet transfusion in patients who underwent PBSCT using a nonmyeloablative conditioning regimen. Although ABH antigens are also expressed on platelets,[17,18] most of the previous studies did not show an association between major/bidirectional ABO incompatibility and delayed platelet engraftment or increased platelet transfusion requirement after SCT. The reason for the delayed platelet engraftment in major/bidirectional ABO-incompatible CBT could not be clearly explained.

Most previous studies have shown no significant effect of major/bidirectional ABO incompatibility on survival after SCT. However, some investigators have shown that recipients of bidirectional ABO-incompatible SCT had a lower survival rate.[19,20] In the present study, because all patients with bidirectional ABO-incompatible CBT had high-risk diseases (Table 1), we could not obtain a clear result regarding an association between bidirectional ABO incompatibility and survival after CBT (data not shown). Further studies including larger patient numbers are required to elucidate the impact of ABO incompatibility on clinical outcome of CBT.

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Letter to the Editor

Early-Onset Pulmonary Complication Showing Organizing Pneumonia Pattern following Cord Blood Transplantation in Adults

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Bronchiolitis obliterans organizing pneumonia (BOOP) is a well-known pulmonary complication after hematopoietic stem cell transplantation (SCT) [1-4]. BOOP generally occurs approximately 100 days or later after SCT. We describe 4 patients who developed a pulmonary disorder with a histologic pattern of OP in the early period after cord blood transplantation (CBT).

Patient 1 was a 32-year-old man with acute myelogenous leukemia. In October 2003, he received 2 antigen–mismatched CB grafts that contained 2.11×10^7/kg total nucleated cells (TNCs) before freezing. The conditioning regimen included 12 Gy total body irradiation (TBI), 120 mg/kg cyclophosphamide, and 12 g/m^2 cytarabine, along with granulocyte colony-stimulating factor [5]. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate. A neutrophil count consistently greater than 500/μL (neutrophil engraftment) was achieved on day +21. Grade II acute GVHD involving the skin occurred from day +23. On day +32, the patient presented with cough, dyspnea, and fever. An arterial blood gas analysis showed a PO_2 of 57.3 mm Hg and a PCO_2 of 37.2 mm Hg. Chest computed tomography (CT) scans showed a diffuse ground-glass opacity in the lungs (Figure 1A). No causative infectious agents were identified in bronchoalveolar lavage fluid (BALF). Transbronchial lung biopsy (TBLB) specimens obtained on day +33 showed a histologic pattern of OP (Figure 2A). On day +34, we initiated prednisolone therapy (1 mg/kg per day), which led to rapid improvement of the symptoms. CT scans on day +57 showed almost complete resolution of the lesions. The patient is currently well without pulmonary symptoms.

Patient 2 was a 35-year-old man with myelodysplastic syndrome. In August 2004, he received 2 antigen–mismatched CB grafts containing 2.39×10^7/kg TNCs. Conditioning and GVHD prophylaxis were the same as for patient 1. Neutrophil engraftment was achieved on day +30. Grade I acute GVHD involving the skin occurred but spontaneously resolved. On day +60, the patient presented with fever without cough and dyspnea. Arterial blood PO_2 and PCO_2 values were 73.6 mm Hg and 39.3 mm Hg, respectively. CT scans on day +62 showed patchy consolidation in the lungs (Figure 1B). No causative infectious agents were identified in the BALF. TBLB specimens taken on day +63 showed the OP pattern (Figure 2B). On day +64, prednisolone therapy (0.5 mg/kg per day) was initiated. CT scans on day +69 showed that the lung lesions were tending to resolve. Because of leukemia relapse, we discontinued cyclosporine administration and reduced the prednisolone dosage on day +82. The consolidation in the lungs did not completely resolve. The patient died of relapse on day +195.

Patient 3 was a 46-year-old man with myelodysplastic syndrome. In June 2005, he received 2 antigen–mismatched CB grafts containing 2.36×10^7/kg TNCs. The patient also had pulmonary alveolar proteinosis, as reported previously [6].

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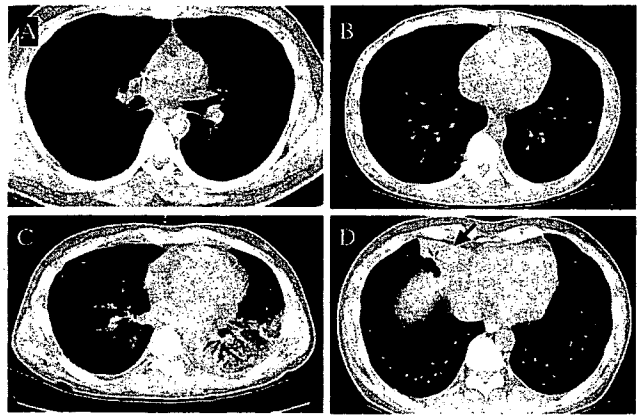

Figure 1. Chest computed tomography (CT) scans at the onset of organizing pneumonia. A, Chest CT scan on day +32 in patient 1. Diffuse ground-glass opacity in the lungs is shown. B, Chest CT scan on day +62 in patient 2. Patchy consolidation in the lungs is evident. C, Chest CT scan on day +50 in patient 3. Extensive consolidation is shown with air bronchograms of the left lung. D, Chest CT scan on day +49 in patient 4. Extensive consolidation is evident with air bronchograms of the right lung.

Conditioning and GVHD prophylaxis were the same as described above. Neutrophil engraftment was achieved on day +34. Grade II acute GVHD involving the skin occurred on day +18 but spontaneously resolved. On day +49, the patient presented with dyspnea and fever without cough. Arterial blood PO_2 and PCO_2 values were 60.3 mm Hg and 31.2 mm Hg, respectively. CT scans on day +50 showed extensive consolidation with air bronchograms of the left lung (Figure 1C). Cytomegalovirus DNA was detected in BALF at 600 copies/mL (normal range, <200 copies/mL), but other infectious agents were not identified. TBLB specimens examined on day +51 showed an OP pattern (Figure 2C). Specific staining did not suggest cytomegalovirus infection. On day +53, we initiated prednisolone therapy (2 mg/kg per day), which led to remarkable improvement of the symptoms. CT scans on day +81 showed almost complete resolution of the consolidation. The patient is currently well without pulmonary symptoms.

Patient 4 was a 38-year-old man with acute lymphoblastic leukemia. In August 2006, he received 2 antigen–mismatched CB grafts containing 1.87×10^7/kg TNCs. The conditioning regimen included 12 Gy TBI, 120 mg/kg cyclophosphamide, and 12 g/m^2 cytarabine. GVHD prophylaxis was the same as described above. Neutrophil engraftment was achieved on day +24. Grade II acute GVHD involving the skin occurred on day +31 but spontaneously resolved. On day +45, the patient presented with cough and fever. Arterial blood PO_2 and PCO_2 values were 75.4 mm Hg and 38.9 mm Hg, respectively. CT scans on day +49 showed extensive consolidation with air bronchograms of the right lung (Figure 1D). Cytomegalovirus DNA was detected in BALF at 200 copies/mL, but other infectious agents were not identified. TBLB specimens taken on day +53 showed a typical OP pattern, as manifested by fibrous-plug formation (Figure 2D). Specific staining did not suggest cytomegalovirus infection. We initiated prednisolone therapy (1 mg/kg per day) on day +54, which led to improvement of the symptoms. CT scans on day +77 showed substantial resolution of the consolidation. The patient is well without pulmonary symptoms.

BOOP is a clinicopathologic syndrome [7]. BOOP without identifiable causes is also termed cryptogenic OP (COP) [8]. The characteristic histologic feature is the presence of buds of fibrous granulation tissue in the distal airspaces [7-9]. TBLB specimens from our patients showed an OP pattern. The typical histologic feature of BOOP or COP was observed in patient 4; however, the degrees of organization in the alveoli were mild in patients 2 and 3, suggesting that OP in the patients might be in the early or immature stages.

Previous studies showed an association between chronic GVHD and BOOP after SCT [2-4]. In addition, Freudenberger et al indicated that prior occurrence of acute GVHD was associated with the subsequent development of BOOP [2]. In our study, OP in patient 1 occurred concomitantly with the presence of acute GVHD, but OP in patients 2 to 4 occurred after the resolution of acute GVHD. Later, limited-type chronic GVHD occurred in patients 1 and 4, and extensive-type chronic GVHD occurred in patient 3. Although the role of alloimmunity in the development of OP was not determined in our patients, steroid therapy resolved the pulmonary lesions in all of the patients to varying degrees.

BOOP is generally recognized as a late complication in SCT patients [1,2]. Of 112 adult patients in our institution who underwent CBT following a conditioning regimen containing

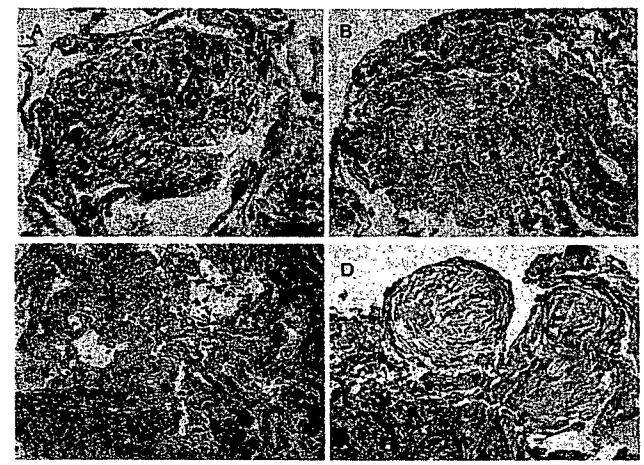

Figure 2. Microscopical features of organizing pneumonia (OP) in transbronchial lung biopsy (TBLB) specimens. A, TBLB specimen obtained from patient 1 on day +33 (hematoxylin and eosin [H&E], original magnification ×200). The TBLB specimen shows the accumulation of foamy macrophages and granulation tissue with fibroblasts in alveolar spaces. B, TBLB specimen obtained from patient 2 on day +63 (H&E, original magnification ×100). The OP pattern observed in patient 1 is evident. C, TBLB specimen obtained from patient 3 on day +51 (H&E, original magnification ×100). The OP pattern observed in patients 1 and 2 is evident. D, TBLB specimen obtained from patient 4 on day +53 (H&E, original magnification ×200). The TBLB specimen shows an intraluminal fibrous plug typical of OP.

12 Gy TBI, OP was histologically diagnosed in 7 patients. The 4 patients in the present study showed an OP pattern on days +33, +51, +53, and +63 after CBT. The remaining 3 patients developed OP on days +257, +432, and +636 after CBT. At the onset of late OP, 1 patient had limited-type chronic GVHD after the occurrence of grade I acute GVHD, and 2 patients had extensive-type chronic GVHD after the occurrence of grade II acute GVHD. We identified no obvious differences in clinical features between early-onset and late-onset OP in our patients after CBT. This study has shown that OP can occur during very early periods after CBT. The features of BOOP after CBT, including the association of GVHD, should be investigated further in a large number of patients.

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