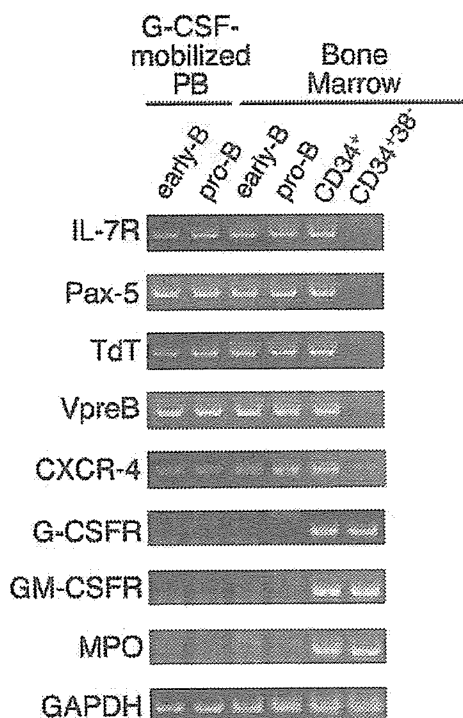

FIGURE 6. Differential expression of hematopoiesis-affiliated genes in BM and G-CSF-mobilized lymphoid progenitors as shown by RT-PCR analysis. BM $CD34^+$ cells were used as controls. In both BM and G-CSF-mobilized PB, lymphoid progenitors expressed lymphoid-affiliated genes but not myeloid-affiliated genes. MPO, myeloperoxidase; GM-CSFR, GM-CSF receptor.

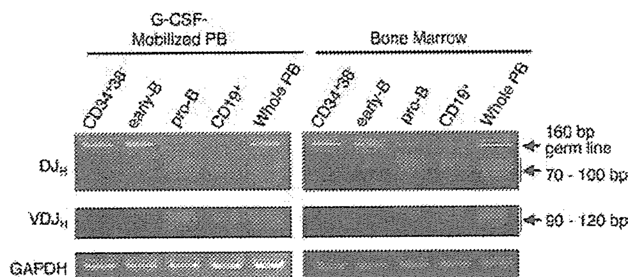

FIGURE 7. PCR analysis of DJ_H and VDJ_H genes rearrangement on DNA from BM and G-CSF-mobilized lymphoid progenitors. $CD34^-CD19^+$ cells were used as controls. None of the Ig genes rearrangements was observed in $CD34^+CD38^-$ cells. Partial DJ_H rearrangement initiated at the stage of $CD34^+CD10^+CD19^-$ early B cells followed by the rearrangement of VDJ_H genes at $CD34^+CD10^+CD19^+$ pro-B cells along with B cell development pathway. G-CSF-mobilized B lymphoid progenitor displayed the same pattern of Ig rearrangement status as its BM counterpart.

than their BM counterparts under physiological conditions, or might be *trans*-differentiated from other lineages. In our analysis, expression of B lineage-specific differentiation programs was preserved, and no myeloid genes were activated in G-CSF-mobilized lymphoid progenitors. By limiting dilution assay, we also demonstrated that the B cell differentiation potential of G-CSF-mobilized lymphoid progenitors was equivalent to that of their BM counterparts. Thus, G-CSF can mobilize B lymphoid progenitors without loss or alteration of the original characteristics of B lymphoid progenitors in BM. For that reason, B lymphoid progenitors, as opposed to all $CD34^+$ cells or myeloid cells, represent a good population for analysis of mechanisms of G-CSF-induced mobilization, because, lacking the receptor, lymphoid progenitors would be less affected by G-CSF signals during mobilization.

Recent insights have increased understanding of the important role of the BM microenvironment, or niche, in retention and development of HPC within the BM. Regulation of cell-fate determination and trafficking of the primitive HPC may be governed by complex interactions between HPC and the surrounding BM niche (47, 48). As discussed above, SDF-1/CXCR-4 signaling is crucial for retention of B lymphoid progenitors within the BM, which can support further B cell development within the BM microenvironment (22, 39, 40). However, whether G-CSF can change BM microenvironments themselves to promote or inhibit HPC mobilization remains largely unknown. Our findings indicated that G-CSF can mobilize cell populations that do not possess G-CSFR from the BM into the circulation. Accordingly, G-CSF-mobilized blood cells can include a variety of populations such as mesenchymal stem and progenitor cells, which can differentiate into nonhematopoietic cells such as vascular endothelial cells, cardiac muscle cells, and hepatocytes. Such mobilized blood cells conceivably might serve as a therapeutic agent in the treatment of various degenerative disorders as opposed to BM cells as a stem cell source (49). Up to now, G-CSF has been the HPC mobilizer of choice in clinical settings, based upon its potency and safety. However, poor mobilization has been reported in ~10–20% of healthy donors, representing a major problem (50, 51). To address these unresolved issues, further investigation of mechanisms of G-CSF-induced mobilization may lead to more effective and safer mobilization methods and agents, and clarify the usefulness of G-CSF-mobilized PB cells as an alternative source of a variety of cells for regenerative medicine.

In summary, our data provide further evidence for an indirect effect of G-CSF on human HPC mobilization by demonstrating mobilization of lymphoid progenitors. Lineage-independent modulation of adhesion molecules such as VLA-4 and CXCR-4 might be involved in G-CSF-induced mobilization. These findings suggest that G-CSF can mobilize not only HPC but also nonhematopoietic cells residing in the BM by indirect effects involving multiple *trans*-acting signals that affect cell interactions with the marrow microenvironment.

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Disclosures

The authors have no financial conflict of interest.

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Cidofovir for treating adenoviral hemorrhagic cystitis in hematopoietic stem cell transplant recipients

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Summary:

Adenovirus (AdV) infection is an important cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients. We treated 16 patients with AdV hemorrhagic cystitis (HC) following HSCT with cidofovir (CDV; 1 mg/kg/day, three times weekly for 3 weeks). Patients included 10 males and six females with a median age of 50 years (range 10–62). Two of the 16 patients were unevaluable because of early death from nonadenoviral causes. CDV therapy cleared AdV from urine in 12 of 14 patients (86%). Of 14 patients, 10 (71%) showed clinical improvements in HC. Among 14 patients, seven (50%) had avoided renal damage, the most important CDV toxicity. One patient previously treated with foscarnet for cytomegalovirus (CMV) required hemodialysis, and CDV treatment was discontinued. In another patient, CDV treatment was discontinued because of grade 2 nephrotoxicity. Four patients became positive for CMV antigenemia while being treated with CDV, and two developed herpes simplex virus (HSV) stomatitis while being treated with CDV. CDV proved effective in treating AdV HC in transplant patients. However, CDV at 1 mg/kg/day given three times weekly failed to prevent breakthrough infection with CMV and HSV in some patients.
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Adenovirus (AdV) infections including hemorrhagic cystitis (HC)[1–3] are emerging as life-threatening complications in hematopoietic stem cell transplant patients.[4] Reported occurrence rates of AdV infection complicating allogeneic hematopoietic stem cell transplantation (HSCT) vary from 5 to 21%,[1,5–8] and reported mortality rates have ranged from 7.7 to 38%.[6,9–11]

For treatment of AdV infection, reduction of immunosuppression[8] or infusion of donor lymphocytes[12] have been proposed. However, since AdV infections often occur in the presence of severe graft-versus-host disease (GVHD), immunotherapy may not be feasible. While specific anti-AdV therapy is therefore needed, no presently available drug has been proven to be effective, although some treatment success with ganciclovir (GCV),[13] vidarabine (AraA)[14] and ribavirin[15,16] have been reported. Unfortunately, these results could not be reproduced.[17] Cidofovir (CDV), a monophosphate nucleotide analogue of cytosine that inhibits viral DNA polymerase, demonstrates *in vitro* and *in vivo* activity against several viruses including herpesviruses, AdV, papilloma viruses, polyoma viruses, and poxvirus.[18] Several reports have described the effectiveness of CDV in post-transplant AdV disease.[19,20] The dose-limiting toxicity of intravenous CDV, when given at the recommended dose of 5 mg/kg once weekly, is nephrotoxicity.[18] Recently, a smaller, more frequent dose of CDV, 1 mg/kg/day three times weekly, demonstrated efficacy for treatment of post-transplant AdV infection.[20] In the present study, we have prospectively evaluated both toxicity and efficacy of CDV treatment for AdV HC in transplant patients. In all, 16 transplant patients were treated with CDV at a dose of 1 mg/kg, three times weekly for 3 weeks.

Materials and methods

Diagnosis of AdV HC

To exclude regimen-related HC, only patients who developed macroscopic hematuria with clinical signs of cystitis newly appearing *de novo* at least 10 days after HSCT and also had no tendency toward generalized bleeding or bacteriuria were considered to have HC.[21] According to previously reported criteria with minor modifications,[22] the severity of HC was graded as mild, sustained microscopic

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hematuria; moderate, gross hematuria and dysuria without clots; severe, gross hematuria and dysuria with clots. At the onset of HC, a urine specimen was obtained for viral culture and polymerase chain reaction (PCR). For rapid diagnosis, immunochromatography was performed (Adenocheck; Santen, Osaka, Japan). All patients underwent all the three diagnostic modalities (viral culture, PCR, and immunochromatography). When AdV was detected by one or more of these methods, a diagnosis of AdV HC was made.

Viral culture from urine

A 2 ml volume of urine was centrifuged overnight at 20 000 *g* and the sediment was added to culture of Hep-2 cells for up to 4 weeks. When a cytopathic effect of viral infection was observed, viral species were identified using monoclonal antibodies against AdV. Viral culture was carried out before the initiation of CDV treatment and 1 week after the last dose of CDV. The clearance of AdV was defined as the negative viral culture after treatment.

PCR of urine samples

After 2 ml of a urine sample was centrifuged at 15 000 *g* for 1 h at 4°C, the sediment was resuspended in 100 μl of PBS. DNA was purified using QIAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Next, 5 μl of purified DNA was subjected to PCR assay using a GeneAmp Kit and a GeneAmp PCR System 9600 (Perkin-Elmer, Boston, MA, USA). Primers used to screen for AdV infection were AD185S (5′-tccagcaacttcatgtccatgg-3′) and AD 185A (5′-tcgatgacgccgcggt-3). The size of the final products was confirmed by 3% agarose gel electrophoresis.[21]

Patient characteristics

In total, 16 patients were treated with CDV (10 males and six females with a median age of 50 years, ranging from 10 to 62). All patients had AdV HC. In all, 14 patients underwent allogeneic HSCT for acute myelogenous leukemia (AML) (n = 1), acute lymphoblastic leukemia (ALL) (n = 2), adult T-cell leukemia/lymphoma (ATL) (n = 2), chronic myelogenous leukemia (n = 1), myelodysplastic syndrome (MDS) (n = 1), multiple myeloma (MM) (n = 2), malignant lymphoma (ML) (n = 1), and severe aplastic anemia (SAA) (n = 3). Two other patients with systemic sclerosis underwent CD34+ cell autologous HSCT. Among allogeneic transplants, sources of stem cells were as follows: three from HLA-identical family donors, one from a DR-mismatch family donor, two from haplo-identical family donors, five from unrelated donors, and three from unrelated cord bloods. Patients No. 3 and no. 12 received antithymocyte globulin (ATG) as part of conditioning (Table 1).

Patient No. 2 died of cerebral infarction 5 days after initiation of CDV treatment, while patient No. 13 died from fungal pneumonia 11 days after initiation of CDV treatment. As these two patients with early death unrelated to AdV were excluded from analysis, 14 patients were evaluable. All patients received immunosuppressive therapy including cyclosporine, tacrolimus, and a steroid, as shown in Table 2. Serotypes of AdV isolated from urine were type 11 (11 patients), type 35 (one patient), or not determined (two patients). Onset of AdV HC ranged from 17 to 142 days after post transplantation (median, 37). Intervals between the onset of AdV HC and CDV treatment ranged from 0 to 56 days (median, 3). Two patients received AraA for treatment of AdV HC (Table 2).

CDV treatment

All of the patients gave their written informed consent in accordance with the requirements of the Institutional Review Board. The treatment regimen consisted of CDV, 1 mg/kg per day three times weekly for 3 weeks. Oral Probenecid (2 g) was given 3 h before CDV administration,

Table 1 Characteristics of patients

Patient no.	*Sex/age*	*Diagnosis*	*Transplant*	*Stem cell source*	*Use of ATG*	*Recipient CMV Ab*	*Donor CMV Ab*	*Recipient HSV Ab*
1	F/20	ALL/2CR	UBMT	DR mismatch	No	Positive	Negative	Positive
2	F/51	ATL/CR	CBT	B, DR mismatch	No	Positive	NE	Positive
3	M/32	SAA	UBMT	Match	Yes	Positive	Positive	Positive
4	F/54	SSc	autoPBSCT	CD34	No	Positive	NE	Positive
5	M/51	ML	alloPBSCT	Haplo-identical	No	Positive	Positive	Positive
6	F/41	ATL/Ref	alloPBSCT	Identical	No	Positive	Positive	Positive
7	F/52	MM	alloPBSCT	DR mismatch	No	Positive	Positive	Positive
8	M/47	ATL	UBMT	Match	No	Positive	Positive	Positive
9	M/17	SAA	BMT	Haplo	No	Positive	Positive	Positive
10	M/10	ALL/Ref	CBT	Three-loci mismatch	No	Positive	NE	Positive
11	M/36	CML/BC	alloPBSCT	Identical	No	Positive	Positive	Positive
12	M/45	SAA	UBMT	DR mismatch	Yes	Positive	Negative	Positive
13	M/62	MM	UBMT	B, DR mismatch	No	Positive	Negative	Positive
14	M/61	MDS	alloPBSCT	Identical	No	Positive	Positive	NE
15	M/50	AML/Ref	CBT	A, B mismatch	No	Positive	NE	NE
16	F/49	SSc	autoPBSCT	CD34	No	Positive	NE	Positive

ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; ATL = adult T-cell leukemia/lymphoma; SAA = severe aplastic anemia; ML = malignant lymphoma; CML = chronic myelogenous leukemia; CR = complete remission; Ref = refractory; BC = blastic crisis; MM = multiple myeloma; MDS = myelodysplastic syndrome; SSc = systemic sclerosis; BMT = bone marrow transplantation; UBMT = unrelated BMT; CBT = cord blood transplantation; PBSCT = peripheral blood stem cell transplantation; auto = autologous; allo = allogeneic; ATG = anti-thymocyte globulin; CMV = cytomegalovirus; HSV = herpes simplex virus; Ab = antibody; NE = not evaluated.

Table 2 characteristics of AdV disease

Patient no.	*GVHD*	*Immunosuppressive at HC onset*	*Serotype of AdV*	*Viral study at the onset of HC culture/PCR/IC*	*Onset of HC (days from transplant)*	*Start of CDV administration (days from transplant)*	*Interval from onset to CDV treatment (days)*	*Prior therapy for HC*
1	Grade II	FK506/PSL	11	+/+/+	40	59	19	AraA
3	No	Cs	11	+/+/+	29	30	1	No
4	NE	PSL	11	+/+/+	63	66	3	No
5	No	FK506/PSL	35	+/+/+	17	17	0	No
6	Grade II	Cs/PSL-FK506/mPSL	ND	+/+/+	18	19	1	No
7	Grade II	Cs/PSL	ND	+/+/+	80	83	3	No
8	Grade II	PSL	11	+/+/+	53	109	56	AraA
9	No	FK506/PSL	11	+/+/+	25	25	0	No
10	Grade III	FK506	11	+/+/+	26	39	13	No
11	Chronic lung	FK506/PSL	11	+/+/+	142	149	7	No
12	No	FK506	11	+/+/+	34	34	0	No
14	Grade II	FK506/PSL	11	+/+/+	126	129	3	No
15	Grade II	Cs/PSL	11	+/+/+	50	64	14	No
16	NE	PSL	11	+/+/+	31	31	0	No

AdV = adenovirus; HC = hemorrhagic cystitis; CDV = cidofovir; IC = immunochromatography; NE = not evaluable; ND = not determined; GVHD = graft-versus-host disease; FK506 = tacrolimus; Cs = cyclosporine; PSL = prednisolone; AraA = vidaravin.

Table 3 Outcome of CDV treatment

Patient no.	*Improvement of HC*	*Onset of effect (days)*	*Eradication of ADV from the urine**	*Initial creat (mg/dl)*	*Max creat (mg/dl)*	*Final creat (mg/dl)*	*Renal toxicity (NCI-CTC)*	*Previous PFA treatment*	*Activation of herpesviruses during CDV treatment*
1	Effective	6	Effective	1.38	1.38	1.02	1→1	No	None
3	No	—	Effective	0.54	0.83	1.02	0→0	No	None
4	Effective	7	Effective	0.76	1.02	0.59	0→1	No	None
5	No	—	Effective	1.2	2.38	1.79	1→2	No	CMV antigenemia, HSV stomatitis
6	Effective	13	Effective	0.54	0.59	0.59	0→0	No	CMV antigenemia
7	Effective	12	Effective	0.89	0.97	0.59	0→0	No	None
8	No	—	No	1.2	5.3	5.3	1→3	Yes	None
9	Effective	9	Effective	0.56	1.21	0.83	0→1	No	None
10	Effective	9	Effective	0.35	0.41	0.41	0→0	No	None
11	No	—	No	1.3	2.8	2.8	1→2	No	None
12	Effective	14	Effective	1	1.38	1.03	0→1	Yes	CMV antigenemia, HSV stomatitis
14	Effective	14	Effective	0.8	1.2	1	0→1	No	None
15	Effective	10	Effective	1.4	1.4	0.9	1→1	No	None
16	Effective	8	Effective	1.05	1.34	0.67	1→1	No	CMV antigenemia

*The eradication of AdV was defined by negative culture for AdV 1 week after the last dose of CDV.
Initial creat = serum creatine when starting CDV treatment; Max creat = maximal serum creatine during CDV treatment; Final creat = serum creatine upon completing CDV treatment; CDV = cidofovir; PFA = foscarnet; HC = hemorrhagic cystitis; AdV = adenovirus; CMV = cytomegalovirus; HSV = herpes simplex virus.

while 1 g was given 1 and 8 h afterward. Intravenous hydration with normal saline also was given. Patients were followed up for 2 months after the completion of CDV treatment.

Results

Outcome of CDV therapy

CDV therapy was successful in clearing AdV from the urine in 12 of 14 patients (86%), as defined by negative culture for AdV 1 week after the last dose of CDV. Of 14 patients, 10 (71%) showed clinical improvement in HC (Table 3). Median time to improvement of HC grade after CDV therapy was 9.5 days (range, 6–14; Table 3 and Figure 1). Patients No. 3 and No. 5 had persistent symptoms of HC despite eradication of AdV in the urine.

Toxicity

Serum creatinine concentration for all patients, at the time of initiation and termination of CDV treatment, as well as the maximum serum creatinine concentration during CDV treatment, are shown in Table 3. Renal toxicity was graded according to the Common Toxcity Criteria of National Cancer Institute (NCI-CTC Version 2.0; April 30, 1999). Among 14 patients, seven (50%) had no renal toxicity.

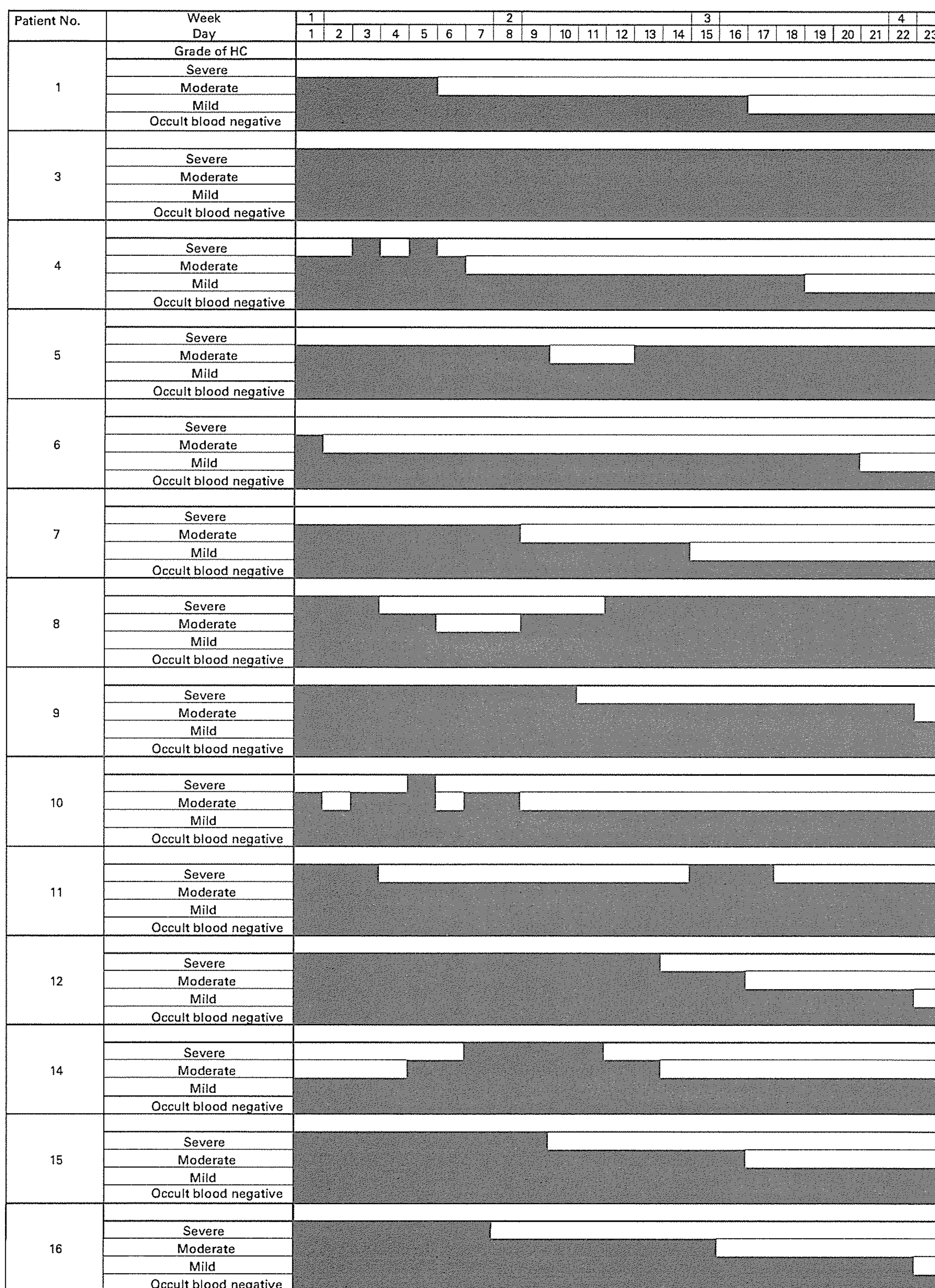

Figure 1 Clinical courses of 14 patients with adenoviral HC, who received cidofovir treatment.

Patient No. 8, who had been treated with foscarnet for CMV antigenemia, required hemodialysis and discontinuation of CDV treatment. Patient No. 11 had grade 2 renal toxicity and CDV treatment was terminated. In contrast, patient No. 5 had grade 2 renal toxicity, but could continue CDV treatment. Patient No. 10 developed veno-occlusive disease (VOD) during CDV treatment.

Virally associated findings during the treatment with CDV

Table 1 showed donor/recipient CMV and recipient HSV serostatus. As CDV has been reported to have significant anti-CMV and anti-HSV activity, concurrent use of acyclovir (ACV) or GCV was avoided to reduce renal toxicity. Patient No. 5 developed CMV antigenemia when CDV treatment was started, and CMV antigenemia persisted during CDV treatment. After completion of CDV treatment, he was treated with GCV, which abolished CMV antigenemia. Before CDV treatment, patient No. 6 was treated with GCV for CMV antigenemia that persisted throughout CDV therapy. Patient No. 12 developed CMV antigenemia during CDV treatment. After completion of CDV treatment, CMV antigenemia was abolished by treatment with foscarnet. Patient No. 16 developed CMV antigenemia during CDV treatment, and because of an increase in CMV antigenemia GCV was added. During CDV treatment, patients No. 5 and No. 12 developed HSV-1 stomatitis, which was treated successfully with ACV (Table 3).

Discussion

The present study reports the outcome in AdV HC treated with CDV. As expected, the main toxicity of CDV treatment was renal. Among 14 evaluable patients, two developed severe renal toxicity, resulting in discontinuation of CDV treatment. One of these patients who required hemodialysis had a history of foscarnet treatment. Previous treatment with foscarnet has been reported to exacerbate CDV renal toxicity,[23] which was proved for this patient. CDV renal toxicity complicating treatment of AdV HC is difficult to evaluate. Many other nephrotoxic agents, including cyclosporine, tacrolimus, and amphotericin B, are frequently administered to HSCT patients; furthermore, AdV infection itself can cause renal damage such as nephritis[16] and obstructive nephropathy.[24] In this study, six patients (Nos. 5, 9, 12, 14, 15, and 16) experienced increased level of serum creatinine concentrations, but continued CDV treatment, with improvement in terms of both AdV HC and renal function (Table 3). Thus, AdV HC itself may have contributed to the increase in serum creatinine during CDV treatment. Use of CDV before emergence of renal damage from AdV infection would be desirable. Patient No. 10 developed VOD, which has not been reported previously as a form of CDV toxicity. More information is necessary to determine whether or not VOD is among CDV toxicities.

Among 14 evaluable patients, 10 (71%) showed clinical improvement of AdV HC, which is similar to a success rate of 63% reported in patients with definite AdV disease reported by the European Group for Blood and Marrow Transplantation.[25] A long delay between AdV infection and treatment has been linked to a greater risk of treatment failure.[17] For rapid diagnosis, we used immunochromatography. At the onset of HC, all patients in the study were positive for AdV by this method. Positivity was confirmed later both by PCR result and by isolation of AdV from urine. Thus, immunochromatography appears reliable for rapid diagnosis of AdV HC. Since post-transplant AdV infection causes significant mortality[6,9–11] and HC causes considerable patient discomfort, CDV would appear to be beneficial treatment while maintaining an acceptable toxicity profile.

At a dose of 5 mg/kg/week, CDV has been reported to have significant anti-CMV and anti-HSV activity.[26] Indeed, CDV is considered a second-line treatment for GCV-refractory CMV disease.[27] Among our patients, two had persistent CMV antigenemia and two developed CMV antigenemia during treatment with CDV. In addition, two patients developed HSV-1 stomatitis. Thus, CDV at a dose of 1 mg/kg/day three times weekly may be insufficient to prevent or treat CMV or HSV disease. Alternatively, patients who develop AdV HC might be immune compromised to the extent that for them CDV treatment may not be effective against CMV or HSV. Vigilance against infection by and/or additional prophylaxis agents for herpesviruses, therefore, is important during CDV treatment with 1 mg/kg three times weekly.

In conclusion, CDV at a dose of 1 mg/kg/day, three times weekly could be administered with acceptable toxicity for effective treatment of AdV HC. Prospective randomized trials are necessary to further study the use of CDV for AdV HC.

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Reconstitution of HLA-A*2402–Restricted Cytomegalovirus-Specific T-Cells following Stem Cell Transplantation

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Abstract

Cytomegalovirus (CMV)-specific immune reconstitution early after stem cell transplantation (SCT) was evaluated prospectively by detecting CD8+ T-cells, which recognize the peptide QYDPVAALF in the context of HLA-A*2402. Fifteen allogeneic SCT recipients were included in the study. All recipients and donors were seropositive for CMV and had the HLA-A*2402 allele. CMV-specific T-cells were detected as early as 1 month after transplantation, and their numbers increased to peak levels 2 to 5 months after transplantation. The numbers of CMV-specific T-cells in patients who developed grade II to IV acute graft-versus-host disease (GVHD) and received corticosteroids for acute GVHD were low in the early period after allogeneic SCT. There was a trend toward earlier reconstitution of CMV-specific CD8+ T-cells in allogeneic peripheral blood SCT (PBSCT) patients than in allogeneic bone marrow transplantation patients. The contribution of T-cells in the graft to the recovery of CMV-specific immune responses was also suggested by the finding that the reconstitution of CMV-specific CD8+ T-cells was delayed in CD34-selected autologous PBSCT compared with unpurged autologous PBSCT. The reconstitution of CMV-specific CD8+ T-cells was delayed in patients with CMV disease or recurrent CMV reactivation. These observations suggest that the detection of CMV-specific T-cells with an HLA-peptide tetramer is useful to assess immune reconstitution against CMV and to identify patients at risk for CMV disease or recurrent CMV reactivation after SCT.

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Key words: HLA-A*2402; Cytomegalovirus; CMV-specific T-cells; Stem cell transplantation; Tetramer

1. Introduction

Cytomegalovirus (CMV) disease is a significant cause of morbidity and mortality after stem cell transplantation (SCT) [1]. Preemptive or prophylactic therapy with ganciclovir reduces the incidence and severity of CMV disease after transplantation [1,2]. Preemptive therapy calls for ganciclovir to be given only to patients who are at high risk for CMV disease based on the detection of CMV. However, a higher incidence of CMV disease has been demonstrated with this approach than with the prophylactic ganciclovir regimen [2]. On the other hand, prophylactic ganciclovir therapy begun at engraftment has resulted in effective prevention of CMV disease in the first 100 days after transplantation, but it has also been associated with invasive fungal infections and late CMV disease [2]. A delay in the recovery of CMV-specific T-cell responses as a result of ganciclovir prophylaxis has been suggested to contribute to the occurrence of late CMV disease [3].

CMV disease remains a major concern in SCT recipients, especially when it does not respond to ganciclovir

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therapy. Ganciclovir-resistant CMV is rarely isolated from SCT recipients, compared with acquired immunodeficiency syndrome patients [4-6]. CMV disease refractory to ganciclovir therapy is considered predominantly due to the profound immunodeficiency inherent in SCT recipients [6-8].

Major histocompatibility complex (MHC)-restricted and CMV-specific cytotoxic T-lymphocytes play an important role in protection against CMV disease [1,9-11]. Adoptive transfer of CMV-specific T-cells provides persistent reconstitution of CMV-specific T-cell responses and is effective in the treatment of patients who experience persistent or recurrent CMV infection [9-11]. A functional assay using the enzyme-linked immunospot method and flow cytometric analysis was developed to detect CMV-specific T-cells producing intracellular cytokine [12-15]. Recently, direct visualization of CMV-specific $CD8^+$ T-cells was introduced with the development of fluorescently labeled tetrameric MHC-peptide complexes [16,17]. This assay is highly sensitive and rapid for monitoring CMV-specific T-cells [18]. The HLA-A*0201–restricted NLVPMVATV epitope and the HLA-B*0702–restricted TPRVTGGGAM epitope have been used to monitor CMV-specific T-cells following allogeneic SCT [16,17]. A CMV-specific cytotoxic T-lymphocyte epitope, QYDPVAALF, was recently identified in the amino acid sequence of the 65 kd phosphoprotein (pp65) presented by the HLA-A*2402 molecule [14]. HLA-A24 is one of the most common alleles among the Japanese, and more than 95% of the alleles among the Japanese are A*2402 [19-21]. In this study, we prospectively monitored CMV-specific $CD8^+$ T-cells that recognize the short peptide, QYDPVAALF, presented by HLA-A*2402 and evaluated CMV-specific T-cell reconstitution in HLA-A*2402 recipients who received SCT.

2. Materials and Methods

2.1. Patients

Between September 2001 and December 2002, 15 consecutive patients who were HLA-A*2402 positive and had received allogeneic SCT for hematologic malignancy were enrolled in the study. All recipients and donors were seropositive for CMV before transplantation and were positive for the HLA-A*2402 allele. The characteristics of the allogeneic SCT patients are shown in Table 1.

Four patients who received unpurged autologous peripheral blood SCT (PBSCT) and 3 patients who received CD34-selected autologous PBSCT also were studied. Two of the 4 patients who received unpurged autologous PBSCT had acute nonlymphoblastic leukemia, and the remaining 2 patients had non-Hodgkin's lymphoma. Two of the 3 patients who received CD34-selected autologous PBSCT had systemic sclerosis with interstitial pneumonia, and 1 patient had dermatomyositis with interstitial pneumonia. All patients achieved sustained engraftment and survived for more than 100 days after transplantation. Informed consent was obtained from the patients or responsible family members.

Table 1.
Characteristics of Allogeneic Stem Cell Transplantation Patients*

Male/female sex, n	8/7
Median age (range), y	36 (22-68)
Underlying disease, n	
Acute nonlymphoblastic leukemia	5
Acute lymphoblastic leukemia	2
Chronic myelogenous leukemia	2
Myelodysplastic syndrome	3
Non-Hodgkin's lymphoma	2
Natural killer cell lymphoma	1
Donor and HLA disparity, n	
Related/identical	5
Related/nonidentical†	3
Unrelated/identical‡	6
Unrelated/nonidentical§	1
Stem cell source, n	
Bone marrow	8
Peripheral blood	7
Pretransplantation conditioning, n	
Myeloablative	10
Nonmyeloablative	5
GVHD prophylaxis, n	
Cyclosporine/methotrexate	6
Tacrolimus/methotrexate	9
Grades of acute GVHD, n	
0	3
I	3
II	7
III, IV	2

*GVHD indicates graft-versus-host disease.
†One patient with a donor mismatched for the HLA-B antigen, 1 patient mismatched for HLA-A and HLA-B antigens, and 1 patient with a haploidentical donor.
‡HLA-A–, HLA-B–, and HLA-DRB1–identical unrelated donors.
§An HLA-B antigen–mismatched unrelated donor.

2.2. Stem Cell Transplantation

The stem cell sources, the regimens for pretransplantation conditioning, and prophylaxis for graft-versus-host disease (GVHD) in allogeneic SCT are shown in Table 1. Eight patients underwent bone marrow transplantation (BMT), and 7 patients had PBSCT. The myeloablative conditioning regimen consisted of 12 Gy total body irradiation and 120 mg/kg cyclophosphamide (CY) in 4 patients and 12 Gy total body irradiation, 120 mg/kg CY, and 8 g/m^2 cytosine arabinoside (Ara-C) in 2 patients. Four patients were administered 16 mg/kg busulfan and 120 mg/kg CY. Nonmyeloablative conditioning regimens included 180 mg/m^2 fludarabine and 8 mg/kg busulfan in 2 patients and 125 mg/m^2 fludarabine and 60 mg/kg CY in 3 patients. To prevent GVHD, we administered 3 mg/kg cyclosporine per day as a continuous intravenous infusion in 6 patients in combination with 10 mg/m^2 methotrexate on day 1 and 7 mg/m^2 on days 3 and 6, and we administered 0.03 mg/kg tacrolimus per day by continuous intravenous infusion to another 9 patients in combination with the same methotrexate regimen. The diagnosis and grading of acute GVHD was based on clinical criteria with histologic confirmation obtained as required [22]. Complete chimerism was confirmed 1 month after transplantation in all allogeneic SCT patients.

Autologous PBSCT for acute nonlymphoblastic leukemia was performed according to the protocol described previously, with minor modifications [23]. PBSC were collected during the hematopoietic recovery period after consolidation chemotherapy and cryopreserved without ex vivo purging until transplantation. The pretransplantation conditioning regimen consisted of 16 mg/kg busulfan, 40 mg/kg etoposide (VP-16), 700 mg/m^2 Ara-C, and 12 g/m^2 Ara-C. Granulocyte colony-stimulating factor (G-CSF) (filgrastim) was combined with this regimen. For non-Hodgkin's lymphoma patients, 500 mg/m^2 VP-16 was administered for 3 days after 3 courses of induction chemotherapy with a CHOP regimen (CY, hydroxydaunomycin, vincristine [Oncovin], and prednisone). PBSC were collected during the hematopoietic recovery with G-CSF. Then, 3 more courses of induction chemotherapy were performed with the CHOP regimen. The pretransplantation conditioning regimen consisted of 400 mg/m^2 ranimustine, 1200 mg/m^2 carboplatin, 1500 mg/m^2 VP-16, and 100 mg/kg CY. For autologous CD34-selected PBSCT, mobilization of PBSC was performed with 2 g/m^2 CY for 2 days followed by G-CSF administration. CD34$^+$ cells were selected with the CliniMACS device (Miltenyi Biotec, Bergisch-Gladbach, Germany) and cryopreserved until transplantation. The pretransplantation conditioning regimen for CD34-selected autologous PBSCT consisted of 200 mg/kg CY.

Each patient was isolated in a room with laminar air flow, and a standard decontamination procedure was followed. Prophylaxis for bacterial, fungal, and *Pneumocystis carinii* infections consisted of fluconazole, ciprofloxacin, and sulfamethoxazole/trimethoprim. All patients were given 1000 mg/day acyclovir orally from day −7 to day 35 for the prevention of herpes simplex virus infection. All blood products from random donors were irradiated and filtered.

2.3. Monitoring of CMV Reactivation

CMV reactivation was monitored at least once a week after engraftment with the antigenemia assay and quantitative real-time polymerase chain reaction (PCR) analysis. The CMV antigenemia assay was performed according to the method described previously [24]. The degree of antigenemia was expressed as the number of CMV antigen–positive cells per 5 × 10^4 leukocytes. The extraction and amplification of CMV DNA were performed according to the method described by Machida et al [25]. Viral DNA was extracted from plasma with a QIAamp Blood minikit (Qiagen, Valencia, CA, USA) and then subjected to the PCR. The PCR was performed with a TaqMan Universal PCR master mix (PE Biosystems, Tokyo, Japan).

2.4. Diagnosis of CMV Reactivation and Disease

CMV reactivation was defined as the presence of 1 or more antigen-positive cells or ≥2 × 10^2 CMV DNA copies/mL. For the diagnosis of CMV disease, such as pneumonia, colitis, or hepatitis, positive CMV-reactivation results had to be accompanied by clinical symptoms, signs, and histologic confirmation [26].

2.5. Preemptive Therapy with Ganciclovir for the Prevention of CMV Disease

The decision to use preemptive therapy was based on positive results in the antigenemia test (≥1 antigen-positive cells per 50,000 white blood cells); intravenous infusion of ganciclovir at a dose of 10 mg/kg per day was started and continued for as long as the antigenemia persisted. G-CSF was administered when the absolute neutrophil count was <500/μL.

2.6. Tetramer Staining

MHC-peptide tetramers were produced as described previously [14]. We used a CMV-specific T-cell epitope, QYDPVAALF, in the amino acid sequence of pp65 presented by HLA-A*2402 molecules. Peripheral blood mononuclear cells (2 × 10^6), which were drawn monthly after transplantation, were stained at 37°C for 15 minutes with Tricolor anti-CD8 monoclonal antibody (Caltag Laboratories, Burlingame, CA, USA) and a tetramer concentration of 0.1 mg/mL. The stained cells were washed twice and fixed with 0.5% paraformaldehyde before flow cytometric analysis. The peripheral blood lymphocyte count was used to determine the absolute number of CMV-specific CD8$^+$ T-cells.

2.7. Statistical Analysis

The percentages and absolute numbers of CD8$^+$ T-cells binding the HLA-A*2402 QYDPVAALF tetramer in the peripheral blood were compared by means of the Spearman rank correlation test. The absolute numbers of CMV-specific CD8$^+$ T-cells after transplantation were evaluated with the Mann-Whitney *U* test. A *P* value <.05 was considered statistically significant.

3. Results

3.1. Detection of CD8$^+$ T-Cells Binding the HLA-A*2402 QYDPVAALF Tetramer in the Peripheral Blood after Allogeneic SCT

CMV-specific T-lymphocytes, which recognize the peptide QYDPVAALF in the context of HLA-A*2402 molecules, were monitored monthly for a year after transplantation in the first 7 patients and for 3 months after transplantation in the next 8 patients (Figure 1 and Table 2). The median percentage of HLA-peptide tetramer–positive cells in the present study was 0.08% (range, 0%-2.85%) of CD8$^+$ T-cells, in contrast to approximately 0.1% in normal CMV-seropositive individuals [14,27]. The percentages and absolute numbers of CMV-specific CD8$^+$ T-cells in the peripheral blood were strongly correlated (r = 0.92; P < .0001 by the Spearman rank correlation test [n = 108]). Therefore, we express the results as the absolute number in the following sections. The median number of CMV-specific CD8$^+$ T-cells in the first 7 patients was 0.08 × 10^6/L (range, 0-16.19 × 10^6/L) at 1 month posttransplantation. The number then increased to 0.25 × 10^6/L (range, 0-41.53 × 10^6/L) at 2 months, 0.18 × 10^6/L (range, 0.01-13.61 × 10^6/L) at 3 months, 0.48 × 10^6/L (range, 0-8.45 ×

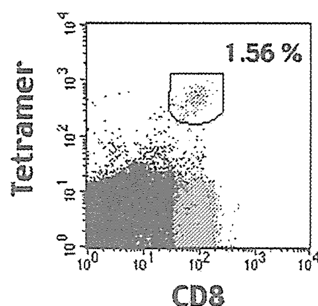

Figure 1. Staining of cytomegalovirus-specific CD8+ T-cells with an HLA-A*2402 QYDPAALF tetramer. The percentage of CD8+ T-cells binding an HLA-peptide tetramer was 1.56% of the CD8+ population.

10^6/L) at 4 months, and 0.3 × 10^6/L (range, 0-5.18 × 10^6/L) at 5 months posttransplantation and gradually decreased thereafter (Table 2). There was a trend toward higher numbers of CMV-specific CD8+ T-cells at 2 months after transplantation than at 1 month after transplantation among the 15 patients (P = .08 by the Mann-Whitney U test; Figure 2). Two of the 15 patients (unique patient numbers [UPN] 4 and 5) did not develop CMV reactivation; however, the numbers of CMV-specific T-cells in these 2 patients were increased at 2 or 3 months after transplantation (from 0.01 × 10^6/L at 1 month to 0.18 × 10^6/L at 3 months posttransplantation [UPN 4] and from 0.16 × 10^6/L at 1 month to 0.25 × 10^6/L at 2 months posttransplantation [UPN 5]) (Table 2). Thus, the increment in CMV-specific T-cells was not necessarily accompanied by detectable CMV reactivation. CMV-specific CD8+ T-cells from 2 patients were sorted by fluorescence-activated cell sorting, and PCR analysis of short tandem repeats revealed that all CMV-specific CD8+ T-cells were derived from the donor (data not shown).

3.2. Reconstitution of CMV-Specific CD8+ T-Cells in Recipients Who Developed Acute GVHD

Grades I, II, and III to IV acute GVHD developed in 3, 7, and 2 patients, respectively (Table 1). The reconstitution of CMV-specific CD8+ T-cells was significantly delayed in the patients who developed grade II to IV acute GVHD after transplantation ($P < .05$ at 1 month, $P < .01$ at 2 months, and $P < .05$ at 3 months; Mann-Whitney U test) (Figure 3). Moreover, the delayed recovery of CMV-specific CD8+ T-cells was most prominent in the patients who received ≥2 mg/kg corticosteroids for the treatment of acute GVHD ($P < .01$ at 1 month, $P < .01$ at 2 months, and $P < .01$ at 3 months posttransplantation; Mann-Whitney U test) (Figure 3). There was no difference in recovery between the patients with myeloablative conditioning regimens and those with nonmyeloablative regimens (data not shown).

3.3. Relationship between the Number of CMV-Specific CD8+ T-Cells and Stem Cell Source

Eight patients received allogeneic BMT, and 7 patients had allogeneic PBSCT. There was a trend toward an earlier reconstitution of CMV-specific CD8+ T-cells in PBSCT patients than in BMT patients (P = .07, Mann-Whitney U test) (Figure 4). To determine the influence of T-cells infused at transplantation on the reconstitution of CMV-specific T-cell responses, we monitored CMV-specific CD8+ T-cells in 4 CMV-seropositive patients who received unpurged autologous PBSCT and in 3 CMV-seropositive patients who received autologous PBSCT with selected CD34+ cells (Table 3). The median number of infused CD3+ cells was 11.0 × 10^7/kg (range, 8.8-12.8 × 10^7/kg) in the recipients who received unpurged autologous PBSCT and 3.3 × 10^3/kg (range, 3.0-5.0 × 10^3/kg) in the patients who received CD34-selected autologous PBSCT. The reconstitution of CMV-specific CD8+ T-cells in the patients who underwent CD34-selected autologous transplantation was delayed compared with those who underwent unpurged autologous PBSCT (Figure 4).

3.4. Reconstitution of CMV-Specific CD8+ T-Cells in Recipients Who Developed CMV Disease or Recurrent CMV Reactivation

One patient who received a BMT from an HLA-identical unrelated donor developed CMV colitis on day 26 posttransplantation. The recurrence of CMV reactivation requiring preemptive ganciclovir therapy was observed before day 100 in 3 patients. The recovery of CMV-specific CD8+ T-cells was delayed in patients with CMV disease or recurrent CMV reactivation ($P < .05$, Mann-Whitney U test) (Figure 5).

4. Discussion

The reconstitution of CMV-specific T-cell responses has previously been studied with the HLA-A*0201 and HLA-B*0702 tetramers [16-18]. In the present study, the HLA-A*2402 QYDPVAALF tetramer was used to detect CMV-specific CD8+ T-cells. HLA-A24 is one of the most common alleles among the Japanese [19-21]. CMV-specific CD8+ T-cells were detected as early as 1 month after transplantation in the peripheral blood of approximately half of allogeneic SCT recipients, including unrelated BMT recipients. Cwynarski et al [16] reported that the recovery of CMV-specific T-cells after allogeneic SCT from unrelated donors was delayed, and tetramer-binding cells were not detectable before day 100 after transplantation. In these investigators' studies, recipients of unrelated bone marrow were treated in vivo with Campath-1H as part of GVHD prophylaxis. Campath-1H induces marked lymphocytopenia after treatment. Moreover, these patients also were given ganciclovir prophylaxis, which has been reported to be correlated with failure to recover CMV-specific T-cell responses in the first 90 days after transplantation. Ganciclovir suppression of CMV replication to preclude in vivo priming and expansion of CMV-specific T-cell precursors and the inhibition of antigen-induced T-cell proliferation due to ganciclovir's effects on cellular DNA synthesis have been speculated as possible mechanisms [3]. Neither immunosuppressive drugs such as Campath-1H and antithymocyte globulin nor prophylactic ganciclovir was administered in our study. These

Table 2.
Kinetics of HLA-A*2402–Restricted Cytomegalovirus (CMV)-Specific T-Cells following Allogeneic Stem Cell Transplantation*

								CMV-Specific CD8+ T-Cells after Transplantation, ×10⁶/L											
						CMV	CMV	Months after Transplantation											
UPN	Age/Sex	Disease	Stem Cells	GVHD	Steroids†	Disease	Reactivation‡	1	2	3	4	5	6	7	8	9	10	11	12
1	68/M	ANLL	PBSC	0	(–)	(–)	1	16.19	41.53	13.61	8.45	2.45	3.21	2.00	2.77	1.55	2.20	3.27	1.51
2	50/M	CML	BM (UR)	II	(+)	Colitis	1	0	0.02	0.05	0.48	0.37	0.19	0.04	0	0.05	0.01	0.04	0.02
3	38/M	NHL	PBSC	0	(–)	(–)	1	0.08	15.22	8.04	6.74	5.18	16.15	11.69	5.27	6.60	5.90	2.48	2.92
4	25/F	CML	PBSC	II	(–)	(–)	0	0.01	0.05	0.18	0.06	0.09	0	0.05	0	0	0	0.08	0
5	63/F	MDS	PBSC	0	(–)	(–)	0	0.16	0.25	0.17	0.16	0.30	0	0.09	0.06	0.16	0.23	0.03	0.06
6	32/F	ALL	PBSC	I	(–)	(–)	1	1.07	1.34	0.51	0.74	0.26	0.14	0.14	0.47	0.45	0.37	0.82	1.23
7	32/M	ANLL	BM (UR)	IV	(+)	(–)	2	0	0	0.01	0	0	0	0	0	0	0.03	0.07	0.07
8	22/F	ANLL	BM (UR)	II	(–)	(–)	1	2.20	0.77	0.31									
9	37/F	ALL	BM (UR)	I	(–)	(–)	1	0	0.08	0.04									
10	36/M	MDS	BM (UR)	II	(+)	(–)	2	0	0.08	0.01									
11	32/M	NK-Ly	BM	III	(+)	(–)	2	0	0.04	0.04									
12	28/M	ANLL	PBSC	II	(+)	(–)	1	0	0.03	0.01									
13	27/F	MDS	BM (UR)	I	(–)	(–)	1	0.07	2.09	0.71									
14	49/F	NHL	PBSC	II	(–)	(–)	1	0.06	0.15	0.06									
15	48/M	ANLL	BM (UR)	II	(–)	(–)	1	0.01	0.03	0.02									

*UPN indicates unique patient number; GVHD, graft-versus-host disease; M, male; ANLL, acute nonlymphoblastic leukemia; PBSC, peripheral blood stem cells; CML, chronic myelogenous leukemia; BM, bone marrow; UR, unrelated; NHL, non-Hodgkin's lymphoma; F, female; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; NK-Ly, natural killer cell lymphoma.

†Corticosteroids ≥2 mg/kg.

‡No. of times CMV reactivation required preemptive ganciclovir therapy.

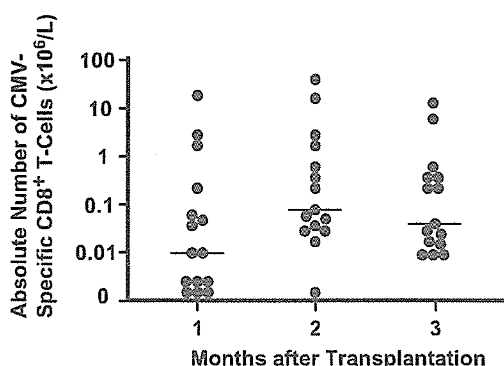

Figure 2. Reconstitution of cytomegalovirus (CMV)-specific CD8+ T-cells following allogeneic stem cell transplantation. Horizontal bars indicate median values.

factors may explain why our study detected HLA-A*2402–restricted CMV-specific CD8+ T-cells in the early period after transplantation. CMV reactivation and CMV disease frequently develop before day 100, although late-onset CMV disease is also a concern after allogeneic SCT [2,28]. Monitoring CMV-specific CD8+ T-cells in the early period after transplantation may be important to assess the immunologic recovery against CMV.

CMV reactivation has been demonstrated to precede an increase in the number of CMV-specific CD8+ T-cells, indicating a significant predictor of CMV-specific T-cell responses [16,17]. The presence of CMV in SCT recipients is also necessary for the posttransplantation reconstitution of CMV-specific T-cells [16,17]. Two of 15 patients in the present study did not develop CMV reactivation, but the recipients mounted measurable cellular immune responses against CMV at 2 months and 3 months after transplantation. The levels of CMV reactivation in the present study may also have been below the detection limits of the antigenemia assay and the quantitative real-time PCR assay, as has been suggested by Cwynarski et al [16]. Otherwise, an increase in the number of CMV-specific CD8+ T-cells may have been a result of a normal homeostatic mechanism expanding the entire T-cell pool.

The reconstitution of CMV-specific CD8+ T-cells was delayed in recipients who developed grade II to IV acute GVHD, compared with those with grade I acute GVHD or without acute GVHD. Corticosteroid administration for the treatment of acute GVHD also affected the recovery of CMV-specific CD8+ T-cells. GVHD and corticosteroids are well-recognized risk factors for CMV disease after allogeneic SCT [1,29]. The results of this study support these findings [16,17]. Careful management to prevent CMV disease is important for recipients with acute GVHD and for those who receive corticosteroids for the treatment of acute GVHD after allogeneic SCT.

CMV-specific CD8+ T-cells have been demonstrated to be derived from the donor [30]. Recurrent CMV antigenemia or CMV disease occurs more frequently after transplantation from a CMV-seronegative donor than from a seropositive donor [3,17]. Gratama et al [17] also showed that the number of CMV-specific T-cells infused into CMV-seropositive SCT recipients was inversely correlated with the number of recurrent CMV infections after transplantation. These results indicate that the number of CMV-specific CD8+ T-cells in the grafts may exert an influence on the recovery of CMV-specific T-cell responses after transplantation. In the present study, there was a trend toward an earlier reconstitution of CMV-specific CD8+ T-cells in recipients of PBSC than in bone marrow recipients. PBSC contain 5 to 10 times more T-cells than bone marrow, possibly contributing to an earlier reconstitution of CMV-specific T-cell responses in PBSCT recipients, although the exact number of T-cells infused at transplantation was not determined in this study. Hakki et al [29] demonstrated in a multivariate analysis that using bone marrow as a source of stem cells was associated with impaired CD8+ T-cell function. Moreover, the delayed recon-

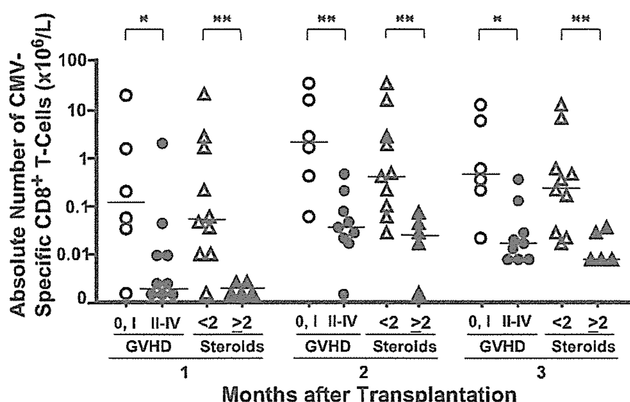

Figure 3. Reconstitution of cytomegalovirus (CMV)-specific CD8+ T-cells in patients who developed acute graft-versus-host disease (GVHD) and in those who received corticosteroids following allogeneic stem cell transplantation. Indicated are patients who received <2 mg/kg (<2) and ≥2 mg/kg (≥2) corticosteroids. Horizontal bars indicate median values (*, $P < .05$; **, $P < .01$).

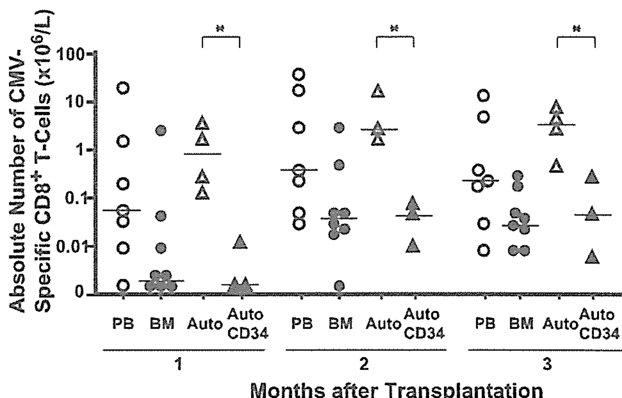

Figure 4. Reconstitution of cytomegalovirus (CMV)-specific CD8+ T-cells in patients who received peripheral blood (PB) or bone marrow (BM) and in patients who received an unpurged autologous PB stem cell transplant (PBSCT) (Auto) or CD34-selected autologous PBSCT (Auto CD34). Horizontal bars indicate median values (*, $P < .05$).

Table 3.
Characteristics of Autologous Peripheral Blood Stem Cell (PBSC) Transplantation Patients*

UPN	Age, y/Sex	Disease/Status	Stem Cells	CD34⁺ Cell Selection†	Pretransplantation Conditioning§	CD34⁺ Cells Infused, /kg	CD3⁺ Cells Infused, /kg
1	57/Male	NHL/1Rel	PBSC	(–)	MCEC	1.9×10^6	NE
2	52/Male	ANLL/1CR	PBSC	(–)	G-BEA	8.1×10^6	11.0×10^7
3	44/Female	NHL/1CR	PBSC	(–)	MCEC	24.7×10^6	8.8×10^7
4	30/Female	ANLL/1CR	PBSC	(–)	G-BEA	6.0×10^6	12.8×10^7
5	53/Female	SLE, SSc, IP	PBSC	(+)	CY	21.0×10^6	3.3×10^3
6	54/Female	DM, IP	PBSC	(+)	CY	4.9×10^6	5.0×10^3
7	55/Male	SSc, IP	PBSC	(+)	CY	6.6×10^6	3.0×10^3

*UPN indicates unique patient number; NHL, non-Hodgkin's lymphoma; 1Rel, first relapse; MCEC, regimen of 400 mg/m^2 ranimustine, 1200 mg/m^2 carboplatin, 1500 mg/m^2 etoposide, and 100 mg/kg cyclophosphamide; NE, not examined; ANLL, acute nonlymphoblastic leukemia; 1CR, first complete remission; G-BEA, regimen of 16 mg/kg busulfan, 40 mg/kg etoposide, and 700 mg/m^2 and 12 g/m^2 cytosine arabinoside, combined with granulocyte colony-stimulating factor; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; IP, interstitial pneumonia; CY, 200 mg/kg cyclophosphamide; DM, dermatomyositis.

†CD34⁺ cells were selected with the CliniMACS device.

stitution in the present study of CMV-specific CD8⁺ T-cells in the CD34-selected autologous PBSCT recipients compared with the unpurged autologous PBSCT recipients suggests that CMV-specific T-cells in the grafts contribute to the recovery of CMV-specific T-cell responses in the early period after SCT. Further study, such as an examination of the levels of the T-cell receptor excision circle, may determine whether T-cells in the graft repopulate the thymus dependently or independently in the early period after SCT [31].

The reconstitution of CMV-specific CD8⁺ T-cells was delayed in patients who developed CMV disease or recurrent CMV reactivation, suggesting that CMV-specific CD8⁺ T-cells confer protection against CMV disease or recurrent high-level CMV reactivation, although CMV-specific CD8⁺ T-cells have been reported to be heterogeneous in healthy seropositive donors, with only a portion of these cells correlating to functional virus-specific cells [32]. The HLA-peptide tetramer assay is useful to identify patients at risk for CMV disease or recurrent CMV reactivation requiring preemptive ganciclovir therapy.

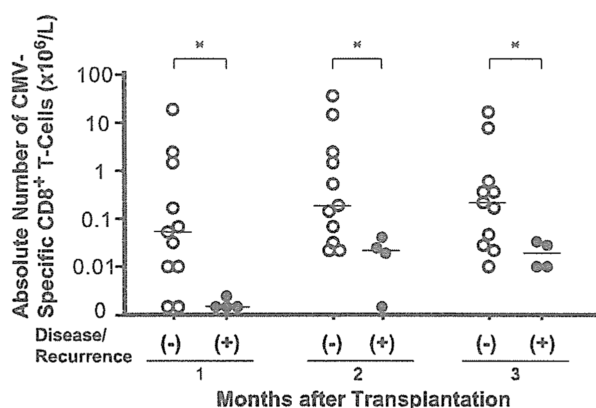

Figure 5. Reconstitution of cytomegalovirus (CMV)-specific CD8⁺ T-cells in patients who developed CMV disease or recurrent CMV reactivation following allogeneic stem cell transplantation. O indicates patients who did not develop CMV disease or recurrent CMV reactivation; ●, patients who developed CMV disease or recurrent CMV reactivation. Horizontal bars indicate median values (*, $P < .05$).

CD34-selected autologous transplantation delays immune reconstitution and causes an increased incidence of infectious complications [33-36]. An increased incidence of CMV disease has been reported in patients who received CD34-selected autologous transplantation [33,34,37]. In the analysis of 31 CMV-seropositive patients who received CD34-selected autologous PBSCT, Holmberg et al [37] reported that 7 patients (22.6%) developed CMV disease and that 4 (12.9%) died from the complications of their infection. In the present study, the reconstitution of CMV-specific CD8⁺ T-cells in CD34-selected autologous transplantation was delayed, a response similar to that of patients who developed grade II to IV acute GVHD or received ≥2 mg/kg corticosteroids. Infection surveillance, diagnostic work-up, and prevention strategies similar to those used with allogeneic transplant recipients may be required in CD34-selected autologous PBSCT recipients, as Crippa et al have recommended [34].

In the present study, the HLA-A*2402 QYDPVAALF tetramer was used to evaluate 1 component of the total CMV-specific T-cell immune response [18,27]. A multivariate analysis to identify factors influencing immunologic recovery against CMV could not be performed because of the limited number of patients in this study. However, our observations suggest that the detection of CMV-specific T-cells with the HLA-peptide tetramer is useful to assess immune reconstitution against CMV and to identify patients at risk for CMV disease or recurrent CMV reactivation after SCT.

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Case Report

Successful Treatment of Minimal Residual Disease–Positive Philadelphia Chromosome–Positive Acute Lymphoblastic Leukemia with Imatinib Followed by Reduced-Intensity Unrelated Cord Blood Transplantation after Allogeneic Peripheral Blood Stem Cell Transplantation

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Abstract

We describe a 35-year-old woman with Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph⁺ ALL) who received allogeneic sibling donor peripheral blood stem cell transplantation (PBSCT) and entered a second complete remission. Upon detection of BCR-ABL transcripts after PBSCT, the patient received imatinib, leading to molecular remission. Following the failure of donor leukocyte infusions, she underwent reduced-intensity unrelated cord blood transplantation (RI-UCBT), and has continued durable molecular remission for more than 30 months without substantial graft-versus-host disease. Because of a lack of adverse effects of imatinib on transplantation outcome, a treatment strategy consisting of molecular monitoring–guided initiation of imatinib followed by RI-UCBT may be promising in the management of Ph⁺ ALL after allogeneic SCT.

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Key words: Philadelphia chromosome–positive acute lymphoblastic leukemia; Unrelated cord blood transplantation; Imatinib; Minimal residual disease

1. Introduction

The prognosis for adult patients with Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph⁺ ALL) is poor. Although allogeneic stem cell transplantation (SCT) is considered the only potentially curative therapy, a substantial proportion of patients undergoing allogeneic SCT develop hematologic relapse or experience disease progression. In such cases, further treatment is rarely successful [1-5]. Consequently, identification of patients at the highest risk prior to overt hematologic relapse is of great importance. The reverse transcription polymerase chain reaction (RT-PCR) is a sensitive method for detecting low-level transcripts of the breakpoint cluster region–Abelson oncogene locus (BCR-ABL) to assess minimal residual disease (MRD) in Ph⁺ ALL [6]. Detection of BCR-ABL transcripts after allogeneic SCT is associated with a probability of hematologic relapse exceeding 90% [6,7].

Another important goal is to prevent MRD after allogeneic SCT from developing hematologic relapse. Imatinib (Glivec, STI571; Novartis Pharmaceuticals, East Hanover, NJ, USA), a selective protein tyrosine kinase inhibitor of BCR-ABL, has pronounced but brief antileukemic activity in patients with advanced Ph⁺ ALL, including those with failing SCT [4,5]. Patients with Ph⁺ ALL receiving imatinib after SCT on the basis of BCR-ABL transcript positivity have been shown to have a decreased rate of hematologic relapse and to experience prolonged disease-free survival (DFS) [8]. However, sustained molecular remissions are almost never expected with single-agent imatinib [4,5,8]. Despite the need

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for additional treatment options, no effective therapies are available at present.

Here, we report a Ph⁺ ALL patient with molecular evidence of recurrent leukemia after allogeneic peripheral blood stem cell transplantation (PBSCT) who was successfully treated with an unrelated cord blood transplantation (UCBT) following the induction of molecular remission by imatinib.

2. Case Report

In June 2002, a 35-year-old woman received a diagnosis of Ph⁺ ALL with additional karyotype abnormalities of −7 and der(9;22)(q10;q10). Major BCR-ABL chimeric messenger RNA was detected by RT-PCR. Although the patient achieved complete remission (CR) with an induction therapy consisting of cyclophosphamide, daunorubicin, vincristine, prednisolone, and L-asparaginase, based on the Japan Adult Leukemia Study Group ALL-97 protocol, she underwent hematologic relapse during the first course of consolidation therapy. The patient achieved a second CR, a complete cytogenetic remission but not molecular remission, in October 2002 after receiving imatinib at a daily oral dose of 600 mg for 27 days combined with vincristine and prednisolone. Immediately following conditioning therapy with cyclophosphamide at 120 mg/kg, cytarabine at 8 g/m^2, and total body irradiation with 12 Gy, the patient underwent an HLA-identical PBSCT (17.7 × 10^6/kg body weight CD34⁺ cells) with a brother as the stem cell donor. Cyclosporine A (CSA) and short-term methotrexate were used for prophylaxis against graft-versus-host disease (GVHD). The patient developed acute GVHD with stage 1 liver damage on day 50 but responded well to treatment with prednisolone in addition to CSA. Discontinuation of prednisolone and CSA did not induce GVHD recurrence. As previously described [9], MRD analyses using real-time quantitative RT-PCR (RQ-PCR) analysis of bone marrow samples were performed monthly from the start of the conditioning regimen. The patient gave written informed consent to participate in this study to assess the utility of MRD analysis after allogeneic SCT, which was reviewed and approved by an institutional review board at Kanazawa University Medical Center. The detection threshold of RQ-PCR in this study is 50 copies/1 μg RNA. The predictive value of the BCR-ABL transcript number for hematological relapse of Philadelphia-ALL in this setting is ≥50 copies/1 μg RNA (unpublished data). The MRD study showed the patient attaining molecular remission on day 28, as defined by a decrease in BCR-ABL transcripts below the detection threshold of RQ-PCR (Figure 1). Molecular evidence of recurrent leukemia on day 95 resulted in the re-initiation of treatment with 600 mg imatinib on day 103. Despite the subsequent detection of bone marrow BCR-ABL fusion-positive cells by fluorescence in situ hybridization (FISH), the patient regained molecular remission with imatinib monotherapy on day 120. The patient discontinued imatinib on day 146 due to the development of imatinib-induced pericardial effusion. Donor leukocyte infusions (DLI) on days 168 and 196 containing 1.2 × 10^8/kg and 1.3 × 10^8/kg CD3⁺ cells, respectively, from the same donor resulted in the reappearance of molecular relapse on day 248.

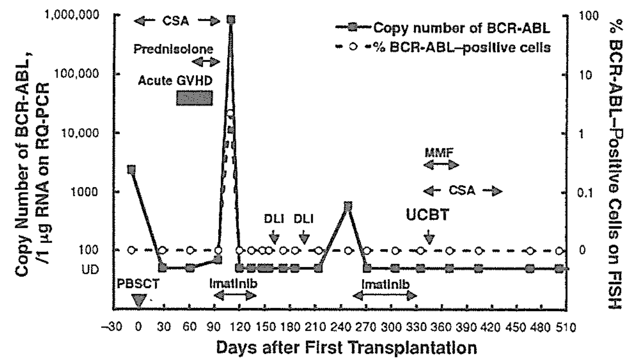

Figure 1. Results of longitudinal RQ-PCR and FISH analyses in bone marrow begun at the start of the preparative regimen for the first transplantation. The detection threshold of BCR-ABL transcripts was 50 copies/μg RNA. UD indicates undetectable levels of transcripts.

Resumption of imatinib on day 257 at a daily oral dose of 400 mg led to molecular remission again with minimal toxicity. In view of the low probability of sustained molecular remission with imatinib [4,5], the urgent need for stem cell transplantation, and the high incidence of regimen-related mortality after conventional second allogeneic SCT in patients with early relapse after first allogeneic SCT [10], reduced-intensity UCBT (RI-UCBT) was planned. The preparative regimen, based on a previous report [11], consisted of cyclophosphamide at 50 mg/kg on day −6, fludarabine at 40 mg/m^2 daily on days −6 to −2, and a single dose of 2 Gy of total body irradiation on day −1. Unrelated cord blood (UCB) that was phenotypically matched and genotypically mismatched at only the DRB1 locus was obtained through the Tohoku Cord Blood Bank. The patient received a UCB graft at a dose of 2.0 × 10^7 nucleated cells/kg of the recipient's body weight in October 2002, 343 days post–first transplantation. To prevent rejection of the graft and GVHD, CSA and mycophenolate mofetil (MMF) were started 3 days before transplantation. Granulocyte colony-stimulating factor was administered from day 1. The patient tolerated the conditioning regimen well, with neutrophil recovery (>5 × 10^8/L) occurring by day 13. Lineage-specific chimerism analysis 25 days posttransplantation showed 100% donor chimerism in both myeloid and T-lymphoid lineages. MMF was discontinued within 3 weeks of UCBT. A gradual tapering of CSA commenced on day 30, and CSA was withdrawn on day 80. On day 140, the patient developed chronic GVHD of the mouth that resolved without treatment. The patient continues to show good performance 30 months after the second transplantation and maintains molecular remission.

3. Discussion

In our patient, the early administration of imatinib, initiated upon molecular evidence of Ph⁺ ALL recurrence after allogeneic SCT, induced molecular remission that has continued after subsequent UCBT with early tapering of CSA. However, the relative contributions of MRD-oriented ima-

tinib treatment, CBT, and early tapering of CSA on her long duration of remission are uncertain.

Concerning imatinib for leukemia relapse after SCT, Wassmann et al [8] reported that in 14 (52%) of 27 Ph^+ ALL patients receiving imatinib upon detection of MRD after SCT, BCR-ABL transcripts became undetectable after a median of 1.5 months. They emphasized that their 48% DFS at 18 months since MRD-triggered imatinib commencement surpassed the 5% DFS in a previous report [5] of imatinib treatment for any Ph^+ ALL relapse after SCT, suggesting a superior response of imatinib in the setting of MRD. However, even with MRD-triggered imatinib after SCT, there is no plateau in survival curves [8]. These findings indicate that imatinib monotherapy is unable to maintain molecular remission in patients with Ph^+ ALL, despite the benefit that treatment with imatinib may provide patients in relapse with a good platform, molecular remission, for subsequent treatment strategies such as SCT.

Takahashi et al [12] showed better outcomes in acute GVHD, treatment-related mortality, and DFS after UCBT than after BMT from unrelated donors. This report may suggest that UCBT could provide the best explanation for the good clinical course in our patient. Thus far, 2 patients with Ph^+ ALL receiving cord blood grafts have been reported [13,14]. Wang et al [14] reported an 11-year-old male patient who received HLA-identical sibling donor CBT during hematologic relapse after chemotherapy. The patient relapsed on day 117 and died of leukemia on day 146. The second was a 3-year-old girl who received HLA 1-antigen-mismatched UCBT during the first hematologic CR [13]. She had maintained long-term remission, but died of leukemia 29 months after UCBT (personal communications). The present case is the first reported case of CBT used to treat a patient with a prior history of SCT. These observations are insufficient to determine the effectiveness of CBT for Ph^+ ALL. However, given that no curative treatment has been established for patients with Ph^+ ALL relapsing after allogeneic SCT [1-4,8], we suggest that CBT could become a promising therapeutic option for the management of such patients.

Our patient was tapered off CSA early after UCBT in an attempt to reduce the chance of relapse due to an enhanced GVL effect. This resulted in successful durable remission without the development of GVHD severe enough to require immunosuppressive therapy. Despite the induction of the GVL effect in some patients with advanced disease, the rapid tapering of CSA could place patients at a risk of developing fatal GVHD [15]. However, the immunological naivety [16] of cord blood lymphocytes may decrease the probability of intractable GVHD after UCBT, allowing the safe reduction of posttransplantation immunosuppression, while the shortened duration of immunosuppression may permit lymphocytes to exert a more potent antileukemic effect. This hypothesis is supported by clinical observations showing similar rates of disease relapse and lower rates of acute and chronic GVHD in adult patients receiving UCBT compared to those receiving allogeneic bone marrow transplantation or PBSCT [11,12,17-19]. In further support, a case report of a child with blast crisis CML was successfully treated with related cord blood transplantation and early withdrawal of CSA [20]. However, it is still unclear whether early tapering of immunosuppression therapy was instrumental in the maintenance of molecular remission in our patient. The correlation of the early tapering of immunosuppression therapy with the sustained molecular remission is only speculative.

Patients undergoing a second allogeneic SCT due to the recurrence of Ph^+ ALL have a very poor prognosis because of increased regimen-related toxicity and a high rate of relapse. With the intention of avoiding severe toxicity, we used a reduced-intensity conditioning regimen that was well-tolerated and achieved durable donor engraftment with minimal GVHD in accordance with results in previous reports of RI-UCBT [11,19].

DLI has a very limited success rate in Ph^+ ALL relapsing after allogeneic SCT [21], likely due in part to a leukemia burden too high at relapse to be eradicated by DLI. Accordingly, the monitoring of MRD after allogeneic SCT is useful for the maximizing antileukemic effects of DLI as well as those of a second transplant. Evidence to this effect can be seen in the reports of 2 patients with MRD levels of leukemia relapse after allogeneic SCT who obtained molecular remission following DLI [22,23]. Recently, Shimori et al [24] reported 2 patients with CML relapsing into lymphoid blast crisis and with Ph^+ ALL, in which the initiation of imatinib led to the elimination of BCR-ABL fusions that was maintained after DLI. However, taking into consideration the persistence of BCR-ABL transcripts after DLI in both patients, the continuation of imatinib treatment, and the short-term follow-up within 5 months of imatinib treatment, it cannot be determined whether these effects are due to imatinib alone rather than the combination of imatinib and DLI. In conjunction with our observation that DLI was ineffective in a patient with molecular remission induced by imatinib prior to DLI, the efficacy of DLI in combination with imatinib remains unclear at present.

The advantages of UCBT are the immediate availability of cells, the absence of a risk to the donor, and a reduced need for HLA compatibility between the donor and recipient [11,2,17-19,25,26]. Because of the establishment of many cord banks, nearly every patient can find a potential cord blood graft, suggesting that a therapeutic approach using imatinib and UCBT guided by molecular monitoring for MRD after SCT could be applied in the majority of patients with Ph^+ ALL. A subsequent study of a large group of patients is required to assess whether imatinib in combination with UCBT is a safe and effective therapy for patients with molecular evidence of recurrent Ph^+ ALL after allogeneic SCT.

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